6-1988

Bovine mammary secretions during involution and the peripartum period: relationship with mastitis pathogen growth

Vijay Kumar Juneja

To the Graduate Council:

I am submitting herewith a thesis written by Vijay Kumar Juneja entitled "Bovine mammary secretions during involution and the peripartum period: relationship with mastitis pathogen growth." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

S.P. Oliver, Major Professor

We have read this thesis and recommend its acceptance:

P.M. Davidson, F.M. Hopkins

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Vijay Kumar Juneja entitled "Bovine Mammary Secretions during Involution and the Peripartum Period:Relationship with Mastitis Pathogen Growth." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Stephen P. Oliver

S.P. Oliver, Major Professor

We have read this thesis
and recommend its acceptance:

P Michael Davidson

Fred W Hopkins

Accepted for the Council:

Lee Minkel

Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master of Science degree at the University of Tennessee, Knoxville, I agree that Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature V.K. Jones

Date 5-17-88

BOVINE MAMMARY SECRETIONS DURING INVOLUTION AND THE PERIPARTUM PERIOD: RELATIONSHIP WITH MASTITIS PATHOGEN GROWTH

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Vijay Kumar Juneja

June 1988

DEDICATION

This thesis is dedicated to my wife, Poonam Juneja. With her love, guidance, and encouragement, anything would be possible.

ACKNOWLEDGMENTS

I would like to express my most sincere thanks and gratitude to Dr. S. P. Oliver whose guidance, encouragement, and patience made this study possible.

I would also like to thank Drs. P. M. Davidson and F. M. Hopkins for their service on my committee and critical evaluation of this thesis.

My thanks go to Kim Yost and Barbara Gillespie for devoting their time in the laboratory to this project.

I thank the Department of Animal Science for granting me the academic and financial opportunity to complete a Master's degree.

I am especially grateful to my family for their support and guidance; for their sacrifices and inspiration which compelled me to achieve and have given me the opportunity to complete this degree.

ABSTRACT

An *in vitro* microassay was used to evaluate growth of eight *Staphylococcus* strains representing five species and five strains of *Corynebacterium bovis* in skims from mammary secretions collected from quarters of five Holstein cows during the nonlactating and peripartum periods. Significant variation in growth among different strains of *Staphylococcus* species and *Corynebacterium bovis* and among cows was observed. All *Staphylococcus* species evaluated followed similar patterns of growth in mammary secretion skims. Mammary secretion skims obtained at 14 and 28 days of involution were poor media for growth of *Staphylococcus* species. Conversely, mammary secretion skims collected at cessation of milking, parturition, and during early lactation supported growth of all species evaluated. *Staphylococcus hyicus* and *Staphylococcus chromogenes* growth was greatest followed by *Staphylococcus epidermidis*, *Staphylococcus xylosus*, and *Staphylococcus hominis*. Similarly, *Corynebacterium bovis* grew well in mammary secretion skims obtained at cessation of milking, at parturition, and 14 days after parturition. However, growth of 4 strains in mammary secretion skims obtained at 14 and 28 day of involution was significantly lower compared to the 5th strain. Growth of *Staphylococcus* species in whey followed a similar pattern, but increased

growth was observed in mammary secretions obtained during involution. Variation in growth of Corynebacterium bovis in mammary secretion skims and whey obtained during involution and peripartum period was not observed. However, growth of Corynebacterium bovis was reduced in whey. Mammary secretion skims were passed through S-200 gel filtration column. Four protein peaks were obtained from each skim sample. Peaks were characterized by two-dimensional polyacrylamide gel electrophoresis. The majority of proteins eluted in peak 1 and 2 from S-200 columns. Protein peaks were evaluated with respect to growth of Staphylococcus species and Corynebacterium bovis. In general, growth of Staphylococcus species and Corynebacterium bovis was reduced in peak 1 and 2 of mammary secretion skims collected during involution. These data suggest that mammary secretion skims support growth of Staphylococcus species and Corynebacterium bovis during lactation but inhibit their growth during the nonlactating period. The presence of high molecular weight biochemical components in mammary secretions are inhibitory to the growth of Staphylococcus species and Corynebacterium bovis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. LITERATURE REVIEW

INTRODUCTION

Mastitis, an inflammation of the mammary gland, is a disease complex that results from the interaction of the cow, the environment, and microorganisms (13). Most mastitis results from the presence of microorganisms within the mammary gland. Mastitis causes large economic losses to the dairy industry resulting from decreased milk production, loss of antibiotic containing milk, cost of veterinary services, cost of extra labor to care for mastitic cows, decreased sale value of cows, increased culling, and loss of genetic material from the herd (34). Reduced milk production associated with subclinical mastitis accounts for the greatest loss (44). Approximately one-half of the dairy cows world-wide have some form of mastitis (61).

Pathogens involved in udder infections have been distinguished into 2 categories: major pathogens, and minor pathogens. Major pathogens are responsible for severe cases of mastitis and can be broadly grouped as contagious and environmental pathogens based on their primary reservoir within a dairy herd. The major contagious pathogens are Staphylococcus aureus and Streptococcus agalactiae with the infected mammary gland

as the major reservoir of these bacteria (21). The environmental pathogens include coliform bacteria (species of Escherichia, Klebsiella, and Enterobacter,) and species of streptococci other than Strep. agalactiae (environmental streptococci). The primary reservoir is the environment in which the cow lives and not the mammary gland (78). Unlike major pathogens, minor pathogens include coagulase-negative staphylococci (CNS) and Corynebacterium bovis. These organisms are rarely associated with marked leukocytosis and clinical manifestations (26).

Minor mastitis pathogens have received little attention from mastitis researchers who have been more concerned with pathogenic microorganisms associated with significant economic loss. Recently, there has been increased interest in minor mastitis pathogens due to: (1) the high prevalence of minor pathogens in herds using mastitis control practices (29); (2) the possible influence of minor pathogens as a natural biological control mechanism against superinfection by major pathogens (7); and (3) the development of new identification systems for staphylococci leading to reevaluation of coagulase-negative staphylococcal data (37,84).

Staphylococcus species

Staphylococcus species are commonly referred to as minor mastitis pathogens and were once thought to be primarily Staph. epidermidis. The attitude towards CNS is perhaps best reflected by a statement in the early 1950's "those staphylococci which produce only narrow zones of alpha haemolysis (1mm or less) are coagulase-negative, weak toxin producers and can be disregarded" (71). However, recent studies have shown that CNS are a heterogenous group of bacteria consisting of several Staphylococcus species (36). Coagulase-negative staphylococci have been considered to be of low pathogenicity and cause mild inflammatory and atrophic lesions (30). Quarters infected with CNS have somatic cell counts (SCC) generally 2-to 3-fold higher than bacteriologically negative quarters. However, the SCC response is of a lower magnitude than that seen with major pathogens (30). Also, SCC in milk from CNS infected mammary glands were higher during early lactation, and increased with advancing age to the fifth lactation (30).

Coagulase-negative staphylococcal infected lactating quarters have been reported to be resistant to superinfection with major pathogens (7). When CNS infected quarters were inoculated with Strep. agalactiae and E. coli, 11 and 62% of quarters became infected, respectively, compared to 91 and 93% of previously

uninfected quarters. Recently, Rainard and Poutrel (63) observed that the frequency of new infections by major pathogens was almost 50% lower in quarters infected with CNS. No information has been reported on the protective role of CNS during the nonlactating period.

Reports on milk production losses as a result of infection with CNS are conflicting (30). Holmberg (33) indicated that greater inflammatory reactions may result from infections by CNS compared to C. bovis. Increased SCC may play a role in restricting new infections, but can also result in marked milk losses because of the negative relationship between milk production and SCC. Timms et al. (82) estimated an 8.7% loss in 305-day milk yield in cows with CNS infections. In another study (35), Staph. epidermidis was positively correlated to herd milk production, suggesting that the organism may not have an adverse effect upon milk yield.

Identification: Previous methods (12,14) for the identification of gram-positive, catalase-positive cocci classified β-hemolytic, coagulase-positive cocci as Staph. aureus. Weakly hemolytic or non-hemolytic, coagulase-negative cocci were identified as Staph. epidermidis or Micrococcus species.

Staphylococci were separated from micrococci on the basis of anaerobic glucose utilization, lysostaphin

susceptibility, anaerobic growth in thioglycolate, and bacitracin susceptibility (2,36). When identification to the species level was attempted on 954 cultures of catalase-positive, clumping-factor and β-hemolysin-negative, gram-positive cocci isolated from the teat and milk of dairy cows, 87% of strains were identified as Staph. xylosus, Staph. epidermidis, Staph. sciuri, Staph. haemolyticus, Staph. hyicus, Staph. chromogenes, Staph. simulans and Staph. cohnii. Nine percent belonged to another group and could not be identified. Only 9 strains were classified as Micrococcus (16). Thus, as a practical approach, tests for separation of staphylococci from micrococci may not be necessary.

Recent procedures for species level identification of staphylococci are modifications of the scheme proposed by Kloos and Schliefer (37,84). Routine identification required numerous media and was time consuming. However, the recent development of commercial identification systems provide a highly suitable method for the identification of staphylococci to the species level. Two systems, the STAPH-Ident and STAPH-Trac systems, have been evaluated and widely used. The STAPH-Ident system only identified 54% of isolates correctly (38). The inability of this system to delineate Staph. chromogenes from Staph. epidermidis was a major problem. However, if additional tests such as acetoin and pigment production were used for

delineation of Staph. hyicus from Staph. epidermidis, 94.3% and 88.1% of mammary gland isolates were identified correctly (84,85). Overall accuracy of the STAPH-Trac system was 91.2% (38). In another study (85), the STAPH-Trac system identified only 67.8% of the isolates correctly. The majority of Staph. hyicus strains were misidentified as Staph. simulans due to a false-negative phosphatase test.

Prevalence and distribution: Staphylococcus species are isolated frequently from nonlactating dairy cows (58) and primigravid heifers (57), teat skin (23) and mammary secretion of lactating cows (30). Coagulase-negative staphylococci are a part of normal teat skin flora and have been found to colonize the teat duct (17). Devriese (17) found these organisms in milk and teat canals, and from teat apexes and teat skin of dairy cows. Variation in the prevalence of IMI caused by specific Staphylococcus species was observed (30). The distribution of Staphylococcus species differed from teat skin, teat canals and mammary secretions (17). Staphylococcus xylosus was isolated frequently from teat skin. On the other hand, Staph. epidermidis and Staph. chromogenes were isolated from the teat canal and milk samples (17). Another study showed that Staph. xylosus and Staph. chromogenes predominated in samples obtained from teat skin and the

most prevalent CNS found in teat ducts and secretions were Staph. chromogenes and Staph. hyicus (5). McDonald (43) observed that the most numerous organism on teat skin was Staph. epidermidis. Recently, Matthews et al. (42) observed that Staph. hyicus was isolated frequently from bovine mammary secretions and accounted for > 50% of Staphylococci isolated during the periparturient period. In the warmer months, Staphylococcus species were isolated more frequently (30). Stage of lactation affected the prevalence of CNS infection and a higher incidence was reported in first lactation cows compared to second lactation cows (30). These data suggest that a heterogeneous population of staphylococci can be isolated from mammary glands of dairy cows, and indicate that the prevalence and distribution of Staphylococcus species may vary from herd to herd.

Staphylococcus species are isolated frequently from milk samples in herds where teat dipping is not practiced (30). These organisms cause a high number of IMI in dairy herds (27,28,35.47). In a herd survey, Jones et al. (35) reported that 27.2% of cows were infected with coagulase-negative staphylococci (CNS). Harmon et al. (30) indicated that 40.3% of cows and 14.3% of quarters were infected with CNS. Postmilking teat dipping reduced the incidence of Staphylococcus species IMI (29). Quarters infected with Staphylococcus species other than Staph.

aureus was 11.0% in a herd when no teat dipping was practiced and 7.2% in herds when teat dipping was practiced (32). Prevalence of Staphylococcus species also differed among herds using different teatdips (32). Prevalence of Staphylococcus species IMI in herds using linear dodecyl benzene sulfonic acid was greater than in herds using chlorhexidine or iodophor teat dips (32). The predominant Staphylococcus species in herds not teat dipping was Staph. epidermidis (37.1%). Teat dipping decreased the prevalence of Staph. epidermidis IMI, but Staph. hyicus IMI increased. On the other hand, when teat dipping was discontinued the prevalence of Staph. hyicus decreased and Staph. epidermidis increased. Somatic cell counts did not differ among quarters with different Staphylococcus species (29,32).

Corynebacterium bovis

Corynebacterium bovis is generally considered a minor mammary gland pathogen (6), and a harmless commensal (10). Corynebacterium bovis has received little research interest because this organism is associated with a mild inflammtory response and apparently is of little economic importance in the dairy industry (10).

Colonization of mammary glands with C. bovis resulted in a slight, but statistically significant, elevation in milk SCC when compared with milk from uninfected glands.

Natural exposure (9) of a large number of strains of C. bovis (9) and experimental study (10) with a single strain resulted in mean SCC of < 200,000/ml, a level not generally considered to be associated with a decrease in milk production or a change in milk composition. Recently, it was confirmed that there was a small but statistically nonsignificant reduction in milk yield and in milk protein and fat composition in quarters colonized by C. bovis (40). However, Natzke et al. (47) observed that quarters harboring C. bovis produced significantly less milk than uninfected quarters.

Prevalence and distribution: Black et al. (4) demonstrated that C. bovis localizes in the teat duct. These observations were confirmed using in vitro techniques which indicated that C. bovis adhered to the squamous epithelium of the teat duct, but not to the distinctly different epithelial cells of the teat cistern or the major ducts of the mammary gland (25). Corynebacterium bovis was isolated from the teat cistern by puncture of the teat wall and removal of milk with a syringe (24). Thus, it appears that although unable to colonize the teat cistern, C. bovis readily gains access to this area.

Corynebacterium bovis is isolated frequently from bovine milk during lactation (4,6). Rates of infection with C. bovis ranged from 9.3% to 85.7% of quarters of

lactating cows (6,9,30). Frequency of C. bovis isolation was reduced significantly in herds using postmilking teat disinfection and dry cow therapy (9). In a herd survey, Jones (35) reported that 20.3% of cows were infected with C. bovis. Bramley (6) observed that 56.6% of quarters were infected with C. bovis before adopting control measures and 5.2% of quarters were infected after 3 years of teat dipping and dry cow therapy. Research has indicated that C. bovis is a highly contagious organism, more so than Staph. aureus or Strep. agalactiae under experimental challenge conditions (60). Corynebacterium bovis colonization of mammary glands persist for long periods. Use of automated backflush systems with an iodophor germicide significantly reduced new C. bovis infections in the absence of teat dipping (31).

Corynebacterium bovis required lipids or Tween 80 for growth (74). Black et al (4) observed that C. bovis died when cultured in trypticase soy broth, but addition of .1% Tween 80 or .1% lipid material increased growth. They (4) suggested that the ability of C. bovis to colonize the streak canal is because of the requirements for nutrients from keratin. Keratin contains a large amount of lipid with long chain fatty acids (1).

Linde et al. (41) observed that quarters colonized with C. bovis were more resistant to induced Strep. dysgalactiae and Staph. aureus IMI than uninfected

quarters. Brooks and Barnum (11) challenged C. bovis colonized quarters of lactating mammary glands by intramammary inoculation of Staph. aureus into the teat sinus and observed increased resistance to infection compared with noncolonized control quarters. However, when Strep. agalactiae was inoculated into the teat sinus, C. bovis colonization did not protect glands against superinfection. Another study (60) indicated that quarters infected with C. bovis were approximately 8.5-fold more susceptible to Strep. agalactiae infection than negative quarters. In contrast, quarters with C. bovis infections were about 53% less susceptible to infection by Staph. aureus than bacteriologically negative quarters. When quarters of lactating mammary glands colonized with C. bovis were inoculated with Strep. uberis, no protection was observed (18). Instead, symptoms of clinical mastitis characterized by increased temperature, elevated SCC in milk, and a marked decrease in milk production was reported. A decreased concentration of the same strain of Strep. uberis caused equally severe mastitis indicating no protection (18).

The protective effect of C. bovis colonization appears to vary depending upon the pathogenic organism involved. Further research is needed to define the mechanism(s) of increased resistance associated with C. bovis colonization. Quarters with an elevated SCC appear

to be less susceptible to infection with pathogens than quarters with a lower SCC (68). Protection of C. bovis colonized mammary gland may be related to slightly higher numbers of somatic cells resulting from C. bovis colonization. Usually IMI occur after a pathogen penetrates the teat canal (50). The protective effect of C. bovis may also be associated with interference in the teat canal because the teat duct is the area in which C. bovis colonizes (4).

DRY PERIOD

The rate of intramammary infections (IMI) during the dry period is usually proportional to the rate of existing infections at drying off (49) and varies depending upon the prevalence of mastitis pathogens in a herd (54,72). The high rate of IMI during the dry period results in more infected glands at parturition than at the end of the previous lactation (55,73). Thus, the high rate of IMI during the dry period is primarily responsible for the high level of IMI in many herds (55).

Neave et al. (48) observed that there was a higher incidence of new IMI throughout the dry period than during lactation and reported that the rate of new IMI during the early dry period period was seven times higher than the rate observed during lactation. Increased susceptibility

during the early dry period has been reported also by others (22,58,70,79). Recent studies on new infections during the dry period revealed that the prepartum period is another time of increased susceptility (22,58,70). Eberhart (22) found that the number of new IMI was highest during the first week postdrying off, decreased to 0 by 4 weeks postdrying off and increased again during the week prior to calving.

Oliver and Mitchell (58) reported on the susceptibility of bovine mammary gland to infection during the dry period in a herd that was Strep. agalactiae-negative and with a low prevalence of Staph. aureus. However, the herd had frequent mastitis caused by environmental streptococci and coliforms. In the absence of antibiotic therapy at drying off, the most commonly encountered bacteria were coagulase-negative staphylococci, streptococcal species other than Strep. agalactiae, C. bovis, and coliforms. Coagulase-negative staphylococcal IMI increased during the dry period and the number was highest at parturition. These results were observed also in heifers at parturition (57). In another study, Oliver (59) reported that the number of quarters infected with CNS increased slightly from late lactation to early involution, was highest at parturition and then decreased during early lactation. Several infections were transient and the rate of spontaneous elimination was high

during the nonlactating period. McDonald (43) indicated that new IMI with Staph. epidermidis was common during the nonlactating period but many IMI were eliminated spontaneously by the cow without therapy during the next lactation. Harmon et al. (27,28) observed that the rate of spontaneous elimination of CNS in quarters not treated with antibiotics at cessation of regular milking was 72.7%. The frequency of isolation of CNS was high at milk cessation, at parturition, and during early lactation, but was low at 7 days of involution and remained low until 14 days before parturition (80). Few studies have shown the efficacy of antibiotic therapy at drying off in the prevention or elimination of Staphylococcus species (6,9,19,29,70). Bramley (6) observed that 13.4% of quarters were infected with CNS before adopting mastitis control measures and observed 6.3% of the quarters were infected with CNS after 3 years of teat dipping and dry cow therapy. Recently, Harmon and Langlois (30) indicated that incidence of CNS averaged 14.3% of quarters and accounted for approximately 60% of all infections in a herd in which teat dipping and dry cow therapy was utilized. Dry cow therapy with novobiocin, cephapirin, or streptomycin-penicillin indicated lowest new infection rate of CNS with cephapirin (29).

McDonald et al. (45) inoculated Staph. aureus directly into the gland sinus at various intervals during

the nonlactating period and observed that 67.3% and 97.3% of the glands became infected during the first and last half of the dry period, respectively. These results were similar to reported rates of new IMI of 73% in glands inoculated with Staph. aureus at cessation of milking (51). When Staph. epidermidis was inoculated, 20.8% and 94.7% of the glands became infected during the first and second half of the dry period, respectively (45). No gland was infected when the organism was inoculated at 7 days after cessation of milking. Thus, mammary glands appear to be susceptible to staphylococcal infection during the dry period particularly during the prepartum period. During early involution, mammary glands appear to be more susceptible to Staph. aureus than Staph. epidermidis.

Oliver (58) observed that the number of quarters infected with C. bovis decreased markedly from late lactation to early involution and further decreased throughout the dry period in the absence of antibiotic therapy at drying off. A marked increase in the number of quarters infected with C. bovis IMI occurred from parturition to early lactation. Mammary glands were highly resistant to C. bovis IMI after early involution and no infections detected at drying off persisted throughout the dry period. Oliver (59) and Harmon et al. (30) reported 96% and 47.6% spontaneous elimination rate, respectively, of C. bovis during the nonlactating period in quarters not

treated with antibiotics at drying off. All quarters became colonized and shed C. bovis throughout the nonlactating and periparturient period when mammary glands were inoculated intracisternally with a streptomycin resistant strain of C. bovis 7 days before cessation of lactation (81). Thus, persistence of C. bovis during the nonlactating period appears to vary among strains of this organism (30,59,81). Eberhart (21) indicated that exposure of teats to contagious pathogens most likely decreases during the dry period due to cessation of regular milking. Rates of elimination of C. bovis IMI was upto 100% in herds using antibiotic therapy at cessation of milking (29).

The rate of new IMI is high when marked physiological transitions occur from lactation to involution and from involution to colostrogenesis (48). The fully involuted gland appears to be highly resistant to new IMI. Significant changes occur in the biochemical composition and antibacterial properties of bovine mammary secretions during physiological transitions of the mammary gland. (53,56,69). As involution progresses, concentrations of lactoferrin (Lf), and immunoglobulin G (IgG) increase, but the concentration of Lf decreases as parturition approaches and remains low during early lactation (53,77). On the other hand, the concentration of citrate, and the citrate to Lf molar ratio decreases as involution

progresses and progressively increases as parturition approaches (53,77). Mammary secretions from fully involuted glands contain elevated numbers of macrophages, polymorphonuclear neutrophils (PMN), and lymphocytes; and high concentrations of Lf and IgG (69,77). All of these components have been implicated as factors of resistance to IMI (8,76,77). Susceptibility or resistance to IMI during the nonlactating period appears to be related to changes in antibacterial components of mammary secretions (8).

Lactoferrin, an iron binding protein with a molecular weight of approximately 78,000, is found in mammary secretion and also in secondary granules of PMN (62,77). Lactoferrin is a minor whey protein in milk and its concentration is .1 to .3mg/ml (69,75). The concentration of Lf increases significantly in dry secretions during involution of the mammary gland (77). The concentration of Lf begins to increase 2 to 4 days after drying off and increases continuously during the first 14 to 21 days of involution (83). The concentration of Lf reaches maximum levels of 20-30mg/ml after 21 to 28 days of involution, though Lf varies from cow to cow and may be in excess of 100 mg /ml (83). Thus, Lf becomes a major whey protein in mammary secretion during the dry period and the concentration is increased approximately 100-fold (76,83). Lactoferrin levels subsequently decrease as parturition

approaches (83) and the concentration is 2-3mg/ml in colostrum (69,83).

One mole of Lf can bind two molecules of iron and when fully saturated, Lf inhibits growth of microorganisms that require iron for their growth (3,15). Bicarbonate is required for binding of iron to Lf (15). The bacteriostatic effect of Lf can be reversed by citrate which chelates iron in a form available for uptake by microbes(3,39). High concentrations of citrate in bovine colostrum and milk can be utilized by a number of enteric bacteria to sequester iron (64) resulting in a reduction of the antibacterial role of Lf (3,65). The ability of bovine mammary secretions to inhibit growth of coliforms was inversely related to the molar ratio of citrate to Lf in the secretions (3,8,52,53). A decrease in the molar ratio of citrate to Lf resulted in a simultaneous increase of the inhibition of the coliform strains (52,53). Bishop et al. (3) observed that a molar ratio of 75 resulted in 50% growth inhibition where as a ratio of >300 resulted in less than 10% growth inhibition. Nonnecke and Smith (53) suggested that a molar ratio of citrate to Lf of > 1000 would be expected to have negligible inhibitory capacity. However, mammary secretion would be expected to be inhibitory to coliform growth during the dry period since the molar ratio of citrate to Lf is generally <20.

Bishop et al. (3) observed that growth of coliforms was inhibited when bovine apo-Lf was added to the growth medium. Inhibition of growth increased when the concentration of apo-Lf increased from .02 to 2mg/ml. However, as the concentration increased further, less inhibition was observed. Nonnecke and Smith (52) indicated that apo-Lf inhibited growth of coliforms, staphylococci and streptococci but the inhibition was greater for coliforms. Growth inhibition increased with increased concentrations of apo-Lf to 5.0mg/ml. A further increase in the concentration of apo-Lf did not result in greater inhibition. Rosenberg and Young (64) reported that decreased growth inhibition at higher concentration of apo-Lf may be due to the ability of coliform bacteria to secret a high affinity iron chelator, enterochelin, in response to iron deprivation. Coliform bacteria have atleast 2 systems for iron acquisition (15). The first system involves the synthesis and secretion of enterochelin into the growth medium. The second iron acquisition system is citrate mediated and is induced by citrate in the growth medium.

Bishop (3) and Nonnecke (52) demonstrated that addition of iron to the growth medium that contained apo-Lf abolished inhibition of coliforms indicating the iron dependent nature of apo-Lf. The addition of increasing concentrations of citrate to the growth medium

containing apo-Lf resulted in increased coliform growth (3,52). Bicarbonate supplementation to the growth medium containing apo-Lf increased inhibition of coliforms (52).

Slight variation in growth of Staph. aureus in mammary secretions collected during the the dry period was observed (20). After a significant reduction in growth in secretions collected between drying off and 7 days after drying off, growth increased throughout the dry and peripartum periods and growth of Staph. aureus was similar to that observed at drying off. Schade (67) observed that Lf had a minimal effect on the growth of Staph. aureus. Staphylococci are susceptible to iron deprivation (66) and under poor iron conditions may produce iron chelators to maintain their iron supplies (46).

Staphylococcus aureus grown in normal rabbit serum synthesized bacterial iron chelators which competed with transferrin and Lf (67). However, Marcelis et al. (46) observed that strains of Staph. epidermidis were highly susceptible and strains of Staph. aureus were comparatively less susceptible to iron deprivation.

SUMMARY

The mammary gland is highly susceptible to new intramammary infections during transitions of the gland from lactation to involution and from involution to colostrogenesis. Factors involved in changing susceptibility are not fully understood and most likely include changes in bacterial populations to which the teat end is exposed, changes in the teat canal, increased fluid accumulation, and changes in the composition of mammary secretions.

The ability of components of mammary secretion to inhibit mastitis pathogens varies during the nonlactating period. Different components of mammary secretions appear to have different effects on bacterial growth. The relationship between minor mastitis pathogen growth and non-cellular components of mammary secretions during the dry period has not been documented. Little is known about the role of minor mastitis pathogens despite the observation that these organisms frequently constitute the greatest proportion of infected quarters in lactating and nonlactating cows. Some studies have shown that quarters infected with minor pathogens during lactation are more resistant to new infections with major mastitis pathogens than noninfected quarters. The importance of these observations in the control of mastitis requires further

study as some studies have shown no benefit of the presence of minor pathogens in preventing new infections by major pathogens. The primary objective of this study was to examine growth of minor mastitis pathogens in mammary secretions collected at various stages of the dry period. In addition, studies were conducted to relate growth of minor mastitis pathogens with antibacterial components of mammary secretions.

REFERENCES

1. Adams, E.W., and C.G. Rickard. 1963. The antistreptococci activity of bovine teat canal keratin. Am. J. Vet. Res. 24:122.

2. Baird-Parker, A.C. 1974. Staphylococcus, p 478-490, In Bergey's Manual of Determinative Bacteriology, 8th ed. R.E. Buchanan and N.E. Gibbons (eds), Willians and Wilkings, Baltimore, MD.

3. Bishop, J.G., F.L. Schanbacher, L.C. Ferguson, and K.L. Smith. 1976. In vitro growth inhibition of mastitis causing coliform bacteria by bovine apo-lactoferrin and reversal of inhibition by citrate and high concentration of apo-lactoferrin. Infect. Immun. 14:911.

4. Black, R.T., R.T. Marshall, and C.T. Bournald. 1972. Locus of mammary gland infections of Corynebacterium bovis. J. Dairy Sci. 55:413.

5. Boddie, R.L., S.C. Nickerson, W.E. Owens, and J.L. Watts. 1987. Udder microflora in nonlactating heifers. Agri-Practice. 8:28.

6. Bramley, A.J. 1975. Infection of the udder with coagulase-negative micrococci and Corynebacterium bovis. page 377 in proc. seminar on Mastitis control. Bull. Doc. 85. Int. Dairy Fed., Brussels, Belgium.

7. Bramley, A.J. 1978. The effect of subclinical Staphylococcus epidermidis infection of the lactating bovine udder on its susceptibility to infection with Streptococcus agalactiae or Escherichia coli. Brit. Vet. J. 134:146.

8. Breau, W.C., and S.P. Oliver. 1986. Growth inhibition of environmental mastitis pathogens during physiologic transitions of the bovine mammary gland. Am. J. Vet. Res. 47:218.

9. Brooks, B.W., D.A. Barnum, and A.H. Meek. 1983. An observational study of Corynebacterium bovis in selected Ontario dairy herds. Can. J. Comp. Med. 47:73.

10. Brooks, B.W., and D.A. Barnum. 1984. Experimental colonization of the bovine teat duct with Corynebacterium bovis and the effect on milk somatic cell counts. Can. J. Comp. Med. 48:141.

11. Brooks, B.W., and D.A. Barnum. 1984. The susceptibility of bovine udder quarters colonized with Corynebacterium bovis to experimental infection with Staphylococcus aureus or Streptococcus agalactiae. Can. J. Comp. Med. 48:146.

12. Brown, R.W., G.E. Morse, F.H.S. Newbould, and L.W. Slanetz. 1969. Staphylococci, p. 14-17. In Microbiological procedures for the diagnosis of bovine mastitis. NMC, Arlington, VA.

13. Brown, R.W., R.J. Eberhart, J.S. McDonald, R.P. Natzke, D.S. Postle, and O.W. Schalm. 1972. Supplement to current concepts on bovine mastitis. National Mastitis Council, Washington. DC.

14. Brown, R.W., D.A. Barnum, D.E. Jasper, J.S. McDonald, and W.D. Schultze. 1981. Staphylococci, p 16-20. In Microbiological Procedures for use in the diagnosis of bovine mastitis. NMC, Arlington, VA.

15. Bullen, J.J., H.J. Rogers, and E. Goriffitus. 1978. Role of iron in bacterial infection. Current Topics in Immunology. 80:1.

16. Devriese, L.A. 1979. Identification of clumping-factor-negative staphylococci isolated from cows' udders. Res. Vet. Sci. 27:313.

17. Devriese, L.A., and H. Dekeyser. 1980. Prevalence of different species of coagulase-negative staphylococci on teats and in milk samples from dairy cows. J. Dairy Res. 47:155.

18. Doane, R.M., S.P. Oliver, R.D. Walker, and E.P. Shull. 1987. Experimental infection of lactating bovine mammary glands with Streptococcus uberis in quarters colonized by Corynebacterium bovis. Am. J. Vet. Res. 48:749.

19. Dodd, F.H., and T.K. Griffin. 1975. The role of antibiotics treatment at drying off in the control of mastitis. pp.282 in Proc. Seminar Mastitis Control. Bull. Doc. 85. Int. Dairy Federation, Brussels, Belgium.

20. Dutt. K.W., R.J. Eberhart, and R.A. Wilson. 1986. In Vitro growth of mastitis pathogens in mammary secretions of the dry and peripartum period. J. Dairy Sci. 69:2408.

21. Eberhart, R.J., and J.M. BuckaLew. 1972. Evaluation of a hygiene and dry period therapy program for mastitis control. J. Dairy Sci. 55:1683.

22. Eberhart, R.J. 1982. New infections in the dry period. 21st Annual Meeting, National Mastitis Council, INC. 1982.

23. Edwards, S.J., and G.W. Jones. 1966. The distribution and characteristics of coagulase-negative staphylococci of the bovine udder. J. Dairy Res. 33:261.

24. Forbes, D. 1970. The pathogenic significance of various intramammary infections. Br. Vet. J. 126:260.

25. Frost, A.J. 1975. Selective adhesion of microorganisms to the ductular epithelium of the bovine mammary gland. Infect. Immun. 12:1154.

26. Griffin, T.K., F.H. Dodd, and F.K. Neave. 1977. A method of diagnosing intramammary infection in dairy cows for large experiments. J. Dairy Res. 44:25.

27. Harmon, R.J., B.E. Langlois, W.L. Christ, and R.W. Wemkin. 1981. Characterization of coagulase-negative staphylococci isolated from quarter milk samples and associated somatic cell counts. J. Dairy Sci. 64 (Suppl. 1):147 (Abstr).

28. Harmon, R.J., B.E. Langlois, W.L. Christ, and R.W. Hemken. 1982. Lactation age, stage of lactation, and somatic cell count relationships associated with coagulase-negative staphylococcal infection of the udder. J. Dairy Sci. 65 (Suppl.1):169 (Abstr).

29. Harmon, R.J., W.L. Crist, R.W. Hemken, and B.E. Langlois. 1986. Prevalence of minor udder pathogens after intramammary dry treatment. J. Dairy Sci. 69:843.

30. Harmon, R.J., and B.E. Langlois. 1986. Prevalence of minor mastitis pathogens and associated somatic cell counts. Proc. NMC.

31. Hogan, J.S., R.J. Harmon, B.E. Langlois, R.W. Hemken, and W.L. Crist. 1984. Efficacy of an iodine backflush for preventing new intramammary infections. J. Dairy Sci. 67:1850.

32. Hogan, J.S., D.G. White, and J.W. Penkey. 1987.

Effects of teat dipping on intramammary infections by staphylococci other than Staphylococcus aureus. J. Dairy Sci. 70:873.

33. Holmberg, O. 1973. Staphylococcus epidermidis isolated from bovine milk:biochemical properties, phage sensitivity and pathogenicity for the udder. Acta Vet. Scand (Suppl.) 45:1.

34. Jainzen, J.J. 1970. Economic losses resulting from mastitis. A review. J. Dairy Sci. 53:1151.

35. Jones, G.M., R.E. Pearson, C.W. Heald, and W.E. Vinson. 1982. Milk Loss, Somatic cell counts and udder infections in virginia herds. Page 31 in Proc. Natl. Mastitis Counc. Annu. Mtg.

36. Kloos, W.E., and K.H. Schliefer. 1975. Simplified scheme for routine identification of human Staphylococcus species. J. Clin. Microbiol. 1:82.

37. Kloos, W.E., and J.F. Wolfshohl. 1982. Identification of Staphylococcus species with the API STAPH-IDENT system. J. Clin. Microbiol. 16:509.

38. Langlois, B.E., R.J. Harmon, and K.A. Kers. 1983. Identification of Staphylococcus species of bovine origin with the API STAPH-Ident system.

39. Law, B.A., and B. Reiter. 1977. The isolation and bacteriostatic properties of lactoferrin from bovine milk whey. J. Dairy Res. 44:595.

40. Levan, P.L., R.J. Eberhart, and E.M. Kesler. 1985. Effects of natural intramammary Corynebacterium bovis infection on milk yield and composition. J. Dairy. Sci. 68:3329.

41. Linde, C., O. Holmberg, and G. Astorm. 1980. The interference between coagulase-negative staphylococci and Corynebacterium bovis and the common udder pathogens in the lactating cow. Nord. Vetinaermed. 32:552.

42. Mathews, K.R., R.J. Harman, and B.E. Langlois. 1988. Prevalence of Staphylococcus species in primiparous and multiparous cows during the periparturient period. proc. NMC.

43. McDonald, J.S. 1977. Streptococcal and Staphylococcal mastitis. Am. J. Vet. Res. 170:1157.

44. McDonald, J.S. 1979. Symposium: Bovine mastitis. J. Dairy Sci. 62:117.

45. McDonald, J.S., and A.J. Anderson. 1983. Intramammary Inoculation of the dairy cow with Staphylococcus aureus and Staphylococcus epidermidis during the nonlactating Period. Am. J. Vet. Res. 44:244.

46. Marcelis, J.H., H.J. Dendass-Slogt, and J.A. Hoogkamp-Korstanje. 1978. Iron requirement and chilator production of Staphylococci, Streptococcus faecalis, and Enterobacteriaceae. Antonic Van Leauwanhock. J. Microbiol. Serol. 44:257.

47. Natze, R.P., R.W. Everett, R.S. Guthrie, J.F. Keown, A.M. Meek, W.G. Merrill, S.J. Roberts, and G.H. Schmidt. 1972. Mastitis control program. Effect on milk production. J. Dairy Sci. 55:1256.

48. Neave, F.K., F.H. Dodd, and E. Henriques. 1950. Udder infections in the dry period. J. Dairy Res. 17:37.

49. Neave, F.K., and J. Oliver. 1962. The relationship between the number of mastitis pathogens placed on the teats of dry cows, their survival and the amount of intramammary infection caused. J. Dairy Res. 29:79.

50. Newbould, F.H.S., and F.K. Neave. 1965. The response of the bovine mammary gland to an infection of staphylococci. J. Dairy Res. 32:163.

51. Newbould, F.H.S. 1979. The use of induced mammary infections for evaluating dry cow treatment products. I. Development of a method. Can. J. Comp. Med. 43:426.

52. Nonnecke, B.J., and K.L. Smith. 1983. Inhibition of mastitis bacteria by bovine milk apo-lactoferrin evaluated by in vitro microassay of bacterial growth. J. Dairy Sci. 67:606.

53. Nonnecke, B.J., and K.L. Smith. 1984. Biochemical and antibacterial properties of bovine mammary secretions during mammary involution and at parturition. J. Dairy Sci. 67:2863.

54. Oliver, J., F.H.Dodd, F.K. Neave, and J.M.Lee. 1956. Udder infections in the dry period.2. The effect of withdrawing secretion from the dry udder on the incidence of infection. J. Dairy Res. 23:194.

55. Oliver, J., F.K. Neave, and M.E. Sharpe. 1962. The

prevention of infection in the dry udder. J. Dairy Res. 29:95.

56. Oliver S.P., and K.L. Smith. 1982. Milk yield and secretion composition following intramammary infusion of colchicine. J. Dairy Sci. 65:204.

57. Oliver, S.P., and B.A. Mitchell. 1983. Intramammary infections in primigravid heifers near parturition. J. Dairy Sci. 66:1180.

58. Oliver, S.P., and B.A. Mitchell. 1983. Susceptibility of bovine mammary glands to infections during the dry period. J. Dairy Sci. 66:1162.

59. Oliver, S.P. 1987. Importance of the dry period in the control of intramammary infections by environmental mastitis pathogens. Proc. NMC.

60. Pankey, J.W., S.C. Nickerson, R.L. Boddie, and J.S. Hogan. 1985. Effects of Corynebacterium bovis infection on susceptibility of major mastitis pathogens. J. Dairy Sci. 68:2684.

61. Philpot, W.N. 1967. Influence of subclinical mastitis on milk production and milk composition. J. Dairy Sci. 50:978.

62. Querinjean, P., P.L. Masson and J.F. Heremans. 1971. Molecular weight, single chain structure and amino acid composition of human bactoferrin Eur. J. Biochem. 20:420.

63. Rainard, P., and B. Poutrel. 1988. Effect of naturally occurring intramammary infections by minor pathogens on new infections by major pathogens in cattle. Am. J. Vet. Res. 49:327.

64. Rosenberg, H., and I. Young. 1974. Iron Transport in the enteric bacteria in Microbial Iron Metabolism, J.B. Neilands, Ed., Academic Press, New York, 67.

65. Reiter, B., J.H. Brock, and E.O. Steel. 1975. Inhibition of Escherichia coli by bovine colostrum and post-colostral milk. II. The bacteriostatic effect of lactoferrin on a serum susceptible and serum resistant strain of E. Coli. Immunology. 28:83.

66. Schade, A.L., C. Pallavicini, and U.Wresmann. 1968. Ekkrinosiderophilin of human milk. Protides Biol. Fluids Proc. Collog. 16:619.

67. Schade, A.L. 1975. Growth of Staphylococcus aureus as controlled by the percentage of iron saturation of Ekkrinosiderophilin (lactoferrin) of human milk, p.266-269. In D. Schlessinger (ed.), Microbiology-1974. American Society for Microbiology, Washington, D.C.

68. Schalm,O.W., J.Lasmanis, and E.J. Caroll. 1964. Effects of pre-existing leucocytosis on experimental coliform (Aerobacter aerogenes) mastitis in cattle. Am.J.Vet.Res.25.83.

69. Schambacher, K.L., and K.L.Smith. 1975. Formation and role of unusual whey proteins and enzymes:relation to mammary function. J. Dairy Sci. 58:1049.

70. Schultze, W.D. 1983. Effects of a selective regimen of dry cow therapy on intramammary infection and on antibiotic sensitivity of surviving pathogens. J. Dairy Sci. 66:892.

71. Slanetz, L.W. and C.H. Bartley. 1953. The diagnosis of Staphylococcal mastitis, with special reference to the characteristics of mastitis staphylococci. J. Infect. Dis. 92:139.

72. Smith, A., D.C. Westgarth, M.R. Jones, F.K. Neave, F.H. Dodd, and G.C. Brander. 1967. Methods of reducing the incidence of udder infection in dry cows. Vet. Rec. 81:504.

73. Smith, A., F.H.Dodd, and F.K.Neave. 1968. The effect of intramammary infection during the dry period on the milk production of the affected quarter at the start of the succeeding lactation. J. Dairy Res. 35:287.

74. Smith, R.F. 1970. Fatty acid requirements of human cutaneous lipophilic Corynebacteria. J. Gen. Microbiol. 60:259.

75. Smith, K.L., and F.L. Schanbacher. 1977. Lactoferrin as a factor of resistance to infection of the bovine mammary gland. J. Am. Vet. Med. Assoc. 170:1224.

76. Smith K.L., and S.P. Oliver. 1981. Lactoferrin: A component of non-specific defense of the involuting bovine mammary gland. In: The Ruminant Immune system. Ed. J.E. Butler. Adv. Exp. Med. and Biol. 137:535.

77. Smith, K.L., and D.A. Todhunter 1982. The physiology

of mammary gland during the dry period and the relationship to infection. In proceedings, Ann. Meet. Natl. Mastitis Council. 87.

78. Smith K.L. 1983. Mastitis control: a discussion. J. Dairy Sci. 66:1790.

79. Smith, K.L., D.A.Todhunter, and P.S. Schoenberger. 1985. Environmental pathogens and intramammary infection during the dry period. J. Dairy Sci. 68:402.

80. Sordillo, L.M., S.C. Nickerson, R.M. Akers, and S.P. Oliver. 1987. Secretion composition during bovine mammary involution and the relationship with mastitis. Int. J. Biochem. 19:1165.

81. Sordillo, L.M., S.P. Oliver, R.M. Doane, E.P. Shull and J.L. Maki. 1988. Duration of experimental Corynebacterium bovis colonization of bovine mammary gland during the lactating, nonlactating, and peripartum periods. Am. J Vet. Res. (accepted for publication)

82. Timms, L.L. 1984. Studies on bovine mastitis: 1) The role of minor pathogens, 2) N-acetyl-B-Dglucosaminidase (NAGase) for monitoring udder health. Ph.D. Thesis. University of Wisconsin-Madison.

83. Welty, F.K., K.L. Smith, and F.L. Schanbacher. 1976. Lactoferrin concentration during involution of the bovine mammary gland. J. Dairy Sci. 59:224.

84. Watts, J.L., J.W. Pankey, and S.C. Nickerson. 1984. Evaluation of the Staph-Ident and STAPHase systems for indentification of staphylococci from bovine intramammary infection. J. Clin. Microbiol. 20:448.

85. Watts, J.L., and S.C. Nickerson. 1985. A comparison of the STAPH-Ident and STAPH-Trac systems to conventional methods in the identification of staphylococci isolated from bovine udder. Vet. Microbiol. 12:179.

II. *STAPHYLOCOCCUS* SPECIES

ABSTRACT

Quarter samples of mammary secretions were collected from 5 cows at 0, 14, and 28 days of involution, at parturition, and 14 days after parturition and used in a microassay to evaluate growth of several *Staphylococcus* species. Significant variation between species and among strains of a species was observed. However, all *Staphylococcus* species evaluated followed similar patterns of growth in bovine mammary secretions. Mammary secretions obtained at 14 and 28 days of involution were poor media for growth of *Staphylococcus* species. Conversely, mammary secretions collected at cessation of milking, parturition, and during early lactation supported growth of all species evaluated. *Staphylococcus hyicus* and *Staphylococcus chromogenes* growth was greatest followed by *Staphylococcus epidermidis*, *Staphylococcus xylosus*, and *Staphylococcus hominis*. Results of this study suggest that the ability of bovine mammary secretions to support or inhibit growth of *Staphylococcus* species is related to the stage of the nonlactating period which may influence the rate of *Staphylococcus* species intramammary infection.

INTRODUCTION

Staphylococcus species have been reported to cause a high proportion of intramammary infections (IMI) in lactating and nonlactating heifers and cows (5,20,21). They are commonly referred to as minor mastitis pathogens and were once thought to be primarily Staphylococcus epidermidis. However, recent studies (13) have shown that coagulase-negative staphylococci are a heterogenous group of bacteria consisting of several Staphylococcus species.

The importance of Staphylococcus species IMI that originate during the nonlactating period is, for the most part, unknown. A recent report by Oliver (23) indicated that in the absence of antibiotic therapy, the number of quarters infected with coagulase-negative staphylococci increased from the last milking of lactation to parturition. However, many quarters infected during the early nonlactating period were not infected at parturition. On the other hand, many new coagulase-negative staphylococcal IMI occurred during the prepartum period. These data suggest that bovine mammary glands are susceptible to new IMI by Staphylococcus species during physiological transitions of the udder and that the rate of spontaneous elimination of Staphylococcus species is high during the nonlactating period.

Several factors may play a role in the susceptibility or resistance of the bovine mammary gland to new IMI by Staphylococcus species during the nonlactating period. These include: changes in bacterial exposure of teats, changes in the survivability of bacteria within the teat canal, and variation in concentrations of antibacterial components of mammary secretions.

Previous studies (4,19) characterized antibacterial components of mammary secretions during the nonlactating period. In vitro growth of mastitis pathogens such as Staphylococcus aureus, Streptococcus species, and coliforms have been reported (7,10,22,27). However, the relationship of changes in mammary secretion composition during the nonlactating period with growth of Staphylococcus species is not known. The objective of this study was to evaluate growth of several Staphylococcus species in bovine mammary secretions collected during different stages of the nonlactating and peripartum periods.

MATERIALS AND METHODS

Experimental animals

Five Holstein cows maintained under identical conditions from the University of Tennessee dairy research herd were used. Mammary secretions from all quarters were

collected at the last milking of lactation, at 14 and 28 days of involution, at parturition, and 14 days after parturition.

Mammary secretion preparation

Samples of mammary secretion were centrifuged at 48,000 x g for 20 min to 1h at 0 C to remove fat and cellular debris. Skim samples were sterilized by filtration through a series of filters with decreasing pore size down to .45um and stored at -20 C. Sterility was determined by plating skim samples on blood agar and incubating for 48h at 37 C.

Bacteria

Eight Staphylococcus strains representing five species and isolated from bovine milk were evaluated:

Staphylococcus hyicus (n=1)
Staphylococcus chromogenes (n=2)
Staphylococcus epidermidis (n=2)
Staphylococcus hominis (n=2)
Staphylococcus xylosus (n=1)

Bacteria were identified to the species level using the Analytical Profile Index Staph-Trac-System(Analytab Products,Plainview,NY)(Table1)(14). Results of tests were recorded and converted to a seven digit profile number. Species identification was performed by comparison of the

Table 1. Identification of *Staphylococcus* species by Analytical Profile Index Staph-Trac system.

Staphylococcus Species	Initial isolation Blood-esculin Pigment	Subculture -P agar Pigment	Lysostaphin Sensitivity	Bacitracin Sensitivity	Novobiocin Sensitivity	Coagulase	No Substrate	D-Glucose	D-Fructose	D-Mannose	Maltose	Lactose	D-Trehalose	D-Mannitol	Xylitol	D-Melibiose	Nitrate	Alkaline Phosphatase	Acetyl-Methyl Carbinol (VP)	Raffinose	D-Xylose	Saccharose	α-Methyl-glucoside	N-acetyl-glucosamine	Arginine dehydrolase	Urase
Staphylococcus epidermidis																										
Strain I	White	White	+	-	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	+	-	-	-	+
Strain II	Cream	White	+	-	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	+	-	-	+	+
Staphylococcus hominis																										
Strain I	Tan	Yellow	+	-	+	-	-	+	+	-	+	+	+	+	-	-	+	-	+	-	-	+	-	-	+	+
Strain II	Tan	Yellow	+	-	+	-	-	+	+	-	+	+	+	+	-	-	+	-	+	-	-	+	-	-	+	+
Staphylococcus chromogenes																										
Strain I	Cream	Tan	+	-	+	-	-	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	-	-	+	+
Strain II	Cream	Yellow	+	-	+	-	-	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	-	+	+	+
Staphylococcus Xylosus	White	White	+	-	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	-	+	-	+
Staphylococcus hyicus	Cream	White	+	-	+	+	-	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	-	+	+	-

+ = utilized; - = did not utilize.

profile number to the Staph-Trac identification codebook. Identification of Staphylococci followed the classification scheme proposed by Kloos and Schleifer (13). All Staphylococcus species were coagulase-negative, except Staph. hyicus, and were sensitive to lysostaphin and resistant to bacitracin. They were also sensitive to novobiocin, except Staph. xylosus. All Staphylococcus species utilized D-glucose-, D-fructose-, lactose-, saccharose-, nitrate, urea (except Staph. hyicus), and α-Naphthyl-phosphate (except Staph. hominis), and did not utilize xylitol-, D-melibiose-, raffinose-, and α-methyl-glucoside.

Two to five colonies of each bacteria grown on blood agar plates were inoculated into 100 ml of sterile ultra high temperature milk and incubated at 37 C until bacteria reached logarithmic growth (approximately 12h). Bacterial cultures were aliquoted into polypropylene vials containing glycerine (15% w/v) and vials were frozen at -80 C.

Microassay of bacterial growth

Frozen vials were thawed and diluted serially in sterile phosphate-buffered saline (PBS;.2M, pH6.8). The number of colony forming units (cfu) per ml was determined by plating dilutions on blood agar and Baird Parker medium prior to conducting the assay.

Sterile microtiter plates (120mmx80mm) with 96 U-shaped wells were used for assays of bacterial growth(4,18,19). Filtered skim (250 ul) or 250 ul of brain heart infusion broth (BHIB controls,Difco Laboratories, Detroit, MI), and 10ul of bacterial inoculum containing about 100 cfu were add to wells. Samples were evaluated in duplicate. Prepared microtiter plates were covered with sterilized lids and incubated at 37 C for 12h. After incubation, contents of individual wells were diluted serially in PBS and four-10ul drops of appropriate dilutions were placed on half plates of Baird Parker medium (Difco Laboratories, Detroit, MI). Some samples (100 ul) were plated on Baird Parker medium without dilution. After incubation at 37 C for 18-24h, colonies were counted using an automated bacterial colony counter. Samples were repeated when the difference between duplicate wells was > .5 cfu($\log_{10}$)/ml.

Statistical analysis

Data were expressed as cfu($\log_{10}$)/ml and analyzed by analysis of variance. Experimental design was split plot using the following model:

$$Y_{ijk} = u + S_i + Q_j + SQ_{ij} + B_k + BQ_{jk} + BS_{ik}(Q)_k + E_{ijk}$$

Y_{ijk} = concentration/ml of bacteria in mammary secretion from an experimental day of the nonlactating period,

u = overall mean,

S =stage of the dry period,

Q =quarter of the gland,

SxQ =stage by quarter interaction,

B =bacterial type,

BxQ =bacterial type by quarter interaction,

BxS(Q)=bacterial type by stage within quarter interaction.

Means were evaluated for significance using Student-Newman Keul's mean separation procedure (25).

RESULTS

Growth of Staphylococcus species in mammary secretions from different quarters of a cow was similar and no significant sampling day by quarter interaction was observed (Table 2). However, mammary secretions from different cows significantly ($P<.05$) influenced growth of Staphylococcus species, but differences tended to be minimal (Table 3).

Staphylococcus species growth in mammary secretions obtained during the nonlactating and periparturient periods is presented in Figure 1. In general, mammary secretions collected at 14 and 28d of involution were poor

Table 2. Growth of Staphylococcus species in mammary secretions from different quarters.

Quarter	$\bar{X}$[1]	SD
Right front	4.57[a]	2.60
Right rear	4.49[a]	2.66
Left rear	4.51[a]	2.67
left front	4.50[a]	2.72

[1] Data expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.

Table 3. Variation of Staphylococcus species growth in mammary secretions from different cows.

Cows	$\bar{X}^{1,2}$	SD
1	4.40^{b}	2.61
2	4.37^{b}	2.87
3	4.68^{a}	2.67
4	4.66^{a}	2.47
5	4.48^{b}	2.67

[1]Data expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.
[2]Means with different superscripts differ ($p<.05$).

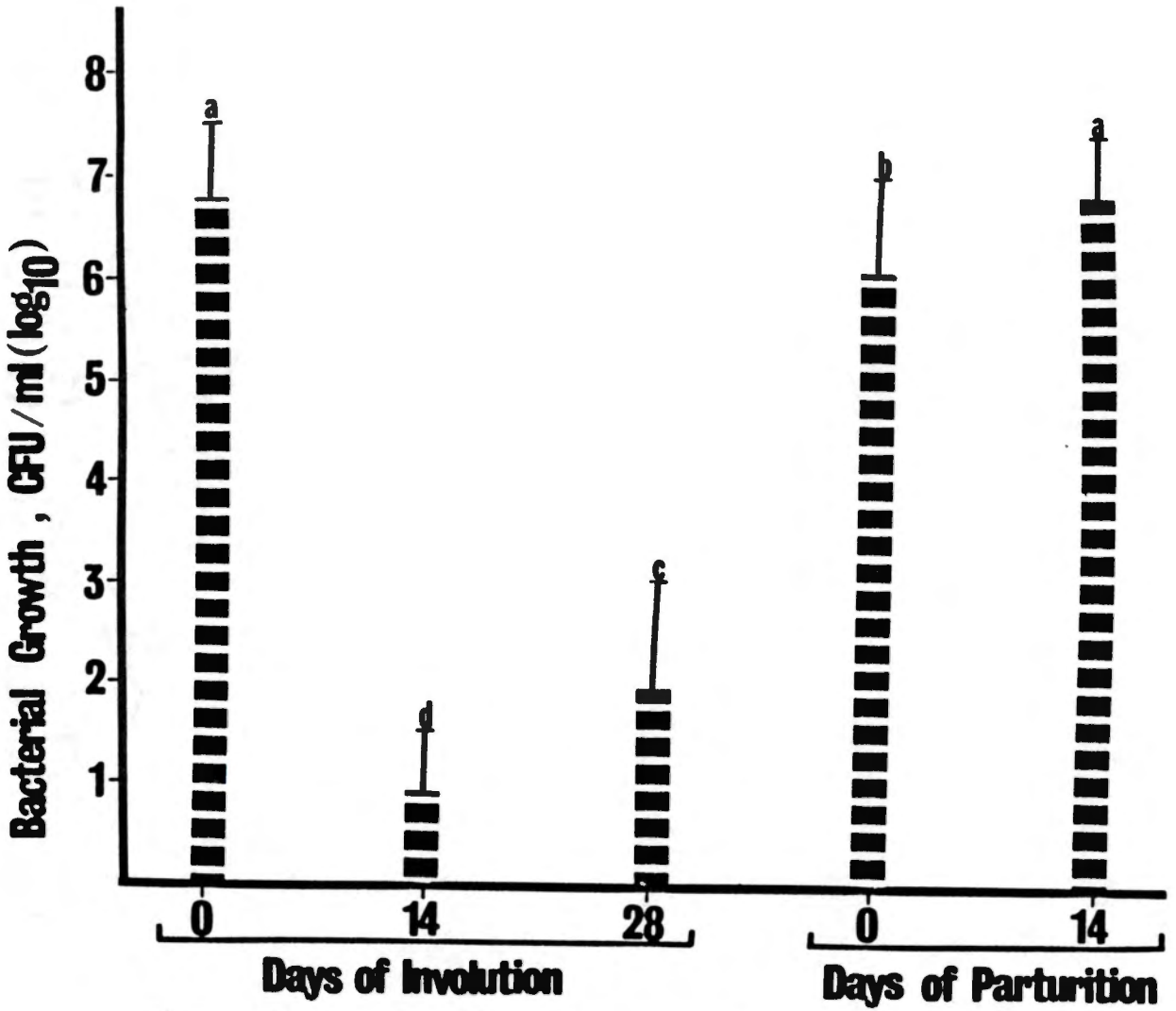

Figure 1. Growth of Staphylococcus species in mammary secretions during the nonlactating and peripartum periods.

media for growth of Staphylococcus species. On the other hand, mammary secretions collected at cessation of milking, at parturition, and during early lactation supported growth of all species evaluated.

Variation in Staphylococcus species growth in mammary secretions and in BHIB control medium are shown in Table 4. Significant ($P<.05$) variation in growth between Staphylococcus species and among strains of a species was observed. In general, one strain of a species tended to grow better in mammary secretions than the other strain. However, differences tended to be slight. Growth of Staphylococcus species in BHIB control medium was consistently 7 to 8.5 cfu ($\log_{10}$) /ml and always exceeded growth in mammary secretions.

Growth of two strains of Staph. epidermidis in bovine mammary secretions collected during the nonlactating and peripartum periods is shown in Figure 2. Mean growth of one strain was significantly higher ($P<.05$) than the other (Table 4). However, both strains of Staph. epidermidis followed a similar pattern of growth and grew well in mammary secretions collected at cessation of milking, parturition, and early lactation. Bacterial growth decreased markedly in mammary secretions obtained during involution. Similar results were obtained with Staph. hominis (Figure 3), Staph. chromogenes (Figure 4), Staph. hyicus (Figure 5), and Staph. xylosus (Figure 5).

Table 4. Growth of Staphylococcus species in bovine mammary secretions and in brain heart infusion broth control medium.

Organisms	Bacterial growth[1,2] mammary secretion	BHIB[3]
Staphylococcus hominis-1	4.05^{d}+2.53	7.14+.06
Staphylococcus hominis-2	4.35^{c}+2.71	7.27+.01
Staphylococcus epidermidis-1	4.40^{c}+2.53	7.19+.02
Staphylococcus epidermidis-2	4.63^{b}+2.43	8.48+.10
Staphylococcus xylosus	4.31^{c}+2.20	7.31+.03
Staphylococcus hyicus	5.00^{a}+3.10	8.17+.09
Staphylococcus chromogenes-1	4.56^{b}+2.91	8.21+.22
Staphylococcus chromogenes-1	4.87^{a}+2.71	8.46+.04

[1]Data expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.
[2]Means with different superscripts differ ($p<.05$).
[3]Brain heart infusion broth.

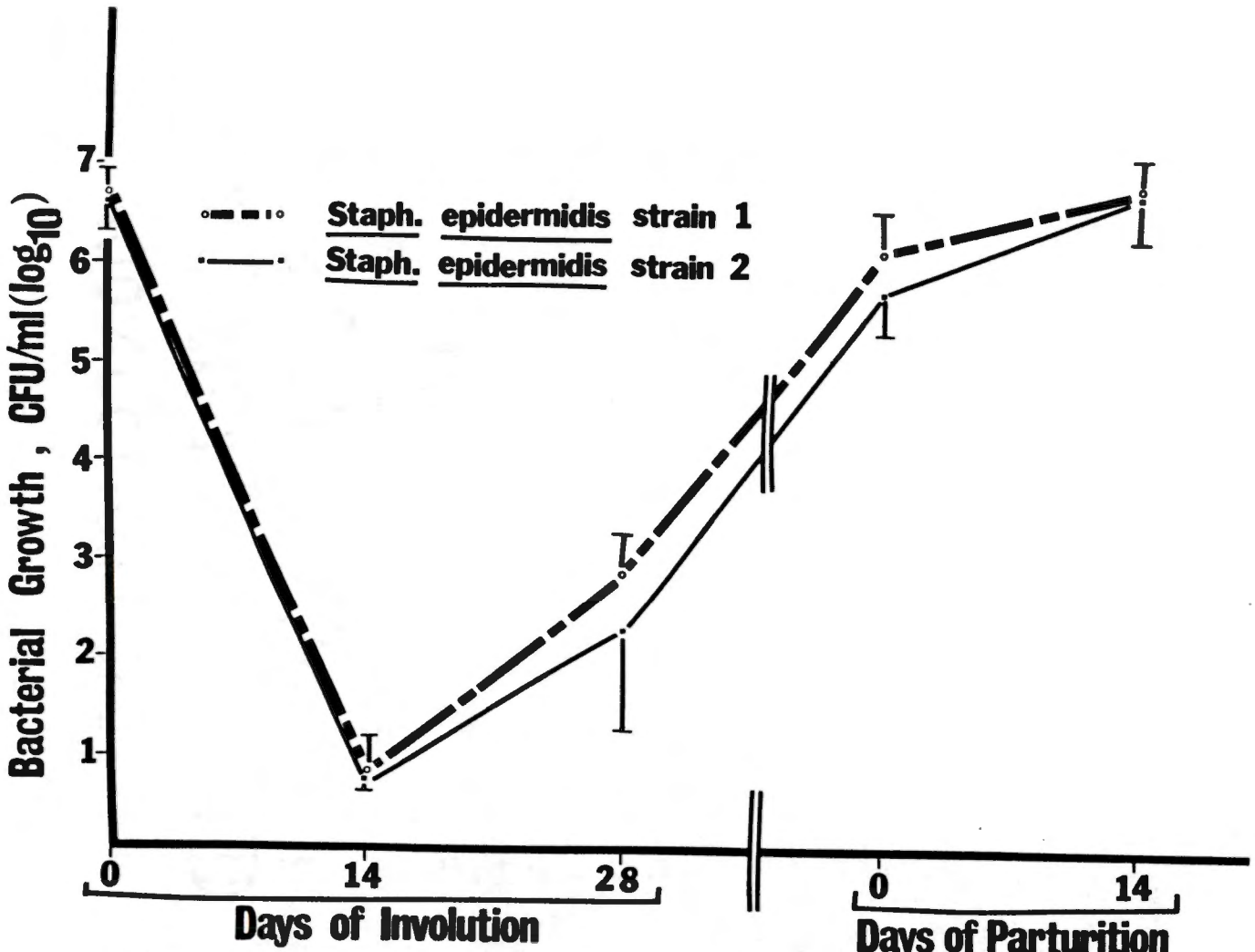

Figure 2. Growth of 2 strains of Staphylococcus epidermidis in bovine mammary secretions during the nonlactating and peripartum periods.

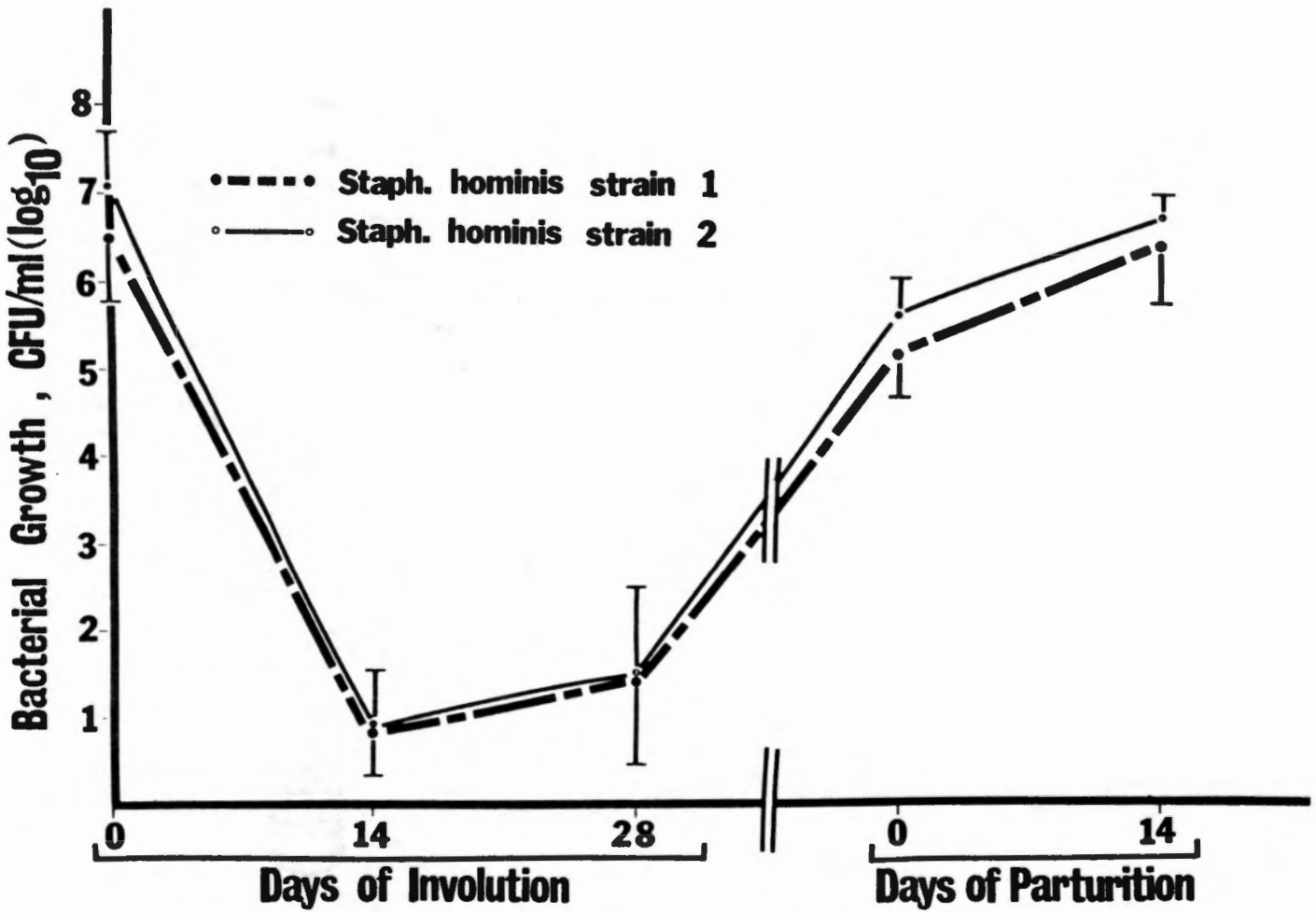

Figure 3. Growth of 2 strains of Staphylococcus hominis in bovine mammary secretions during the nonlactating and peripartum periods.

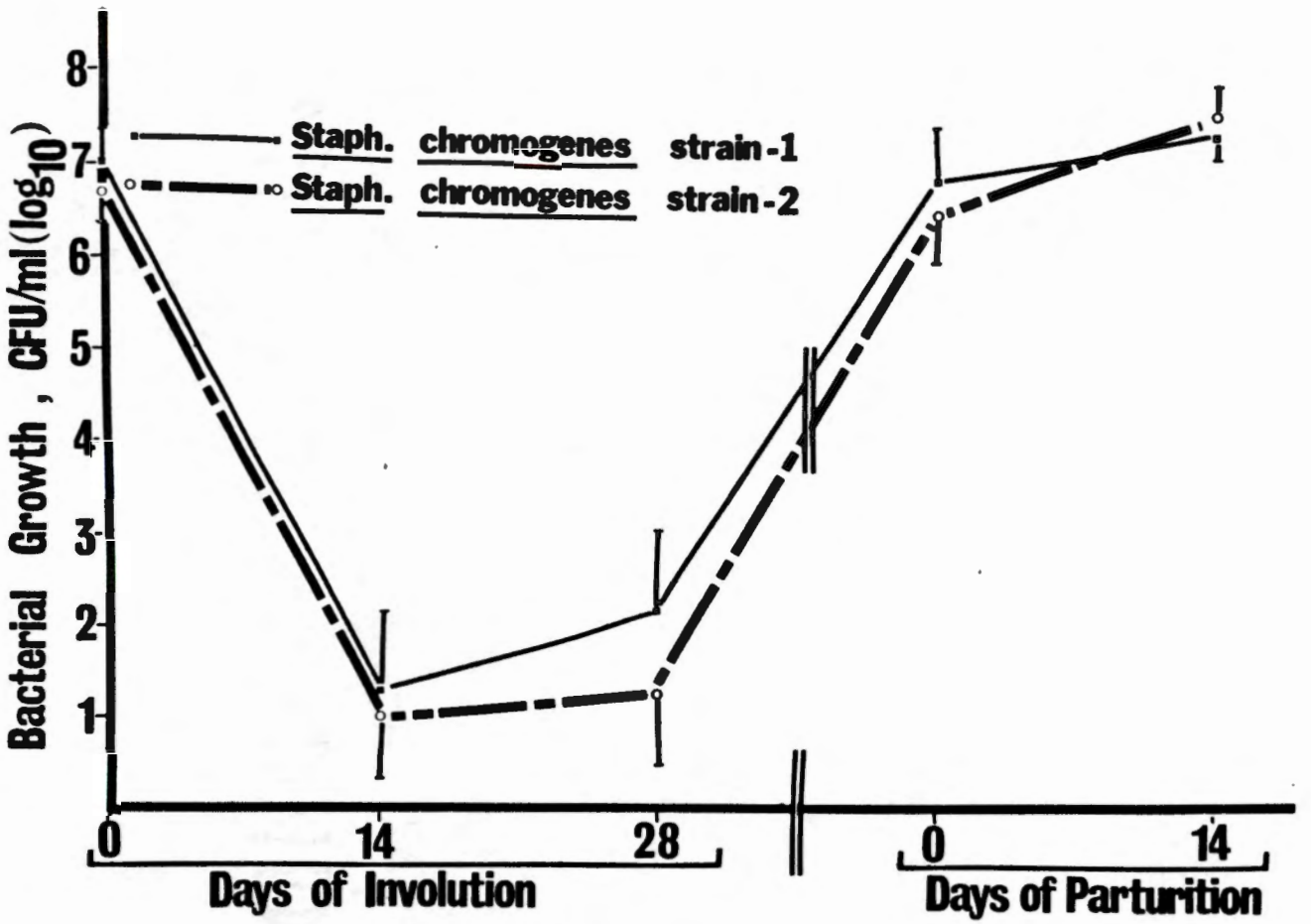

Figure 4. Growth of 2 strains of Staphylococcus chromogenes in bovine mammary secretions during the nonlactating and peripartum periods.

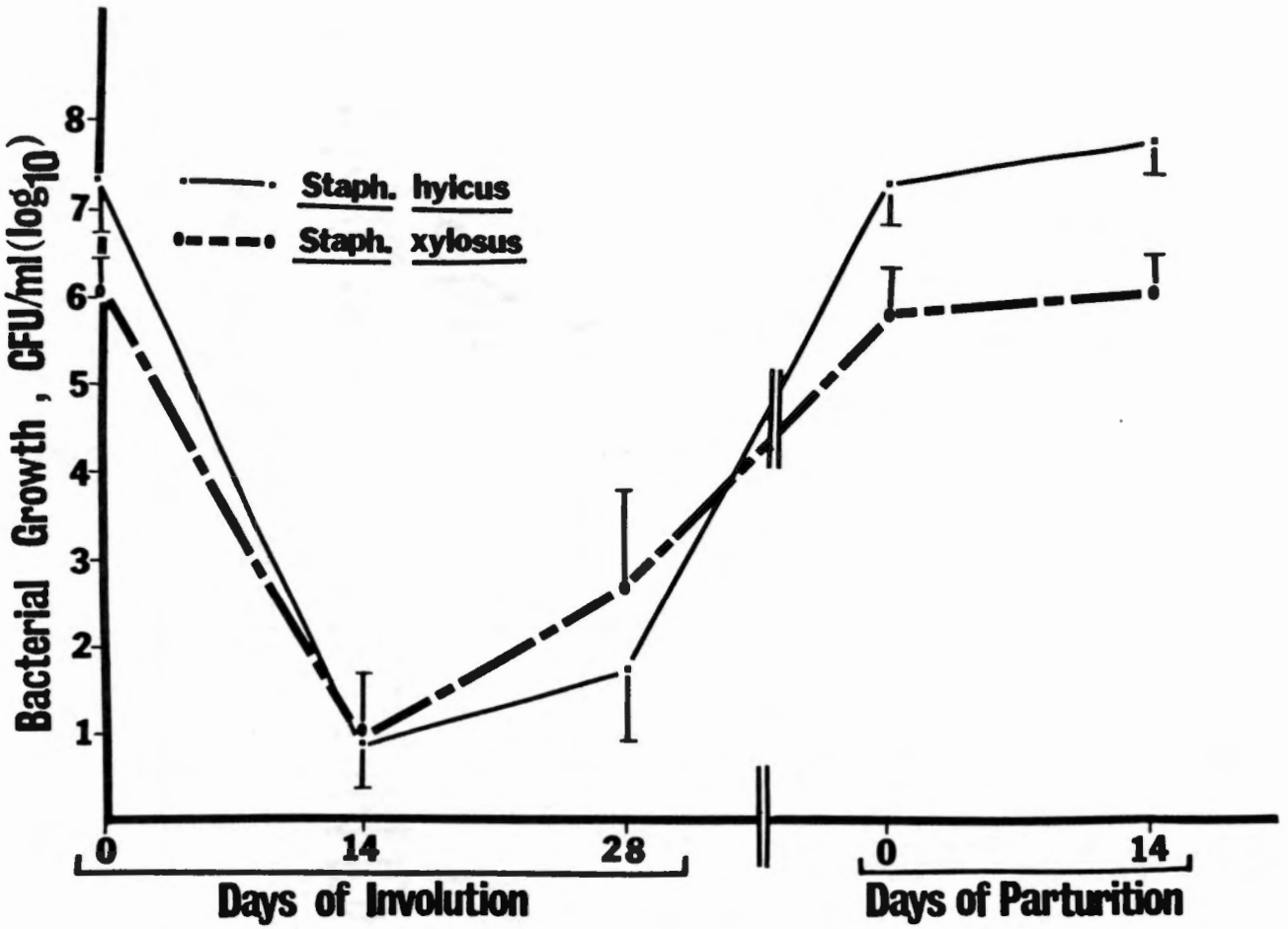

Figure 5. Growth of Staphylococcus xylosus and Staphylococcus hyicus in the mammary secretions during the nonlactating and peripartum periods.

DISCUSSION

Staphylococcus species are isolated frequently in samples of mammary secretions from nonlactating dairy cows (20) and primigravid heifers (21), teat skin (11) and milk from lactating cows (5). Coagulase-negative staphylococci are a part of normal teat skin flora and have been reported to colonize the teat duct (9). Devriese (8) found these organisms in milk and teat canals, and on teat skin of dairy cows. The distribution of Staphylococcus species differed from udder skin, teat skin, teat canals, and mammary secretions (9). Staphylococcus xylosus was isolated frequently from teat skin While Staph. epidermidis and Staph. chromogenes were isolated most frequently from teat canals and mammary secretions, respectively (9). Boddie et al.(2) observed that Staph. xylosus and Staph. chromogenes predominated on teat skin and Staph. chromogenes and Staph. hyicus were most prevalent in teat ducts and mammary secretions of nonlactating heifers. Similar results were reported by Matthews et al.(16). They (16) indicated also that the prevalence of Staphylococcus species after parturition was higher in primiparous than in multiparous cows.

Staphylococcus species evaluated in the present study showed considerable variation in growth in mammary secretions collected during the nonlactating and

periparturient periods. All Staphylococcus species grew well in mammary secretions obtained at cessation of milking, parturition, and during early lactation. On the other hand, mammary secretions obtained at 14 and 28 days of involution were poor media for the growth of Staphylococcus species. The pattern of Staphylococcus species growth in mammary secretions during the nonlactating and peripartum periods is similar to information reported on coliform mastitis pathogens (10,22). But in marked contrast to data published on Staph. aureus (10,24).

Variation in Staphylococcus species growth is most likely associated with changes in the biochemical composition and antibacterial components of bovine mammary secretions. Greatest growth of Staphylococcus species was observed in colostrum and milk which contain high concentrations of citrate, a low concentration of lactoferrin (Lf), and a very high citrate : Lf molar ratio (19,22). Conversely, poorest growth of Staphylococcus species occurred in dry secretions which contain low concentrations of citrate, high concentrations of Lf, and a very low citrate : Lf molar ratio (19,22). Thus, growth of Staphylococcus species in bovine mammary secretions was related positively with reported changes in the concentration of citrate and the citrate : Lf molar ratio,

and related negatively to reported changes in the concentration of Lf.

Iron is an essential component for growth of several microorganisms. However, free ionic iron is maintained at several orders of magnitude below that required for bacterial growth because of iron binding proteins such as Lf, which has a high affinity (K_a,10^{38}) for free iron (6). Citrate, on the other hand, also chelates iron but microorganisms can apparently utilize citrate bound iron (1). Coliform bacteria have atleast 2 genetically distinct systems for iron acquisition: citrate-mediated iron transport system which is induced by citrate in the growth medium, and synthesis and secretion of enterochelin in low iron environments (6). However, mechanisms of iron acquisition for Staphylococcus species have not been well defined.

Schade (26) indicated that Staph. aureus grown in normal rabbit serum sysnthesized iron chelators which competed with transferrin and Lf for iron. However, Marcelis et al. (15) demonstrated that strains of Staph. epidermidis were highly susceptible to iron deprivation, while strains of Staph. aureus were comparatively less susceptible. The iron requirement and ability of Staphylococcus species used in the present study to synthesize and secrete iron chelators is not known. However, all Staphylococcus species followed similar

patterns of growth and were markedly inhibited by bovine mammary secretions at 14 and 28 days of involution, a time when Lf concentrations are elevated and when citrate concentrations are low (19). In contrast, recent studies reported that Staph. aureus tended to grow well in mammary secretions from nonlactating cows (10,24). Staphylococcus species evaluated in the present study may be highly susceptible to iron deprivation resulting in reduced growth during the nonlactating period. However, additional research is required to support this contention.

Growth of Staphylococcus species in bovine mammary secretions throughout the nonlactating and peripartum periods is in general agreement with the pattern of Staphylococcus species IMI during the nonlactating period. Oliver (20) 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. Other studies (16,21) showed that a high proportion of primigravid heifer mammary glands were also infected with coagulase-negative staphylococci near parturition. Harmon et al. (12) demonstrated that a high

rate of spontaneous elimination of Staphylococcus species occurred during the nonlactating period. 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.

Results of this study are also consistent with data on experimental infection of dairy cows with Staph. epidermidis during the nonlactating period. McDonald and Anderson (17) reported that 0 of 9 mammary glands at 7 days of involution, and 2 of 9 mammary glands at 14 days of involution became infected after intracisternal inoculation of Staph. epidermidis. Conversely, 36 of 38 mammary glands became infected after intracisternal inoculation of Staph. epidermidis during the last half of the dry period (17). Consequently, 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 (3,11) suggested that lactating mammary glands infected with coagulase-negative staphylococci were more resistant to superinfection with major mastitis pathogens such as Strep. agalactiae, Escherichia coli, and Staph. aureus. However, the potential protective role of coagulase-negative staphylococcal IMI during the nonlactating period has not been reported. Results of this study and others (12,23)

suggest that protection against superinfection during the early nonlactating period will most likely not be effective since the rate of spontaneous elimination of Staphylococcus species in high and mammary secretions from involuted mammary glands are bactericidal to Staphylococcus species. However, intracisternal inoculation of Staphylococcus species near parturition may provide increased protection at a time when mammary glands are high susceptible to new IMI.

REFERENCES

1. Bishop, J.G., F.L. Schanbacher, L.C. Ferguson, and K.L. Smith. 1976. In Vitro growth inhibition of mastitis causing coliform bacteria by bovine apo-lactoferrin and reversal of inhibition by citrate and high concentration of apo-lactoferrin. Infect. Immun. 14:911.

2. Boddie, R.L., S.C. Nickerson, W.E. Owens, and J.L. Watts. 1987. Udder microflora in nonlactating heifers. Agri-Practice. 8:28.

3. Bramley, A.J. 1978. The effect of subclinical Staphylococcus epidermidis infection of the lactating bovine udder on its susceptibility to infection with Streptococcus agalactiae or Escherichia coli. Brit. Vet. J. 134:146.

4. Breau, W.C., and S.P. Oliver. 1986. Growth inhibition of environmental mastitis pathogens during physiologic transition of the bovine mammary gland. Am.J.Vet.Res. 47:218.

5. Brown, R.W., and R.K. Scherer. 1978. Classification of Staphylococcus epidermidis and Micrococcus strains isolated from bovine milk. Am. J. Vet. Res. 39:767.

6. Bullen, J.J., H.J. Rodgers, and E. Griffiths. 1978. Role of iron in bacterial infection:Current topics in immunology. 80:1.

7. Bushe, T., and S.P. Oliver. 1987. Natural protective factors in bovine mammary secretions following different methods of milk cessation. J. Dairy Sci. 70:696.

8. Devriese, L.A. 1979. Identification of clumping-factor-negative staphylococci isolated from cows udders. Res. Vet. Sci. 27:313.

9. Devriese, L.A., and H. Dekeyser. 1980. Prevalence of different species of coagulase-negative staphylococci on teats and in milk samples from dairy cows. J. Dairy Res. 47:155.

10. Dutt, K.W., R.J. Eberhart, and R.A. Wilson. 1986. In vitro growth of mastitis pathogens in mammary secretions of the dry and peripartum periods. J. Dairy Sci. 69:2408.

11. Edwards, S.J., and G.W. Jones. 1966. The distribution and characteristics of coagulase-negative staphylococci of the bovine udder. J. Dairy Res. 33:261.

12. Harmon, R.J., W.L. Crist, R.W. Hemken, and B.E. Langlois. 1986. Prevalence of minor udder pathogens after intramammary dry treatment. J. Dairy Sci. 69:843.

13. Kloos, W.E., and K.H. Schleifer.1975. Simplified scheme for routine identification of human Staphylococcus species. J. Cli. Micro. 1:82.

14. Langlois, B.E., R.J. Harmon, and K. Akers. 1983. Identification of Staphylococcus species of bovine origin with the API Staph-Ident. System. J. Cli. Micro. 18:1212.

15. Marcelis, J.H., J. Den Daas-Slagt Hanneke, and Jacomina A.A. Hoogk amp-korstanje. 1978. Iron requirement and chelator production of staphylococci, Streptococcus faecalis and enterobacteriaceae. Antonie van leecuwenhoek. 44:257.

16. Matthews, K.R., R.J. Harman, and B.E. Langlois. 1988. Prevalence of Staphylococcus species in primiparous and multiparous cows during the periparturient period. proc. NMC.

17. McDonald, J.S., and A.J. Anderson. 1982. Intramammary inoculation of the dairy cow with Staphylococcus aureus and Staphylococcus epidermidis during the nonlactating period. Am. J. Vet. Res. 44:244.

18. Nonnecke, B.J., and K.L. Smith. 1984. Inhibition of mastitis bacteria by bovine milk apo-lactoferrin evaluated by in vitro microassay of bacterial growth. J. Dairy Sci. 67:606.

19. Nonnecke, B.J., and K.L. Smith. 1984. Biochemical and antibacterial properties of bovine mammary secretions during mammary involution and at parturition. J.Dairy Sci. 67:2863.

20. Oliver, S.P., and B.A. Mitchell. 1983. Susceptibility of bovine mammary glands to infections during the dry period. J.Dairy Sci. 66:1162.

21. Oliver, S.P., and B.A. Mitchell. 1983. Intramammary infections in primigravid heifers near parturition.

J.Dairy Sci.66:1180.

22. Oliver, S.P., and T. Bushe. 1987. Growth inhibition of Escherichia coli and Klebsiella Pneumoniae during involution of the bovine mammary gland:Relation to secretion composition. Am. J. Vet. Res. 48:1669.

23. Oliver, S.P. 1987. Importance of the dry period in the control of intramammary infections by environmental mastitis pathogens. Proc. NMC.

24. Oliver, S.P., and K.A. Hoffman. 1988. Growth of Staphylococcus aureus and streptococci in bovine mammary secretions after intramammary infusion of lipopolysaccharide at drying off. J. Dairy Sci.(Abstr.). In Press.

25. SAS User's Guide: Statistics. version 5 edition.

26. Schade, A.L. 1963. Significance of serum iron for the growth, biological characteristics, and metabolism of Staphylococcus aureus. Biochemische Zeitschrift. 338:140.

27. Todhunter, D.A., K.L. Smith, and P.S. Schoenberger. 1985. In vitro growth of mastitis associated streptococci in bovine mammary secretions. J. Dairy Sci. 68:2337.

III. CORYNEBACTERIUM BOVIS

ABSTRACT

An *in vitro* microassay was used to evaluate growth of several strains of *Corynebacterium bovis* in mammary secretions collected from quarters of five Holstein cows at 0, 14, and 28 days of involution, at parturition, and 14 days after parturition. Significant variation in growth among different strains of *Corynebacterium bovis* and among cows was observed. In addition, *Corynebacterium bovis* growth was influenced significantly by day of sample collection. All strains of *Corynebacterium bovis* grew well in mammary secretions obtained at the last milking of lactation (6.3 cfu($\log_{10}$)/ml), at parturition (5.9cfu($\log_{10}$)/ml), and 14 days after parturition (6.3cfu($\log_{10}$)/ml). However, growth of 4 strains in mammary secretions obtained at 14 and 28 day of involution was significantly lower (2.7 to 4.6 cfu($\log_{10}$)/ml) compared to the 5th strain (5.0 to 5.3 cfu($\log_{10}$/ml). These data suggest that mammary secretions support growth of *Corynebacterium bovis* during lactation but inhibit their growth during the nonlactating period. Inhibition of growth during the nonlactating period may be associated with the high rate of spontaneous elimination of *Corynebacterium bovis* from drying off to parturition.

INTRODUCTION

Corynebacterium bovis is isolated frequently from bovine milk during lactation (1,2). Little is known about C. bovis despite the observation that this organism may constitute the greatest proportion of infected quarters in many dairy herds (3,13). Colonization of the bovine mammary gland by C. bovis does not appear to affect milk yield on composition (3,4). However, the number of somatic cells in milk from C. bovis colonized quarters was increased slightly when compared with milk from uninfected mammary glands (5,12). The increase in somatic cell numbers was considered slight since the geometric mean somatic cell count was <200,000/ml, a value not generally associated with decreased milk production or alterations in milk composition. Consequently, C. bovis is considered a minor mammary gland pathogen (4,13) and a harmless commensal organism (5).

Previous studies have suggested that C. bovis may play an important role in the prevention of IMI by major mastitis pathogens (5,12). Quarters infected with C. bovis were more resistant to induced Streptococcus dysgalactiae and Staphylococcus aureus IMI than uninfected quarters (8), but were not resistant to Strep. agalactiae (5,12) and Strep. uberis experimental infection (6). All of these studies were conducted in lactating cows. The importance

of these observations with respect to protection of nonlactating mammary glands has not been investigated.

A recent study by Oliver (9) showed that in the absence of antibiotic therapy at drying off, quarters infected with C. bovis decreased dramatically from drying off to parturition, but increased during early lactation. Harmon et al. (7) reported that the rate of spontaneous elimination of C. bovis in quarters of cows not treated with antibiotic at drying off was 47.6%, and that antibiotic therapy at drying off eliminated 94.1 to 100% of C. bovis intramammary infections (IMI) depending on the antibiotic used. Reasons for the high rate of spontaneous elimination of C. bovis during the nonlactating period are unknown. Oliver (10) suggested that cellular and / or non-cellular components of nonlactating bovine mammary secretions may be bactericidal to C. bovis. However, further research is needed to confirm this hypothesis. The objective of this study was to determine the influence of noncellular components of bovine mammary secretions on growth of C. bovis. Several strains of C. bovis were evaluated and mammary secretions were obtained during involution and the periparturient period.

MATERIALS AND METHODS

Experimental animals

Five pregnant dairy cows from the University of Tennessee dairy research herd were used in this study. Mammary secretions were collected from all quarters of each animal at the last milking of lactation, 14 and 28d of involution, at parturition and 14d after parturition.

Mammary secretion preparation

Samples of mammary secretion were centrifuged at 48,000 x g for 20min to 1h at 0 C to remove fat and cellular debris. Skim samples were sterilized by filtration through a series of membrane filters with decreasing pore size down to .45um and stored at -20 C. Sterility was determined by plating samples on blood agar and incubating at 37 C for 48h.

Bacteria

Four strains of C. bovis isolated from bovine milk from cows in the University of Tennessee dairy research herds were evaluated. Streptomycin resistance was induced in one of the strains of C. bovis and used also in another study (15) to determine the duration of experimental C. bovis colonization of bovine mammary glands throughout the nonlactating period. In addition, C. bovis 7715 from the

American Type Culture Collection was used. Organisms were identified using the Vitek Automated Microbiologic System's gram-positive identification card. Biochemical and morphological characteristics of all C. bovis strains were consistent with the reference strain. Corynebacterium bovis was identified as gram-positive rods, catalase-positive, urea-positive organisms that exhibited enhanced growth on Brain Heart Infusion (Difco Laboratories, Detroit, MI)(BHI) agar supplemented with .1% Tween80. Corynebacterium bovis was further confirmed as being oxidase-positive at 48h.

Two to five colonies of each strain of C. bovis grown on blood agar were inoculated into 100 ml of sterile ultra high temperature milk and incubated at 37 C until bacteria reached logarithmic growth (approximately 18h). Bacterial cultures were aliquoted into polypropylene vials. Glycerine was added (15%) and vials were frozen at -80 C.

Microassay of bacterial growth

Frozen vials were thawed and serially diluted in sterile phosphate-buffered saline (PBS;.2M,pH6.8). The number of colony forming units (cfu) per ml was determined by plating dilutions on BHI agar supplemented with Tween80 prior to conducting assays. The appropriate dilution yielding 50 to 100 cfu in 10 ul was used.

Microtiter plates containing 96 wells with a capacity of 300 ul per well were used. Each well of a microtiter plate contained 10 ul of the bacterial inoculum containing <100 cfu. Sterile skim samples (250ul) or 250ul BHI broth supplemented with .1% Tween80 (controls) were added also to each well. Plates were covered with sterilized lids and incubated at 37 C for 18h. Samples and controls were evaluated in duplicate. After incubation, contents of individual wells were diluted serially in PBS and four-10 ul drops of appropriate dilutions were placed on half plates of BHI agar supplemented with .1% Tween 80. Plates were incubated for 24-48h at 37 C and bacteria were counted using an automated bacterial colony counter.

Statistical analysis

Data were expressed as cfu ($\log_{10}$)/ml an analyzed by analysis of variance. Experimental design was split plot using the following model:

$$Y_{ijk} = u + S_i + Q_j + SQ_{ij} + B_k + BQ_{jk} + BS_{ik}(Q)_k + E_{ijk}$$

Y_{ijk} = concentration/ml of bacteria in mammary secretion from an experimental day of the nonlactating period,

u = overall mean,

S = stage of the dry perod,

Q = quarter of the gland,

SxQ = stage by quarter interaction,

B = bacterial type,

BxQ = bacterial type by quarter interaction,

BxS(Q) = bacterial type by stage within quarter interaction.

Means were evaluated for significance using Student-Newman Keul's mean separation procedure (16).

RESULTS

Growth of C. bovis in mammary secretions from 4 cows was similar (Table 5). However, growth of C. bovis in mammary secretions from one cow was significantly lower ($P<.05$). No significant quarter effect or sampling day by quarter interaction was observed (Table 6).

Growth of each strain of C. bovis in mammary secretions across all sampling periods and in control medium is shown Table 7. Significant differences ($P<.05$) in growth among the different strains of C. bovis was detected. The overall mean growth of the streptomycin resistant strain, the reference strain, and one wild type strain of C. bovis was similar and did not differ significantly. However, mean growth of two wild type strains of C. bovis was significantly lower ($P<.05$).

Table 5. Variation in growth of Corynebacterium bovis in mammary secretions from different cows.

Cow	$\bar{X}^{1,2}$	SD
1	5.50^{a}	1.09
2	4.88^{b}	1.72
3	5.32^{a}	1.32
4	5.42^{a}	1.18
5	5.34^{a}	1.41

[1] Data are expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.
[2] Means with different superscripts differ ($P<.05$).

Table 6. Growth of Corynebacterium bovis in mammary secretions from different quarters.

Quarter	$\bar{X}$[1]	SD
Left front	5.30	1.36
left rear	5.28	1.45
Right front	5.28	1.33
right rear	5.29	1.38

[1] Data are expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.

Table 7. Growth of Corynebacterium bovis in mammary secretions across all time periods and in brain heart infusion broth control medium.

C. bovis strain	Mammary secretions[1,2]	BHIB[3]
459	5.35^{a}+1.53	7.32+.01
550	5.13^{b}+1.45	7.16+.02
586	5.13^{b}+1.79	7.35+.15
F_6 7715	5.46^{a}+ .42	6.14+.04
	5.38^{a}+1.26	7.18+.04

[1] Data are expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.
[2] Means with different superscripts differ ($P<.05$).
[3] Brain heart infusion broth.

Growth of all strains of C. bovis in control medium always exceeded growth in mammary secretions.

Growth of C. bovis in mammary secretions was affected significantly ($P<.05$) by day of sample collection (Figure 6). Mammary secretions collected at cessation of milking supported growth of C. bovis (6.3 cfu($\log_{10}$)/ml). However, C. bovis growth decreased significantly ($P<.05$) in mammary secretions obtained at 14 days of involution (3.51cfu($\log_{10}$)/ml. Increased growth was observed at 28 days of involution compared to 14 days of involution, but C. bovis growth was still lower ($P<.05$) than that observed in samples of mammary secretion obtained at cessation of milking. Corynebacterium bovis growth increased significantly ($P<.05$) in colostrum and milk obtained during early lactation. Growth of C. bovis in mammary secretions collected 14 days after parturition was similar to growth in secretions obtained at cessation of milking.

Variation in growth of each strain of C. bovis in mammary secretions obtained during the nonlactating and peripartum periods is shown in Figure 7. Growth of 4 of 5 strains of C. bovis followed similar patterns. These 4 strains grew well in secretions collected at cessation of milking (6.4-6.7 cfu($\log_{10}$)/ml). Growth of C. bovis decreased in secretions obtained at 14d of involution (2.7-3.5cfu($\log_{10}$)/ml) and 28d of involution (3.8-4.6

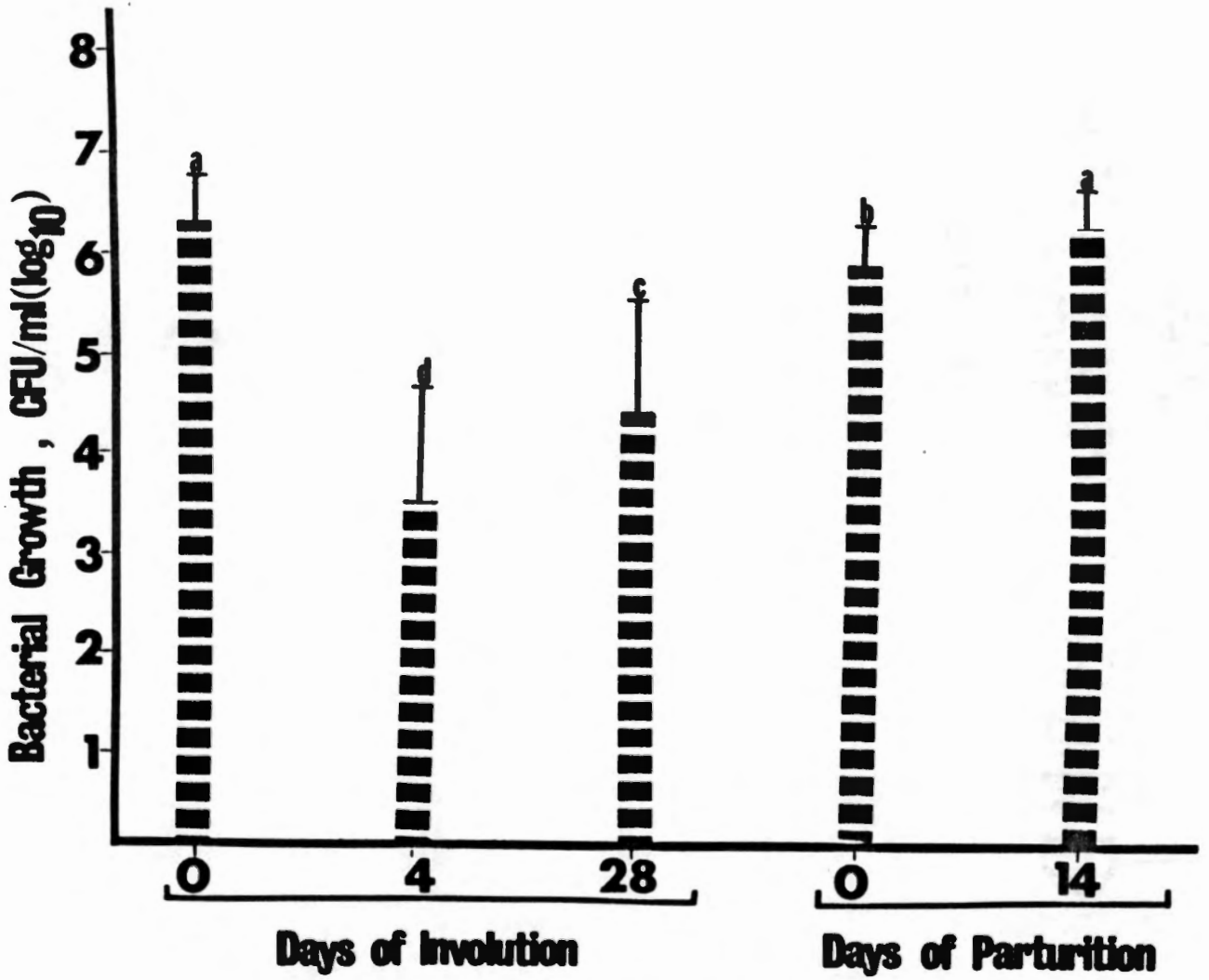

Figure 6. Growth of Corynebacterium bovis in mammary secretions during the nonlactating and peripartum periods.

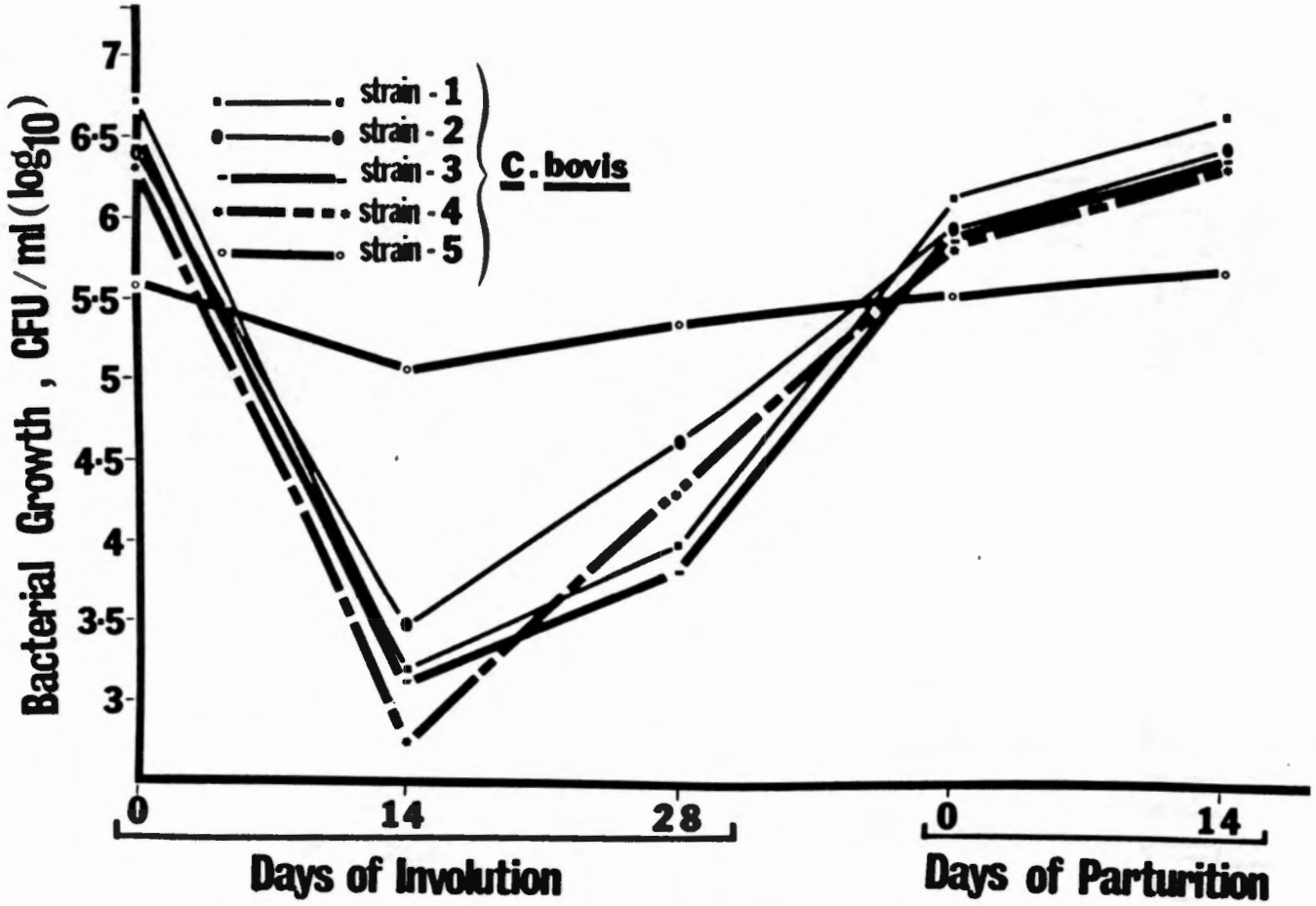

Figure 7. Variation in each strain of Corynebacterium bovis growth during the nonlactating and peripartum periods.

cfu(log_{10})/ml). However, C. bovis growth increased in secretions collected at parturition (5.9-6.2cfu(log_{10})/ml and 14d postpartum (6.4-6.7cfu(log_{10})/ml. The streptomycin resistant strain of C. bovis grew differently from other four strains. This strain grew well in all secretions collected at different stages of nonlactating and peripartum period. Growth was slightly less (5.5-5.7 cfu(log_{10})/ml) than the other four strains (5.9-6.7 cfu(log_{10})/ml) in secretions collected at cessation of milking, parturition and 14d after parturition, but growth was much higher (5.0-5.3cfu(log_{10})/ml) than the other 4 strains (2.7-4.6 cfu(log_{10})/ml) in secretions collected at 14 and 28d of involution.

DISCUSSION

Previous studies (7,10) 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%. Oliver (10) reported that only 9 of 48 quarters infected with C. bovis at drying off were infected during the early dry period, and that only one quarter of 160 cows was infected with C. bovis at parturition. Results of the present study demonstrated that greatest inhibition of C. bovis growth occurred in mammary secretions obtained during involution. Growth of

C. bovis in mammary secretions obtained at 14 days after milk cessation was about 57% lower than that observed in milk obtained at drying off, and about 35% lower in mammary secretions collected at 28 days of involution. Thus, one potential explanation for the high rate of spontaneous elimination of C. bovis during the dry period is that nonlactating cow mammary secretions markedly inhibit their growth. Cellular factors may also play an important role.

Variation in the rate of spontaneous elimination of C. bovis during the nonlactating period as reported by Oliver (10) and Harmon et al. (7) is most likely due to several factors. Significant differences in growth of C. bovis in mammary secretions of individual cows was observed. However, differences were small and mean growth of C. bovis in mammary secretions from all cows evaluated followed a similar pattern. Significant C. bovis strain variability was observed also. Mean growth of streptomycin resistant strain, the ATCC reference strain, and one wild type strain of C. bovis was similar, but significantly higher than mean growth of two other wild type strains of C. bovis. Consequently, enhanced growth of certain strains of C. bovis in mammary secretions may relate significantly to the persistence of IMI by these organisms.

In support of this contention, the streptomycin resistant strain of C. bovis grew well in all mammary

secretions, including secretions from nonlactating mammary glands. The pattern of growth of this organism was in marked contrast with all other strains of C. bovis evaluated. Experimental inoculation of bovine mammary glands during late lactation with the streptomycin resistant strain of C. bovis resulted in IMI that persisted throughout the nonlactating period and into the subsequent lactation (11,15). Similar results were observed when the streptomycin resistant strain of C. bovis was inoculated into quarters of cows during mid-lactation (4). Together, these data suggest that the microassay of bacterial growth used in the present study may be useful in predicting the outcome of experimental challenge of mammary glands with various mastitis pathogens, as well as being useful in determining organisms to use for experimental challenge. Furthermore, growth of mastitis pathogens in bovine mammary secretions may relate significantly to observed rates of IMI and patterns of changing susceptibility during the nonlactating period.

Little is known about the nutritional requirements of C. bovis. Black et al. (1) indicated that C. bovis did not grow well, if at all, in synthetic media but addition of .1% Tween 80 or .1% lipid material enhanced growth. Initially, we attempted to evaluate growth of C. bovis in fat-free cell-free mammary secretions without lipid

supplementation. However, all strains of C. bovis evaluated did not grow. These data agree with other reports that showed that C. bovis requires lipids for growth (14). Supplementation of mammary secretions with .1% Tween 80 resulted in growth of C. bovis. However, growth of C. bovis in the control medium (BHI broth containing .1% Tween 80) always exceeded growth in mammary secretions. Thus, inhibition of C. bovis growth was most likely due to factor(s) present in mammary secretion rather than fatty acid deprivation. Specific components of mammary secretion causing inhibition of C. bovis remains unknown.

Previous studies (5,8,12) indicated that lactating bovine mammary glands infected with C. bovis appeared to be more resistant to IMI by other major mastitis pathogens than uninfected quarters. However, this is somewhat controversial as other reports have shown no benefit (5,6,12). The importance of these observations with respect to prevention of IMI during the nonlactating period has not been reported. However, recent reports have shown that the rate of spontaneous elimination of C. bovis during the nonlactating period in cows not treated with antibiotics at drying off is high (7,13). Furthermore, antibiotic therapy at drying off is very effective in the prevention and control of C. bovis IMI. Thus, based on current information, it is doubtful that C. bovis would

provide protection of mammary glands against more pathogenic mastitis pathogens during the nonlactating period.

REFERENCES

1. Black, R.T., R.T. Marshall, and C.T. Bournald. 1972. Locus of mammary gland infections of Corynebacterium bovis. J. Dairy Sci. 55:413.

2. Bramley, A.J. 1975. Infection of the udder with coagulase-negative micrococci and Corynebacterium bovis. Page 377 in proc. Seminar on Mastitis control. Bull. Doc. 85. Int. Dairy Fed., Brussels, Belgium.

3. Brooks, B.W., D.A. Barnum, and A.H. Meek. 1983. An observational study of Corynebacterium bovis in selected Ontario dairy herds. Can. J. Comp. Med. 47:73.

4. Brooks, B.W., and D.A. Barnum. 1984. Experimental colonization of the bovine teat duct with Corynebacterium bovis and the effect on milk somatic cell counts. Can. J. Comp. Med. 48:141.

5. Brooks, B.W., and D.A. Barnum. 1984. The susceptibility of bovine udder quarters colonized with Corynebacterium bovis to experimental infection with Staphylococcus aureus or Streptococcus agalactiae. Can. J. Comp. Med. 48:146.

6. Doane, R.M., S.P. Oliver, R.D. Walker, and E.P. Shull. 1987. Experimental infection of lactating bovine mammary glands with Streptococcus uberis in quarters colonized by Corynebacterium bovis. Am. J. Vet. Res. 48:749.

7. Harmon, R.J., and B.E. Langlois. 1986. Prevalence of minor mastitis pathogens and associated somatic cell counts. Proc. NMC.

8. Linde, C., O. Holmberg, and G. Astorm. 1980. The interference between coagulase-negative staphylococci and Corynebacterium bovis and the common udder pathogens in the lactating cow. Nord. Vetinaermed. 32:552.

9. Oliver, S.P., and B.A. Mitchell. 1983. Susceptibility of bovine mammary glands to infections during the dry period. J. Dairy Sci. 66:1162.

10. Oliver, S.P. 1987. Importance of the dry period in the control of intramammary infections by environmental mastitis pathogens. Proc. NMC.

11. Oliver,S.P., L.M.Sordillo, J.L. Maki, and E.P.Shull. 1987. Duration of experimental Corynebacterium bovis colonization of bovine mammary glands during the nonlactating and peripartum periods. J.Dairy Sci. (abstr.).P74:128.

12. Pankey, J.W., S.C. Nickerson, R.L. Boddie, and J.S. Hogan. 1985. Effects of Corynebacterium bovis infection on susceptibility of major mastitis pathogens. J. Dairy Sci. 68:2684.

13. Rainard, P., and B. Poutrel. 1982. Dynamics of nonclinical bovine intrammary infections with major and minor pathogens. Am. J. Vet. Res. 43:2143.

14. Smith, R.F. 1970. Fatty acid requirements of human cutaneous lipophilic Corynebacteria. J. Gen. Microbiol. 60:259.

15. Sordillo, L.M., S.P. Oliver, R.M. Doane, E.P. Shull and J.L. Maki. 1988. Duration of experimental Corynebacterium bovis colonization of bovine mammary gland during the lactating, nonlactating, and peripartum periods. Am. J. Vet. Res. (Accepted for publication).

16. SAS User's Guide : Statistics. version 5 edition.

IV. MAMMARY SECRETIONS FRACTIONATION

ABSTRACT

Quarter samples of mammary secretion were collected from 3 cows at 0, 14, and 28 days of involution, at parturition,and 14 days after parturition. Microassay was performed to compare growth of Staphylococcus species and Corynebacterium bovis in skim and whey. Skims from mammary secretion obtained at cessation of milking and during the peripartum period supported growth of all Staphylococcus species. However, skims from mammary secretion obtained during involution were poor media for the growth of Staphylococcus species. Growth of Staphylococcus species in whey followed a similar pattern, but increased growth was observed in mammary secretions obtained during involution. Variation in growth of Corynebacterium bovis in mammary secretion skims and whey obtained during involution and peripartum period was not observed. However, growth of Corynebacterium bovis was reduced in whey. Mammary secretion skims were passed through on S-200 gel filtration column. Four protein peaks were obtained from each skim sample. Peaks were characterized by two-dimensional polyacrylamide gel electrophoresis. The majority of proteins eluted in peak 1 and 2 from S-200 columns. Protein peaks were evaluated with respect to

growth of Staphylococcus species and Corynebacterium bovis. In general, growth of Staphylococcus species and Corynebacterium bovis was reduced in peaks 1 and 2 of mammary secretion skims collected during involution. Results of this study suggest that the presence of high molecular weight biochemical components in mammary secretions are inhibitory to the growth of Staphylococcus species and Corynebacterium bovis.

INTRODUCTION

The incidence of new IMI is highest during the nonlactating period period when marked physiological transitions occur from lactation to involution and from involution to colostrogenesis (9). As a result of these physiological transitions during the early and late stages of involution, significant changes occur in the biochemical composition and antibacterial properties of bovine mammary secretions (11). Mammary secretions during early involution and the peripartum period contain high concentrations of citrate, casein, and lactose,which facilitate growth of pathogens (13). Fully involuted mammary glands contain high concentrations of leukocytes, immunoglobulins, and lactoferrin, which have been implicated as resistance factors to intramammary infections (12).

Results presented in section 1 and 2 indicated that mammary secretions obtained during involution were bactericidal to the growth of Staphylococcus species and inhibited the growth of Corynebacterium bovis. The objectives of this study were to fractionate mammary secretions obtained during involution and the peripartum period and compare growth of Staphylococcus species and C. bovis in skim and whey preparations. In addition, an attempt was made to determine if inhibition of minor mastitis pathogen growth was associated with the protein component of mammary secretions.

MATERIALS AND METHODS

Experimental animals

Three Holstein cows maintained under identical conditions from the University of Tennessee dairy research herd were used in this study. Mammary secretions from all quarters of 3 cows were collected at the last milking of lactation, at 14 and 28 days of involution, at parturition and 14 days after parturition.

Mammary secretion preparation

Samples of mammary secretion were centrifuged at 48,000 x g for 20 min to 1h at O C to remove fat and cellular debris. One-half of skim samples were sterilized

by filtration through a series of filters with decreasing pore size down to .45um and stored at - 20 C until needed. The pH of the remaining skim samples was decreased to 4.5 using glacial acetic acid. Casein was removed by centrifugation at 48,000 x g for 30 min at 0 C and the pH of whey was increased to 6.8 by dropwise addition of 1N sodium hydroxide. Wheys were sterilized and stored in the same manner as described for skims. Sterility was determined by plating skim and whey samples on blood agar and incubating for 48 h at 37 C. Skim and whey preparations from all quarters of each cow were pooled by stage of the dry period.

Bacteria

Growth inhibition of the following mastitis pathogens was evaluated using an in vitro microassay system as described previously in chapter 2:

Staphylococcus chromogenes	(n=1)
Staphylococcus hyicus	(n=1)
Staphylococcus epidermidis	(n=1)
Staphylococcus hominis	(n=1)
Staphylococcus xylosus	(n=1)
Corynebacterium bovis	(n=2)

Column chromatography

Mammary secretion skims from 12 quarters of three cows were pooled by stage of the dry period for column chromatography. A 2.6cm X 100 cm column packed with Sephacryl S-200 (Pharmacia Fine Chemicals, Piscataway, New Jersey) was prepared. The buffer system was 10 mM Tris in .3M NaCl, pH 8.0). Ten ml of mammary secretion skim was loaded onto the column and allowed to elute by gravity. Void volume was approximately 130ml. Fractions (5ml) were collected and optical density at A_{280} was read on a spectrophotometer (Bausch and Lomb spectronic 2000; Bausch and Lomb Analytical systems DIv., Rochester, NY). Four major protein peaks were observed in the chromatographic profiles in each of the 5 skim samples (Figure 8-12). Individual peaks were pooled and dialyzed against 10 mM Tris buffer, pH 8.0. Dialysis was carried out for 72h at 4 C against six changes of buffer. Total protein content of each peak was determined by the Lowry method (6). Volume of sample needed to equal 800 ug of protein was poured into plastic vials and lyophilized.

Two-dimensional polyacrylamide gel electrophoresis

Proteins in each peak were characterized by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) using the procedure of Horst and Roberts (3). Proteins were solubilized in 5mM K_2C0_3 containing 2%

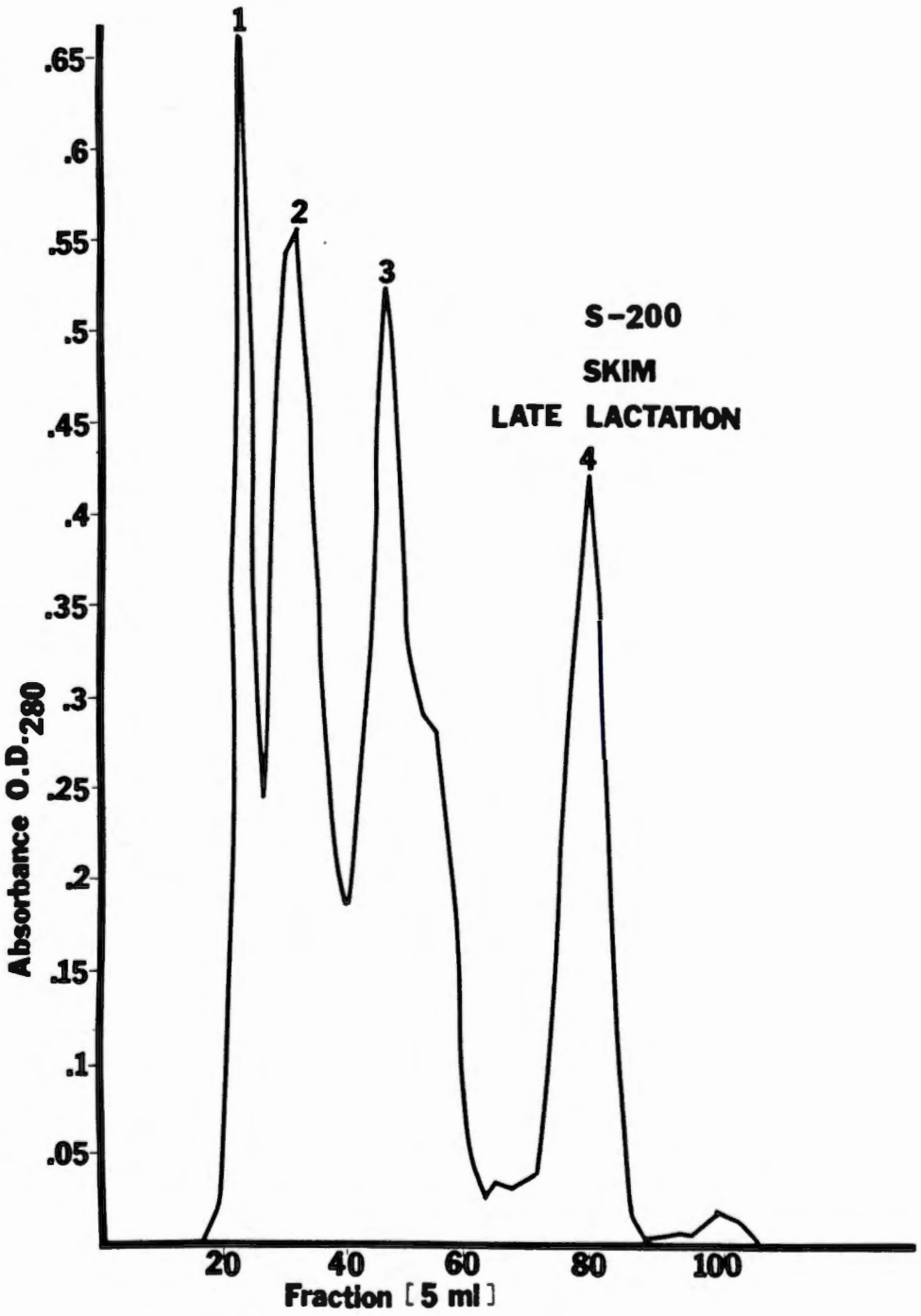

Figure 8. S-200 chromatogram of bovine mammary secretion skim collected at cessation of milking from 3 cows.

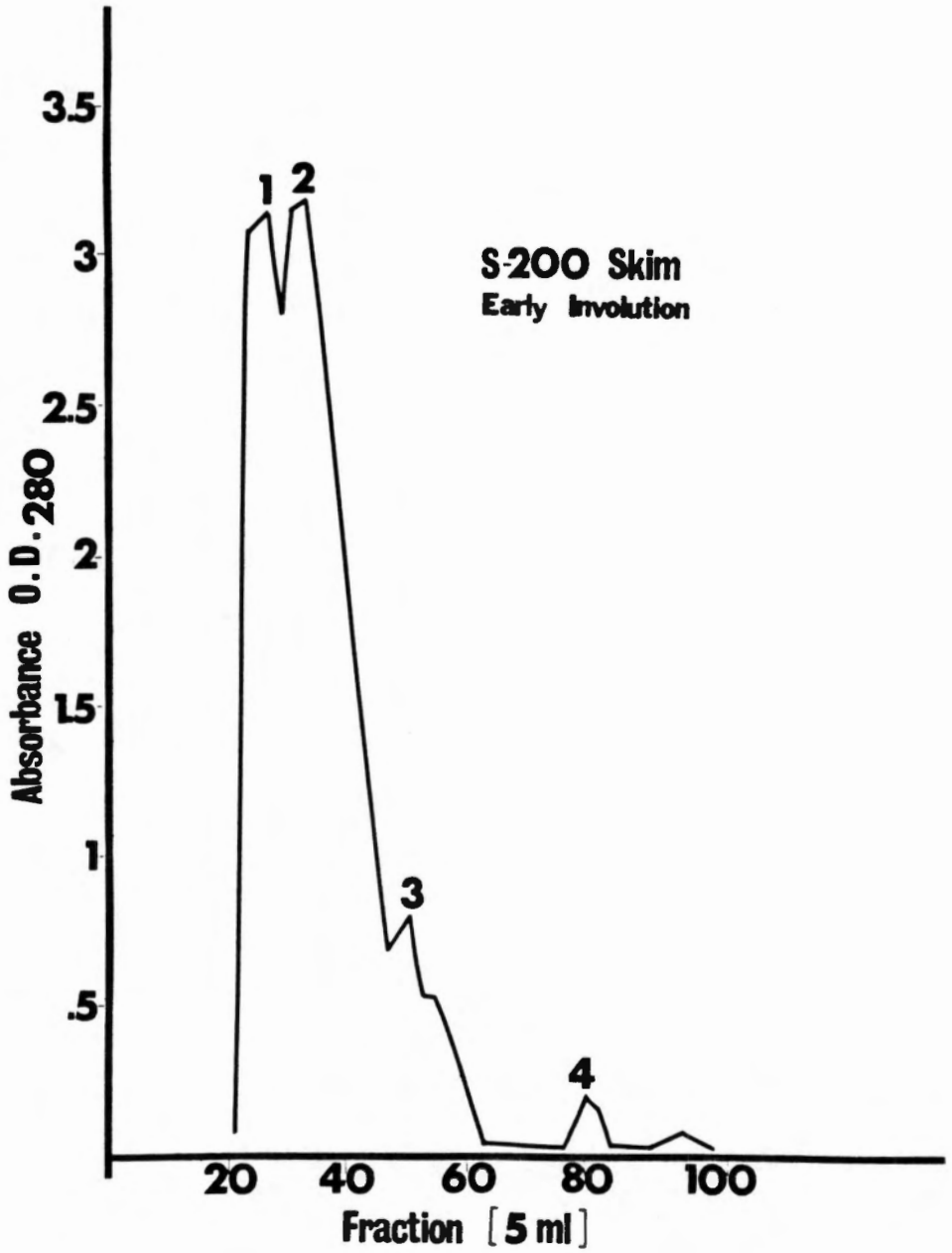

Figure 9. S-200 chromatogram of bovine mammary secretion skim collected at 14 days after cessation of milking from 3 cows.

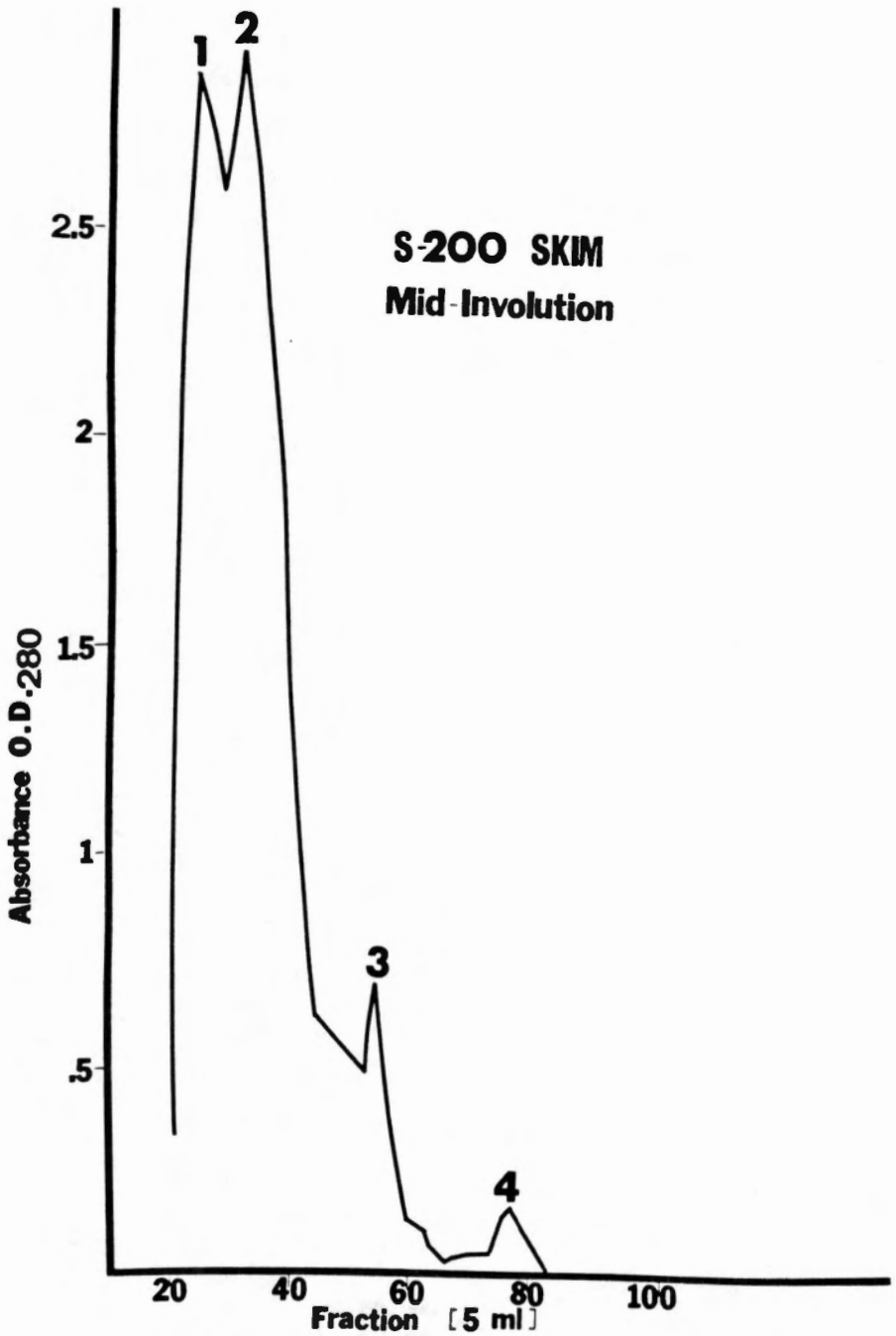

Figure 10. S-200 chromatogram of bovine mammary secretion skim collected at 28 days after cessation of milking from 3 cows.

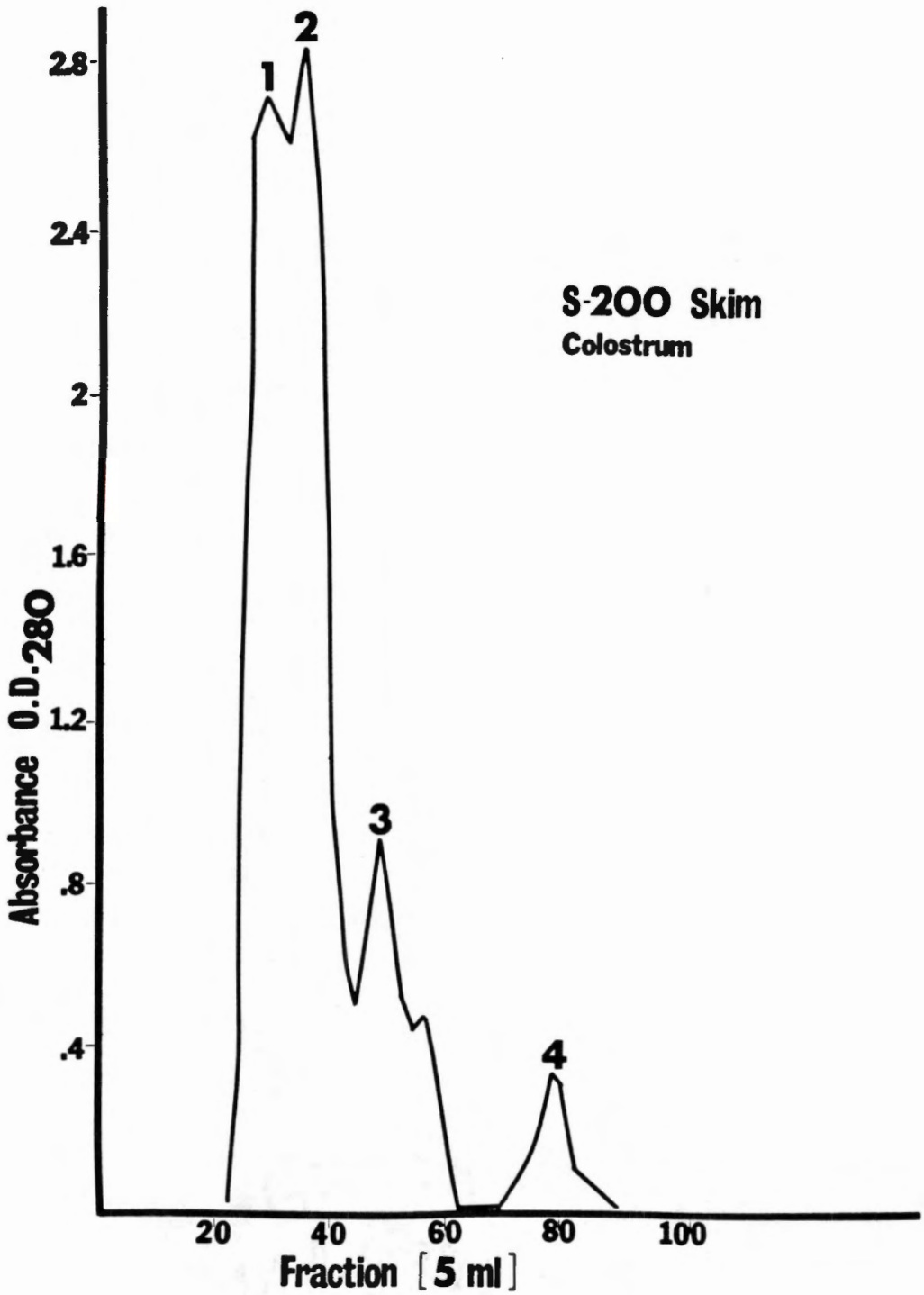

Figure 11. S-200 chromatogram of bovine mammary secretion skim collected at parturition from 3 cows.

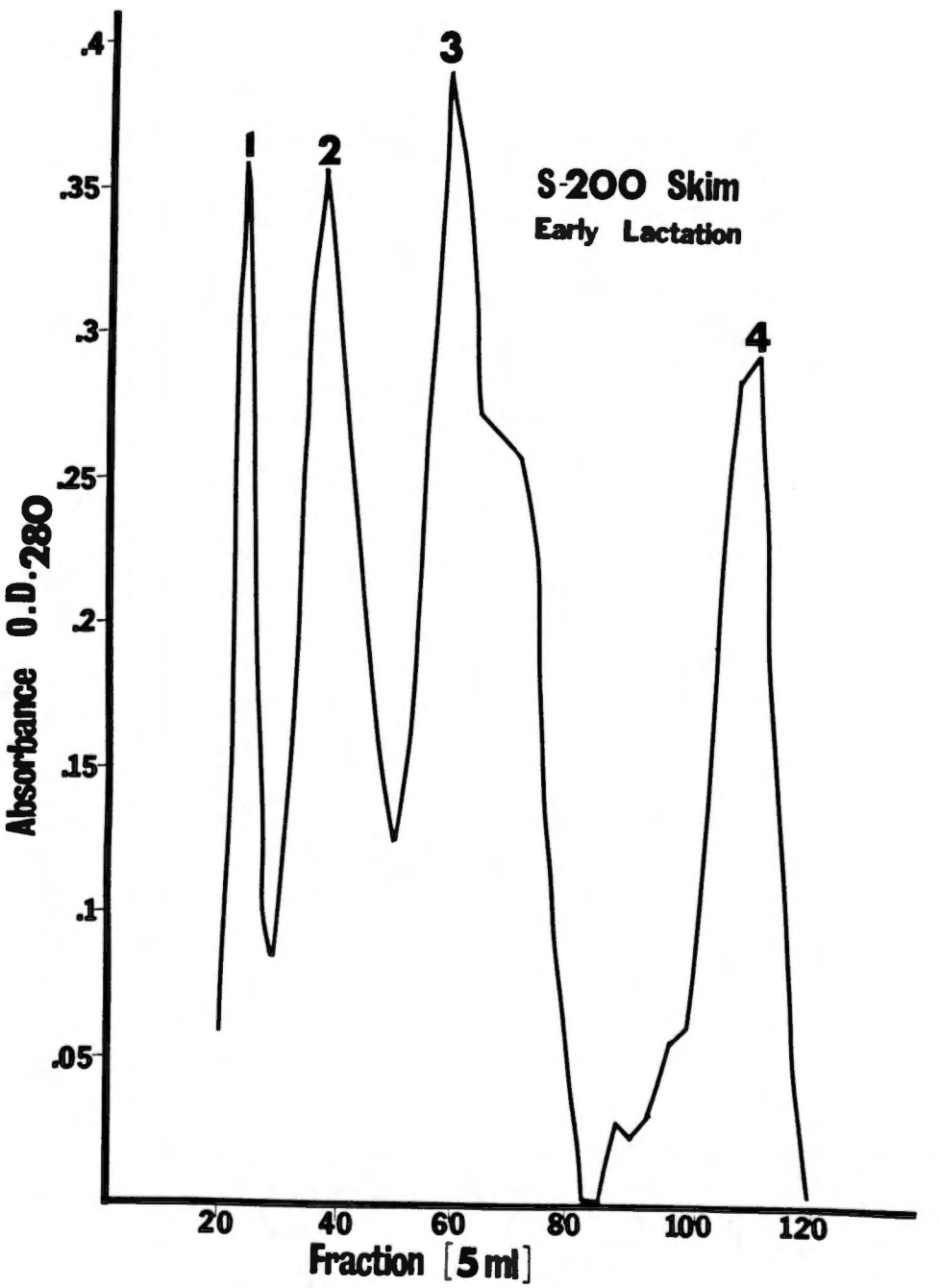

Figure 12. S-200 chromatogram of bovine mammary secretion skim collected at 14 days after parturition from 3 cows.

Nonidet-P40, 9.3M urea and .5% dithiothreitol. Isoelectric focusing in the first dimension used a disc gel electrophoretic procedure (5) which separated proteins by isoelectric point (pI). An average of 400 µg of protein was loaded onto each gel. The pH gradient of disc gel was established by addition of ampholines (PH3.5 to 10.0, PH 5 to 7, and PH 9 to 11) to the unpolymerized gel. Gels were electrophoresed under denaturing conditions in the presence of urea. One additional gel was made to measure pH profiles. The gel was cut into one-cm pieces and each piece was placed in individual vials, minced, and 2 ml of deionized water were added. Vials were capped and left to stand for 2h, then the pH was measured. The pH of slab gels ranged from 3.65 to 8.03. Following electrophoresis, disc gels were equiliberated in .065 M Tris-HCl containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol. Thereafter, disc gels were placed on 10% polyacrylamide slab gels and electrophoresed in the presence of SDS to separate mammary secretion proteins by molecular weight (M_r). Transferrin (M_r 78,000), chymotrypsinogen (M_r 25,000), β-lactoglobulin (M_r 18,000) and α-lactalbumin (M_r 14, 200), were used as molecular weight standards. Slab gels were fixed in 40% ethanol and 7% acetic acid in deionized H_2O, stained with .125% Coomassie Brilliant Blue in ethanol, and destained in a solution of 10% acetic acid and 7% ethanol in deionized H_2O.

Bacteria: Growth of the following bacterial strains was evaluated in individual protein peaks that eluted from S-200 columns:

Staphylococcus chromogenes

Staphylococcus hominis

Staphylococcus epidermidis

Staphylococcus hyicus

Corynebacterium bovis

An in vitro microassay system was used as described previously with few modifications. Samples (245ul) from each protein peak were used and 5ul of brain heart infusion broth (BHIB) was added also. In addition, BHIB was supplemented with .1% Tween80 when C. bovis was evaluated.

RESULTS

Growth of Staphylococcus species and C. bovis in skims and wheys from mammary secretions obtained during the nonlactating and periparturient period is presented in Table 8. Skims from mammary secretions collected at cessation of milking, parturition and during early lactation supported growth of all Staphylococcus species (>6.3 cfu(Log_{10})ml). However, skims from mammary secretions collected at 14 and 28 days of involution were poor media for growth of Staphylococcus species which is

Table 8. Growth of Staphylococcus species and Corynebacterium bovis in skims and whey from mammary secretion obtained during the nonlactating and periparturient periods[1].

	Staphylococcus species[2]	
Experimental period	Skim	Whey
Late lactation	6.92+ .47	5.60+.46
Early involution	.69+ .00	3.70+.76
Mid involution	1.77+1.15	3.37+.52
Colostrum	6.27+ .80	5.26+.48
Early lactation	6.96+ .46	5.40+.60
Mean	$4.52^{a,y}$+2.82	$4.66^{a,x}$+1.09

	Corynebacterium bovis[3]	
Experimental period	Skim	Whey
Late lactation	5.68+.55	4.64+.46
Early involution	4.26+.88	4.52+.74
Mid involution	5.18+.43	5.51+.38
Colostrum	5.82+.40	4.65+.31
Early lactation	5.90+.24	4.73+.52
Mean	$5.36^{a,x}$+.80	$4.81^{b,x}$+.59

^{a,b}Means in a row followed by the same superscript are not significantly different (P>.05).
^{x,y}means in a column followed by the same superscript are not significantly different (P>.05).

[1] Data expressed as $\bar{X}$ ± SD cfu($\log_{10}$)/ml.
[2] Staph. hominis, Staph. epidermidis, Staph. hyicus, Staph. chromogenes.
[3] ATCC reference strain, streptomycin resistant strain.

consistent with results in section 2. Growth of Staphylococcus species in wheys from mammary secretion followed a similar pattern, but growth at 14 and 28 days of involution was 1.6-3cfu(log_{10})/ml higher compared to growth in skim, and growth was 1-1.5cfu(log_{10})/ml lower than in colostrum and milk.

Growth of two strains of C. bovis in skims and wheys from mammary secretion obtained during the nonlactaing and periparturient periods was similar and no marked changes in growth was observed (Table 8). Overall mean growth of all Staphylococcus species in skim and whey was not different significantly, but growth of C. bovis in whey was lower ($P<.05$) than growth in skim. Growth of Staphylococcus species and C. bovis in skims were significantly ($P<.05$) different, but growth in whey was similar.

Variation in growth among Staphylococcus species in skims and whey was observed (Table 9). Staphylococcus chromogenes grew well in skims and wheys from mammary secretions collected at cessation of milking, parturition, and early lactation. However, growth of Staph. chromogenes in skims was greater than in whey. Bacterial growth decreased markedly in skims from mammary secretions obtained during involution. Bacterial growth in whey from mammary secretions obtained during involution was reduced also, but was higher than that observed in skims. Similar

Table 9. Growth of Staphylococcus species in skims and whey from mammary secretion obtained during the nonlactating and peripartum periods[1].

Experimental period	Staphylococcus chromogenes Skim	Whey
Late lactation	7.45+.10	5.97+.09
Early involution	.69+.00	5.97+.09
Mid involution	.69+.00	3.81+.10
Colostrum	6.89+.11	5.08+.14
Early involution	7.33+.30	6.07+.27
Mean	$4.61^{a,y}$+3.32	$4.56^{a,z}$+1.50

Experimental period	Staphylococcus xylosus Skim	Whey
Late lactation	6.64+.11	4.91+.10
Early involution	.69+.00	3.67+.25
Mid involution	2.93+.72	3.56+.46
Colostrum	5.38+.14	5.00+.11
Early involution	6.41+.23	5.03+.08
Mean	$4.41^{a,z}$+2.37	$4.44^{a,z}$+.72

Experimental period	Staphylococcus epidermidis Skim	Whey
Late lactation	6.30+.21	5.47+.71
Early involution	.69+.00	4.55+.44
Mid involution	2.89+.78	3.65+.38
Colostrum	6.59+.16	3.65+.38
Early lactation	6.78+.35	5.18+.19
Mean	$4.65^{a,y}$+2.55	$4.90^{a,x}$+.82

Table 9. Continued.

Experimental period	*Staphylococcus hominis* Skim	*Staphylococcus hominis* Whey
Late lactation	6.95+.29	5.55+.18
Early involution	.69+.00	4.40+.44
Mid involution	1.64+.87	3.64+.82
Colostrum	5.36+.10	4.76+.21
Early lactation	6.85+.30	4.72+.35
Mean	$4.30^{a,z}$+2.75	$4.61^{a,y}$+.75

Experimental period	*Staphylococcus hyicus* Skim	*Staphylococcus hyicus* Whey
Late lactation	7.27+.29	6.28+.30
Early involution	.69+.00	3.02+.15
Mid involution	.69+.00	3.19+.23
Colostrum	7.16+.32	5.80+.15
Early involution	7.42+.26	6.02+.13
Mean	$4.64^{a,y}$+3.34	$4.82^{a,x}$+1.46

^{a,b} Means in a row followed by the same superscript are not significantly different (P>.05).

^{x,y,z} Means in a column followed by the same superscript are not significantly different (P>.05).

[1] Data expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.

results were observed with Staph. xylosus, Staph. epidermidis, Staph. hominis, and Staph. hyicus.

Mean growth of the streptomycin resistant strain of C. bovis in whey was significantly lower ($P<.05$) than growth in skims (Table 10). In general, growth of streptomycin resistant strain of C. bovis was high at all sampling periods, and variation in C. bovis growth in mammary secretion skims and whey obtained during involution and the periparturient period was not detected. Growth of the ATCC reference strain of C. bovis was significantly lower ($P<.05$) in whey than in skims, and less than ($P<.05$) that observed with the streptomycin resistant strain of C. bovis. Growth of the reference strain in mammary secretion skims tended to be lowest during involution which is consistant with results obtained in chapter III. Little variation in growth of the reference strain of C. bovis in whey was observed across all sampling periods.

The protein content in each chromatographic peak from mammary secretion skims collected during involution and peripartum period is presented in Table 11. The protein content of each peak was lowest (21-47µg/100µl) in mammary secretions obtained at cessation of milking and during early lactation. Increased protein content in peak 1 and 2 in mammary secretion collected during involution and at parturition was observed. The protein content in peak 4 in

Table 10. Growth of each strain of Corynebacterium bovis in skims and whey from mammary secretion obtained during the nonlactating and peripartum periods[1].

	Corynebacterium bovis[2]	
Experimental period	Skim	Whey
Late lactation	5.66+.19	4.98+.23
Early involution	5.04+.18	5.19+.05
Mid involution	5.56+.15	5.84+.11
Colostrum	5.53+.30	4.79+.14
Early involution	5.72+.18	5.13+.07
Mean	$5.50^{a,w}$+.30	$5.18^{b,x}$+.38

	Corynebacterium bovis[3]	
Experimental period	Skim	Whey
Late lactation	5.70+.84	4.31+.36
Early lactation	3.48+.27	3.85+.12
Mid involution	4.80+.07	5.19+.21
Colostrum	6.11+.25	4.51+.40
Early lactation	6.08+.11	4.32+.41
Mean	$5.23^{a,x}$+1.09	$4.43^{b,y}$+.53

^{a,b} Means in a row followed by the same superscript are not significantly different (P>.05).
^{w,x,y} Means in a column followed by the same superscript are not significantly different (P>.05).

[1] Data are expressed as $\bar{X} \pm$ SD cfu($\log_{10}$)/ml.
[2] Streptomycin resistant strain.
[3] ATCC reference strain.

Table 11. Protein content in each chromatographic peak obtained from mammary secretion skims collected during the nonlactating and peripartum periods[1].

	Chromatographic peak			
Experimental day	1	2	3	4
Late lactation	34	47	39	<10
Early involution	310	290	44	<10
Mid involution	230	320	49	<10
Colostrum	275	375	89	<10
Early involution	21	32	30	<10

[1]Protein content expressed in µg/100µl.

mammary secretions collected during involution and peripartum period was <10ug/100ul).

Results of 2D-PAGE of fractionated bovine mammary secretion skims are presented in Figures 13-17. Identification of lactoferrin (Lf), bovine serum albumin (BSA), Heavy (IgG_H) and light (IgG_L) chains of IgG, α-lactalbumin (LA), and β-lactoglobulin (LG) was based upon known isoelectric points (pI) and molecular weights (2).The protein band with pI and pH of approximately 8 and Mr similar to that of transferrin (78,000) was presumed to be Lf. Light (IgG_L) and heavy (IgG_H) chains of IgG were identified based upon M_r and protein map patterns as reported (1). Serum albumin was identified based upon reported pI and M_r (2). Casein were observed in the acidic region of the slab gel and was identified based upon pI and M_r of bovine milk caseins (2). α-lactalbumin and β -lactoglobulin were identified based upon pI as reported (2) and M_r as reported (4).

The majority of mammary secretion proteins eluted from S-200 column chromatography in peaks 1 and 2. Relative size and intensity of staining of protein bands and spots indicated that peaks 1 and 2 contained greater amounts of high M_r proteins and the majority of Lf, BSA, IgG_H, and IgG_L eluted from the column in protein peak 1 and 2.

Figure 13. Two-dimensional polyacrylamide gel electrophoresis of fractionated bovine mammary secretion skim collected during late-lactation. (a) 2D-PAGE of protein peak 1; (b) 2D-PAGE of protein peak 2; (3) 2D-PAGE of protein peak 3; BSA, bovine serum albumin; LA, α-lactalbumin; LG, β-lactoglobulin; c, caseins.

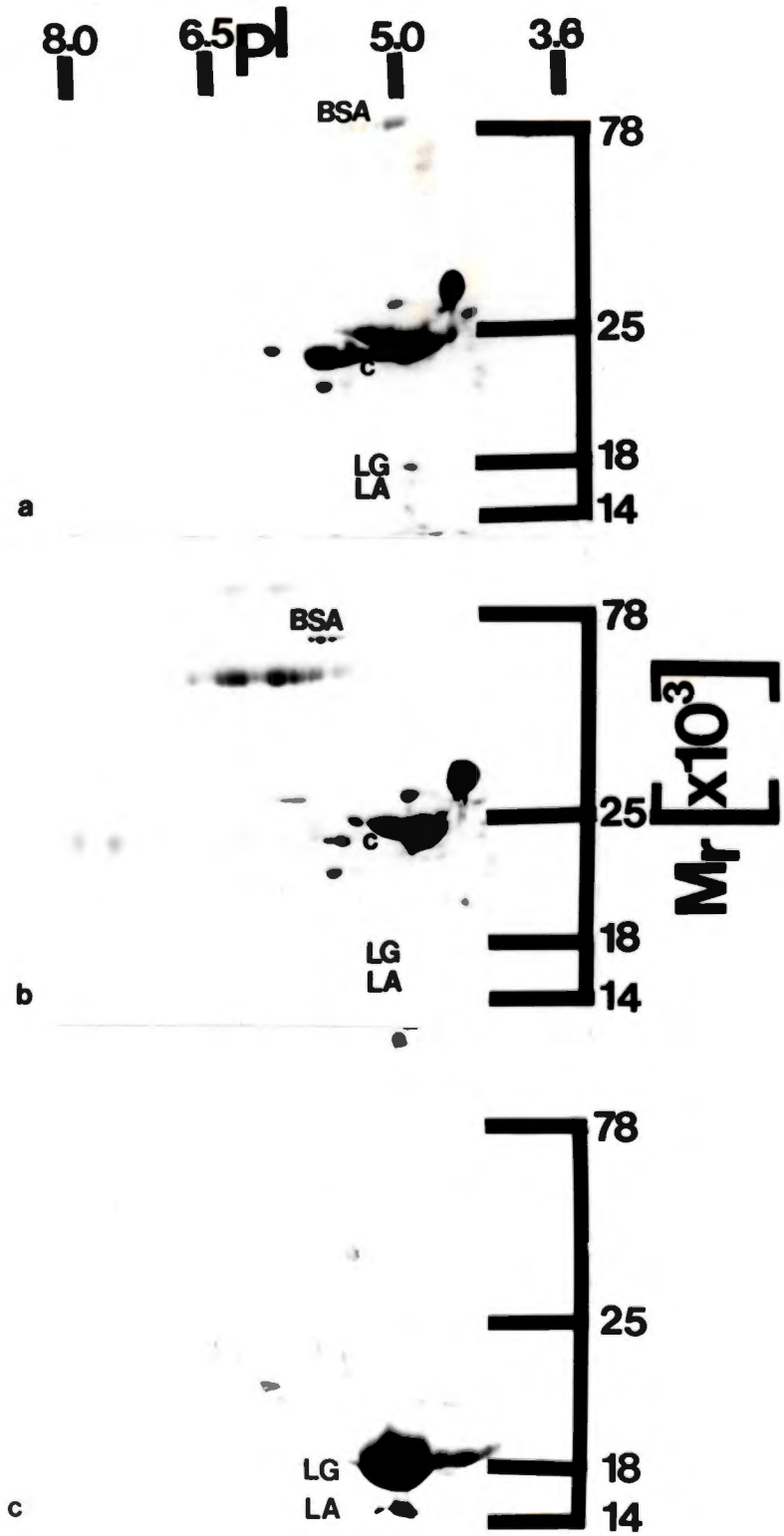
8.0
6.5
pI
5.0
3.6
BSA
78
25
c
18
LG
LA
14
a
BSA
78
25
c
18
LG
LA
14
b
Mr [x10³]
78
25
18
14
LG
LA
c

Figure 13
98
Juneja, 1988

Figure 14. Two-dimensional polyacrylamide gel eletrophoresis of fractionated bovine mammary secretion skim collected during early involution. (a) 2D-PAGE of protein peak 1; (b) 2D-PAGE of protein peak 2; (3) 2D-PAGE of protein peak 3; Lf, lactoferrin; BSA, bovine serum albumin; IgG_H, Heavy chain IgG; IgG_L, light chain IgG, LA, α-lactalbumin; LG, β-lactoglobulin; c, caseins.

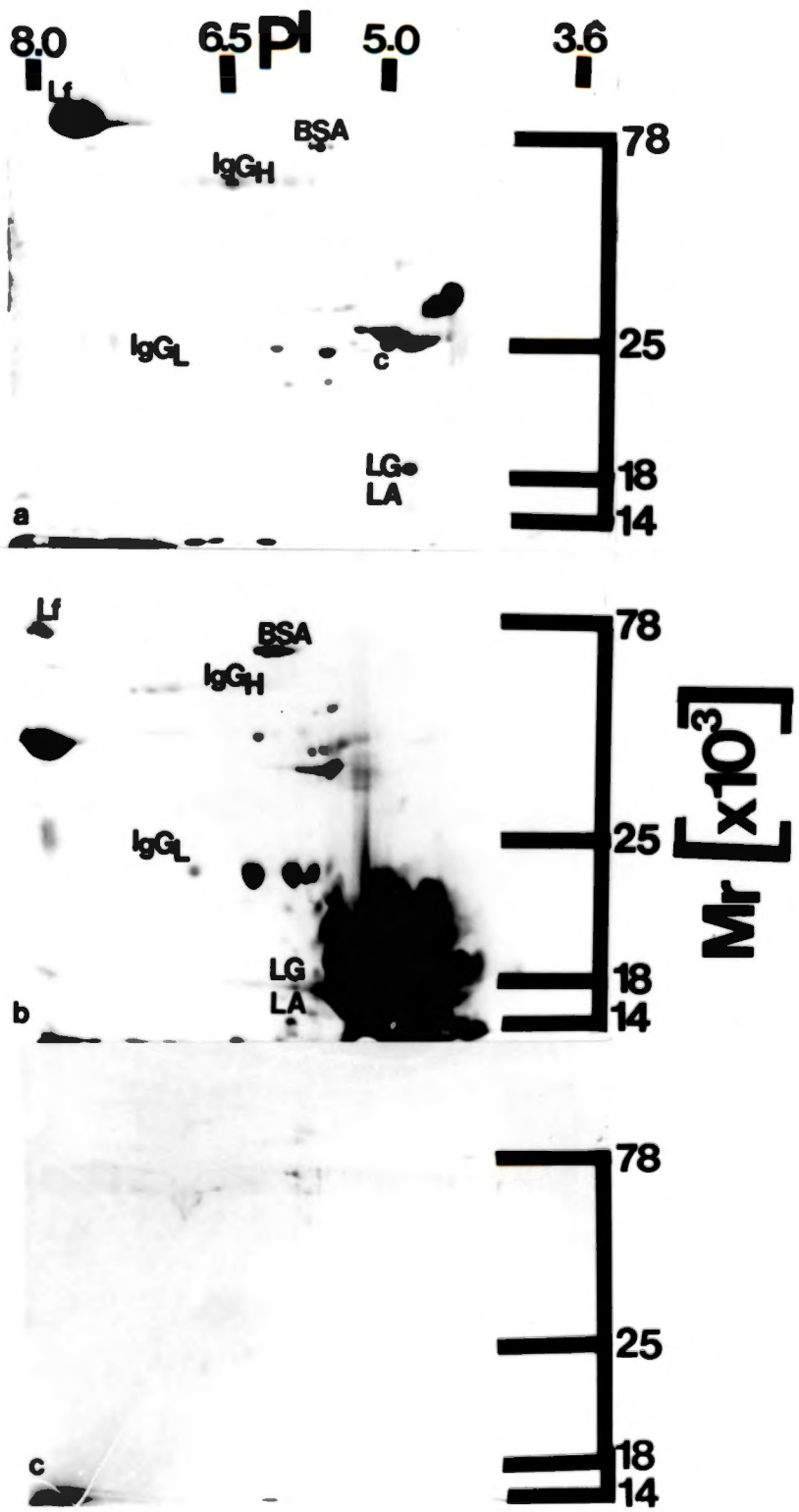
8.0
6.5
pI
5.0
3.6
Lf
BSA
IgG_H
IgG_L
c
LG
LA
a
78
25
18
14
Lf
BSA
IgG_H
IgG_L
LG
LA
b
78
25
18
14
Mr [x10^3]
c
78
25
18
14

Figure 14
100
Juneja, 1988

Figure 15. Two-dimensional polyacrylamide gel electrophoresis of fractionated bovine mammary secretion skim collected during mid-involution. (a) 2D-PAGE of protein peak 1; (b) 2D-PAGE of protein peak 2; (3) 2D-PAGE of protein peak 3; Lf, lactoferrin; BSA, bovine serum albumin; IgG_H, Heavy chain IgG; IgG_L, light chain IgG; LA, α-lactalbumin; LG, β-lactoglobulin; c, caseins.

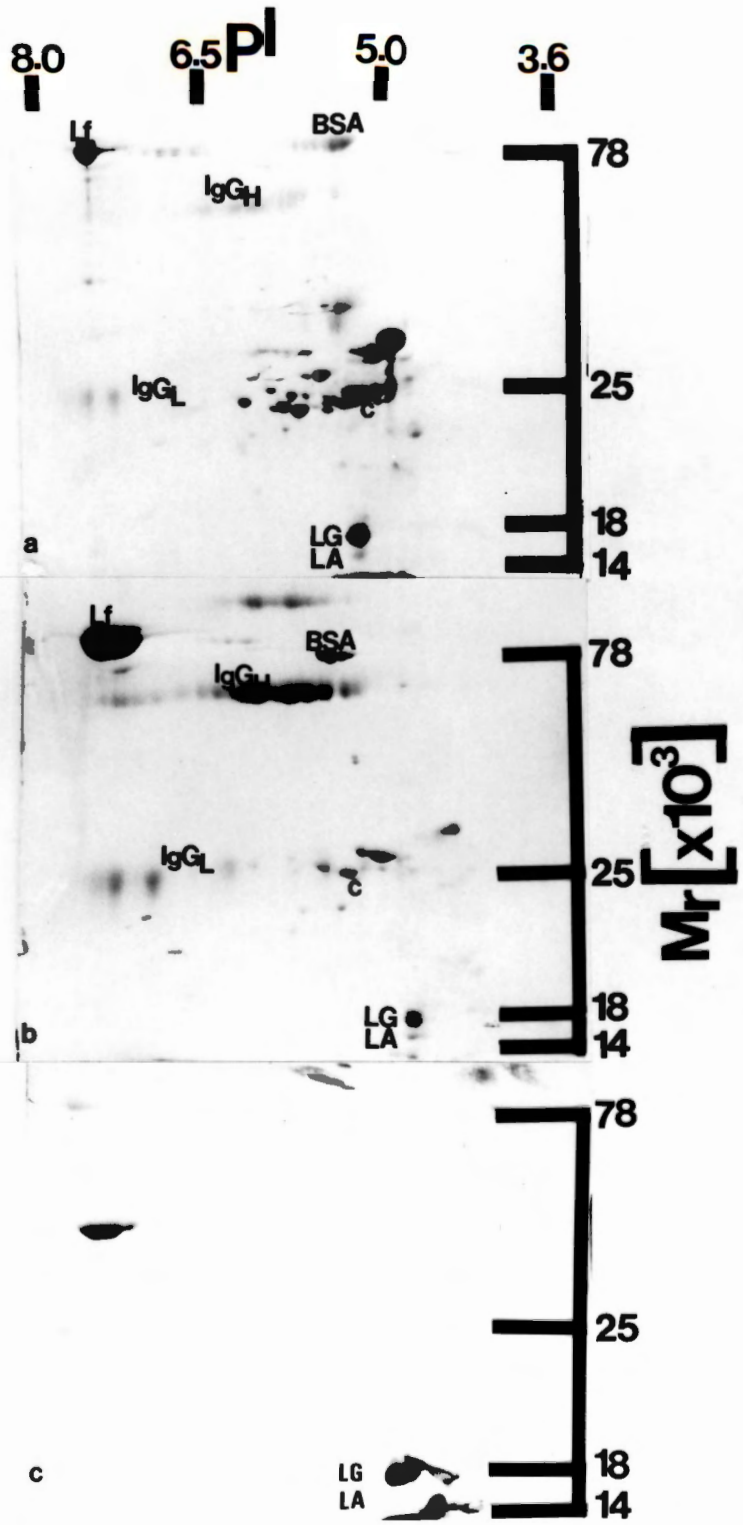
8.0
6.5
pI
5.0
3.6
Lf
BSA
IgG H
IgG L
c
LG
LA
a
b
c
78
25
18
14
Mr [x10³]

Figure 15
102
Juneja, 1988

Figure 16. Two-dimensional polyacrylamide gel electrophoresis of fractionated bovine secretion skim collected at parturition. (a) 2D-PAGE of protein peak 1; (b) 2D-PAGE of protein peak 2; (3) 2D-PAGE of protein peak 3; Lf, lactoferrin; BSA, bovine serum albumin; IgG_H, heavy chain IgG; IgG_L, light chain IgG; LA, α-lactalbumin; LG, β-lactoglobulin; c, caseins.

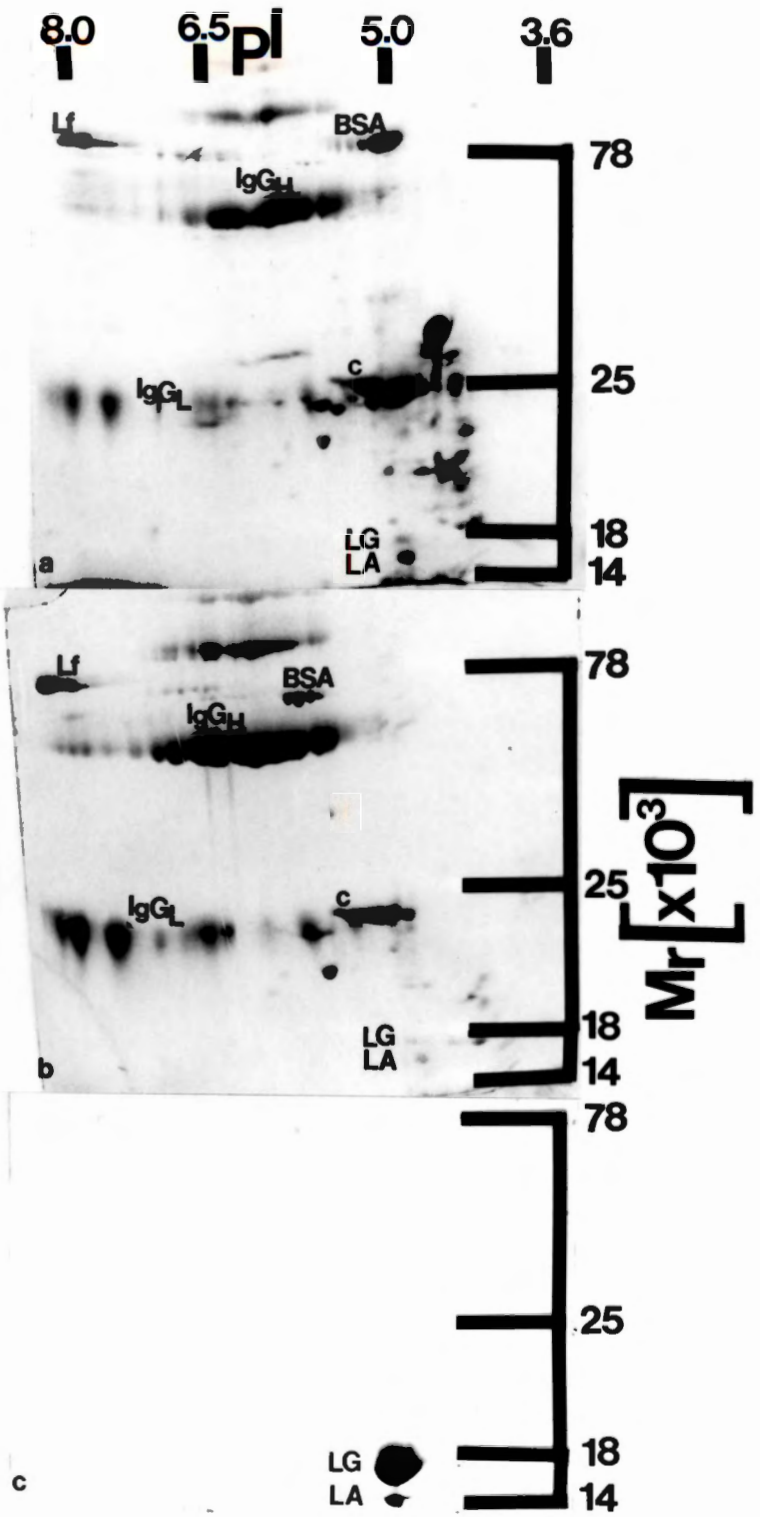
8.0
6.5
pI
5.0
3.6
Lf
BSA
IgG$_H$
IgG$_L$
c
LG
LA
a
b
c
78
25
18
14
Mr [x10^3]

Figure 16
104
Juneja, 1988

Figure 17. Two-dimensional polyacrylamide gel electrophoresis of fractionated bovine mammary secretion skim collected during early-lactation. (a) 2D-PAGE of protein peak 1; (b) 2D-PAGE of protein peak 2; (3) 2D-PAGE of protein peak 3; BSA, bovine serum albumin; LA, α-lactalbumin; LG, β-lactoglobulin; c, caseins.

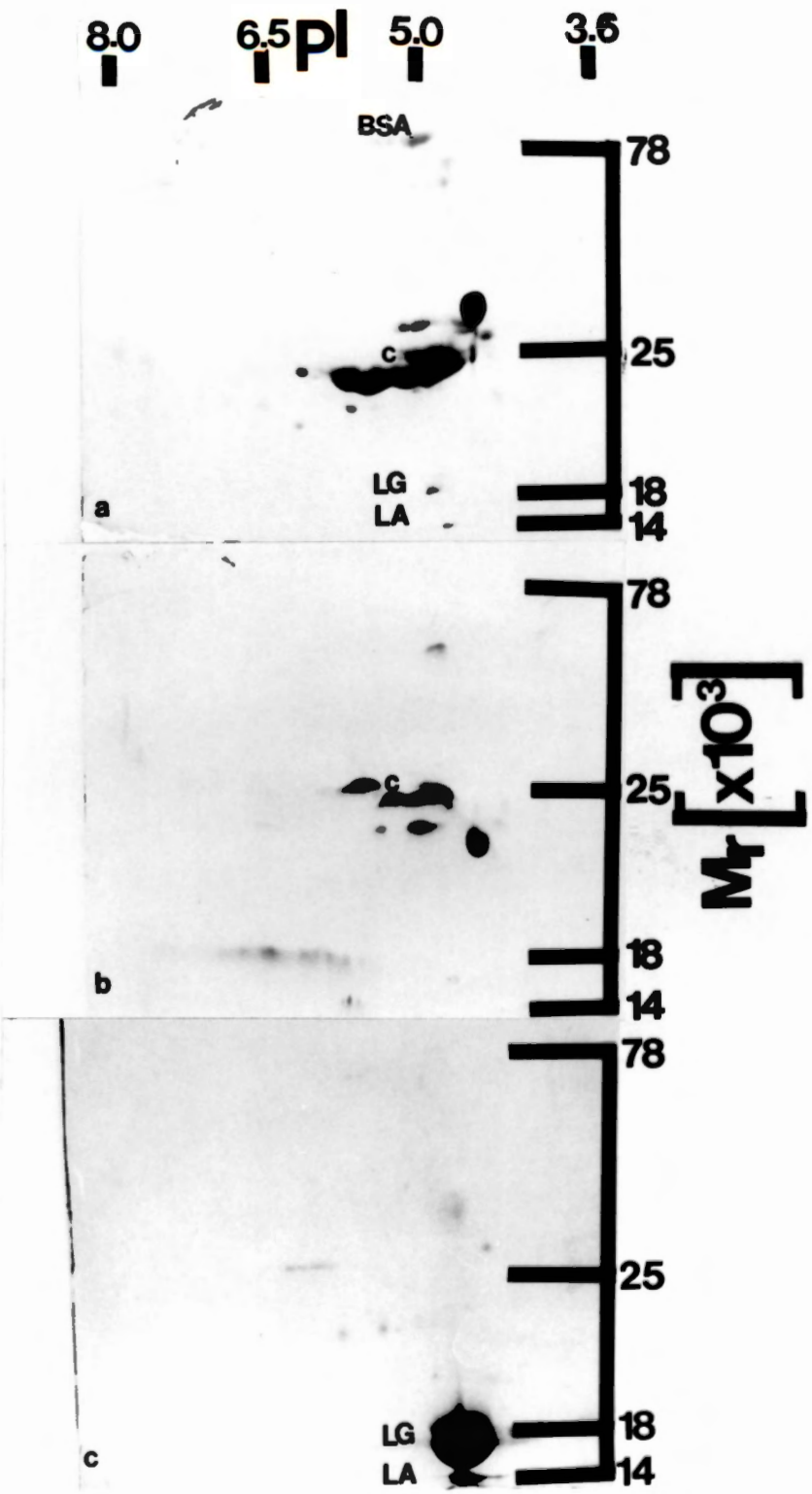
8.0
6.5
pI
5.0
3.6
BSA
78
c
25
LG
18
a
LA
14
78
c
25
Mr [x10³]
18
b
14
78
25
LG
18
c
LA
14

Figure 17
106
Juneja, 1988

Staphylococcus species grew well in peak 3 in mammary secretion obtained during involution and the peripartum period (Table 12).Reduced growth of Staphylococcus species was observed in peak 1 in mammary secretions obtained during involution and at parturition. Also, Staphylococcus species growth was reduced in peak 2 in mammary secretions obtained at 28 days after cessation of milking.

Corynebacterium bovis did not grow well in peak 1 in mammary secretions obtained during involution (Table 12). Also, C. bovis growth was reduced in peak 2 in mammary secretion obtained at 28 days after cessation of milking.

Staphylococcus hominis grew well as compared to other Staphylococcus species in peak 1 through 4 from mammary secretions obtained during involution and peripartum period (Table 13). However, Staph. hominis growth was reduced in peak 1 from mammary secretions obtained during involution and at parturition. Also, growth of Staph. hominis was reduced in peak 2 from mammary secretions obtained 28 days after cessation of milking and at parturition.

Staphylococcus epidermidis growth in peaks 1 and 2 from mammary secretions obtained at cessation of milking, during involution and the peripartum period was similar to Staph. hyicus and Staph. chromogenes (Table 13). Staphylococcus epidermidis growth in peak 3 from mammary secretion obtained during involution and the peripartum

108

Table 12. Growth of *Staphylococcus* species and *Corynebacterium bovis* in each chromatographic peak obtained from mammary secretion skims collected during involution and the peripartum period[1].

	Chromatographic profiles			
Experimental day	1	2	3	4
Staphylococcus[2] species				
Late lactation	4.02+1.79	3.92+1.85	6.48+2.53	4.30+1.93
Early involution	2.41+ .44	4.73+2.04	5.15+2.44	3.82+2.23
Mid involution	3.01+1.72	2,71+ .96	6.21+2.24	2.96+2.88
Colostrum	3.17+ .63	3.54+1.46	6.44+2.08	2.85+3.30
Early lactation	4.37+1.84	4.33+1.77	6.78+2.12	3.52+2.52
Mean	$3.39^{b,y}$+1.46	$3.84^{b,y}$+1.63	$6.21^{a,x}$+2.11	$3.49^{b,y}$+2.39
Corynebacterium[3] *bovis*				
Late lactation	3.35+2.91	5.23+1.62	5.17+2.11	4.64+1.73
Early involution	1.62+ .49	3.70+3.26	4.84+3.68	2.79+1.91
Mid involution	2.21+1.32	1.47+1.35	3.47+3.57	5.48+1.89
Colostrum	3.27+2.38	3.37+1.64	2.81+2.10	2.57+1.64
Early lactation	4.06+3.55	2.94+1.96	4.54+3.34	3.79+3.35
Mean	$2.82^{a,y}$+2.27	$3.14^{a,y}$+2.19	$4.17^{a,y}$+2.73	$2.86^{a,y}$+2.18

^{a,b}Means in a row followed by the same superscript are not significantly different (P>.05).

^{x,y}Means in a column followed by the same superscript are not significantly different (P>.05).

[1] Data expressed as $\bar{X} \pm SD$ cfu($\log_{10}$)/ml.

[2] *Staphylococcus hominis*, *Staphylococcus epidermidis*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*.

[3] *Corynebacterium bovis* wild type. ATCC reference strain, streptomycin resistant strain.

Table 13. Growth of Staphylococcus species in each chromatographic peak obtained from mammary secretion skims collected during involution and the peripartum period.[1]

	Chromatographic peaks			
Experimental day	1	2	3	4
Staphylococcus hominis:				
Late lactation	6.59	6.64	6.73	6.82
Early involution	3.00	6.42	6.04	6.72
Mid involution	5.57	4.09	6.55	6.70
Colostrum	3.96	5.60	6.40	7.66
Early lactation	6.64	6.70	6.76	6.82
Mean	$5.15^{a,x} \pm 1.62$	$5.89^{a,x} \pm 1.10$	$6.50^{a,w} \pm .29$	$6.94^{a,w} \pm .40$
Staphylococcus epidermidis:				
Late lactation	3.00	2.63	2.82	2.23
Early involution	2.49	2.56	1.54	1.54
Mid involution	2.04	2.64	2.94	.69
Colostrum	2.41	3.32	3.56	.69
Early lactation	2.13	2.41	3.79	.69
Mean	$2.41^{a,y} \pm .37$	$3.51^{a,y} \pm 1.73$	$2.93^{a,y} \pm .87$	$1.16^{a,z} \pm .70$

Table 13. (Continued)

	Chromatographic peaks			
Experimental day	1	2	3	4
Staphylococcus hyicus:				
Late lactation	2.63	2.88	8.14	4.56
Early involution	2.16	3.16	6.88	2.75
Mid involution	1.98	2.04	7.68	.69
Colostrum	3.14	2.16	8.34	.69
Early lactation	4.39	4.23	8.29	3.25
Mean	$2.86^{a,y} \pm .97$	$2.89^{a,y} \pm .88$	$7.87^{a,w} \pm .61$	$2.39^{a,y} \pm 1.68$
Staphylococcus chromogenes:				
Late lactation	3.85	3.55	8.22	3.61
Early involution	2.00	2.79	6.16	2.27
Mid involution	2.45	2.08	7.66	3.76
Colostrum	3.16	3.08	7.45	2.35
Early lactation	6.31	4.00	8.27	3.31
Mean	$3.15^{b,y} \pm .95$	$3.10^{b,y} \pm .73$	$7.55^{a,w} \pm .85$	$3.46^{b,y} \pm .71$

^{a,b}Means in a row followed by the same superscript are not significantly different ($p > .05$).

^{x,y,z}Means in a column followed by the same superscript are not significantly different ($p > .05$).

[1]Data expressed as $\bar{X} \pm SD$ cfu($\log_{10}$)/ml.

period was markedly reduced compared to growth of other Staphylococcus species. Staphylococcus epidermidis did not grow in peak 4 from mammary secretions obtained at 28 days after cessation of milking and during the peripartum period. In general, growth of all Staphylococcus species was reduced in peaks 1 and 2 from mammary secretions obtained during involution.

The ATCC reference strain of C. bovis grew well in peaks 1 and 2 from mammary secretion obtained at cessation of milking and during early lactation (Table 14). Growth of the C. bovis reference strain was either reduced or the strain did not grow in peaks 1 and 2 from mammary secretion obtained during involution and at parturition. In general, the reference strain of C. bovis grew better than the other strains of C. bovis evaluated.

The wild type strain of C. bovis did not grow or growth was reduced markedly in peaks 1-4 from mammary secretion obtained during involution and the peripartum period. Variation in growth of the streptomycin resistant strain of C. bovis in peak 1-4 from mammary secretion obtained during involution and peripartum period was observed. Minimum growth was observed in peak 1 from mammary secretion obtained at 14 days after cessation of milking and maximum growth was detected in peak 3 from mammary secretion obtained at 14 days after cessation of milking and 14 days after parturition.

Table 14. Growth of Corynebacterium bovis in each chromatographic peak obtained from mammary secretion skims collected during involution and the peripartum period.[1]

	Chromatographic peak			
Experimental day	1	2	3	4
Corynebacterium bovis[2]:				
Late lactation	.69	4.49	5.40	2.89
Early involution	.69	.69	.69	.69
Mid involution	.69	.69	.69	4.68
Colostrum	.69	.69	.69	.96
Early lactation	.69	3.86	.69	.96
Mean	$.69^{a,z} \pm 0$	$2.08^{a,y} \pm 1.92$	$1.63^{a,z} \pm 2.10$	$1.92^{a,y} \pm 1.80$
Corynebacterium bovis[3]:				
Late lactation	2.91	4.12	2.95	4.69
Early involution	1.54	3.24	6.10	3.27
Mid involution	3.13	3.02	2.27	4.13
Colostrum	3.72	2.45	2.86	3.32
Early lactation	3.72	.69	6.60	7.34
Mean	$3.00^{a,y} \pm .89$	$2.70^{a,y} \pm 1.27$	$4.15^{a,x} \pm 2.02$	$4.55^{a,x} \pm 1.66$
Corynebacterium bovis[4]:				
Late lactation	6.46	7.09	7.15	6.35
Early involution	1.54	4.17	7.72	4.42
Mid involution	2.80	.69	7.51	7.64

Table 14. (Continued)

	Chromatographic peak			
Experimental day	1	2	3	4
Colostrum	5.39	3.96	4.88	3.70
Early lactation	7.77	4.28	6.33	3.35
Mean	$4.80^{a,x} \pm 2.58$	$4.64^{a,x} \pm 2.67$	$6.72^{a,w} \pm 1.16$	$5.09^{a,x} \pm 1.83$

^{a,b}Means in a row followed by the same superscript are not significantly different ($p > .05$).

^{x,y,z}Means in a column followed by the same superscript are not significantly different ($p > .05$).

[1]Data expressed as $\bar{X} \pm SD$ cfu($\log_{10}$)/ml.

[2]Wild type.

[3]Streptomycin resistant strain.

[4]ATCC reference strain.

Growth of Staphylococcus species in skims with BHI was slightly increased compared to growth in skims only (Table 15). Similarly, slight increase in growth of C. bovis in skims containing BHI with Tween80 compared to skims alone was observed. Growth in BHI was slightly higher than in skims or in skims with BHI. Slight variation in growth among Staphylococcus species and among C. bovis strains in BHI, skims, and skims with BHI was observed.

DISCUSSION

Variation in growth of C. bovis in skim and whey may be due to removal of casein at the time of acid precipitation. Corynebacterium bovis may utilize casein for their growth, but Staphylococcus species did not appear to be dependent on casein for their growth. Strain variation was observed with Staphylococcus species and C. bovis.

Growth of Staphylococcus species in skim and whey followed the same pattern during the nonlactating and periparturient periods, but comparatively increased growth was observed in whey obtained from mammary secretions collected at 14 and 28d of involution indicating that some other inhibitory component might have been removed along with casein at the time of acid precipitation.

Table 15. Growth of *Staphylococcus* species and *Corynebacterium bovis* in skims from mammary secretion obtained during involution and the peripartum period and in brain heart infusion broth.[1]

Organism	Late lactation		Early involution		Mid involution		Colostrum		Early lactation		BHI[2]
	Skim 250 µl	Skim 245 µl + 5 µl BHI	Skim 250 µl	Skim 245 µl + 5 µl BHI	Skim 250 µl	Skim 245 µl + 5 µl BHI	Skim 250 µl	Skim 245 µl + 5 µl BHI	Skim 250 µl	Skim 245 µl +5 µl BHI	
Staphylococcus chromogenes	7.74	8.23	3.57	3.92	3.92	4.23	7.54	7.84	7.83	8.30	8.57
Staphylococcus hyicus	7.90	8.36	.69	3.57	3.61	4.39	7.86	8.20	9.94	8.28	8.53
Staphylococcus hominis	6.95	7.26	3.28	3.72	4.52	4.87	6.65	6.94	6.94	7.34	7.36
Staphylococcus epidermidis	7.60	7.96	.69	3.78	4.06	4.81	7.45	7.96	7.70	8.14	8.37
Corynebacterium bovis (wild type strain)	5.41	5.72	2.92	3.38	3.38	3.73	4.25	4.57	5.45	5.77	6.93
Corynebacterium bovis (ATCC reference strain)	6.80	7.10	3.95	4.55	4.14	4.75	5.71	5.96	6.80	7.05	6.92
Corynebacterium bovis (Streptomycin resistant strain)	5.04	5.58	3.73	3.94	3.80	4.00	4.95	5.23	5.05	5.57	6.23

[1]Data expressed as $\bar{X}$ cfu($\log_{10}$)/ml.

[2]Brain heart infusion broth.

Gel filtration separates proteins on the bases of molecular size. Large molecules are excluded from the porous gel grains and emerge from the chromatographic bed in the beginning, whereas smaller molecules penetrate gel pores and emerge later. Bovine mammary secretions contain a variety of proteins with different molecular weights. Thus, based on molecular weight, four protein peaks were obtained from each of the mammary secretion skim samples collected during the nonlactating and periparturient periods.

Differences in growth of Staphylococcus species and C. bovis may be due to changes in the biochemical composition and antibacterial properties of bovine mammary secretion. During physiological transitions of the mammary gland, mammary secretions contain high concentration of fat, casein, lactose, and citrate (13). All of those components have been shown to support bacterial growth. On the other hand, the number of macrophages, neutrophils and lymphocytes and concentrations of immunoglobulins and Lf are low; all of these components have been implicated as resistance factors to intramammary infections (13). Secretions from fully involuted mammary glands contain elevated numbers of macrophages, lymphocytes and neutrophils; and high concentrations of lactoferrin and immunoglobulin (12).

Inhibitory components were in low concentration in mammary secretions collected at cessation of milking and during early lactation, increased growth of organisms was observed in all the four peaks. The growth of the organisms was decreased in peak 1 and 2 of skims collected at 14 and 28d of involution because of high concentration of high molecular weight inhibitory components of mammary secretions in peak 1 and 2.

Previous studies (10) have demonstrated that changes in composition of mammary secretions during involution and peripartum period were correlated with *in vitro* growth of mastitis pathogens. As the concentration of lactoferrin increased, growth of *Escherichia coli*, and *Klebsiella pneumoniae* decreased. On the other hand, a high concentration of citrate and high citrate to Lf molar ratio resulted in increased growth of pathogens. It was suggested that differences in resistance or susceptibility to new IMI may be due to changes in mammary secretion composition leading to increased or decreased bacteriostasis.

The pattern of *Staphylococcus* species growth in peaks from mammary secretions obtained during the nonlactating and periparturient periods is in agreement with the results of studies conducted on coliforms. All *Staphylococcus* species follow similar pattern of growth and the growth was reduced in peak 1 and 2 from mammary

secretions collected at 14 and 28 days of involution, when lactoferrin concentrations are elevated and when citrate concentrations are low. On the other hand, greatest growth of Staphylococcus species was observed in peaks from mammary secretions collected at cessation of milking, and at 14 days after parturition when mammary secretions contain high concentration of citrate and low concentration of Lf,and a very high citrate:Lf molar ratio (13). However, variation in growth among Staphylococcus species in different peaks was observed. Staphylococcus hominis growth was higher than other Staphylococcus species in all 4 peaks across all sampling periods.

Marcelis et al. (7) demonstrated that strains of Staph. epidermidis were highly susceptible to iron deprivation, while strains of Staph. aureus were comparatively less susceptible. The iron requirement of Staphylococcus species evaluated in the present study is not known. Marked decrease in growth of Staphylococcus species in peak 1 and 2 from mammary secretion obtained during involution was observed, a time when Lf concentrations are elevated and when citrate concentrations are low. Staphylococcus species may be highly susceptible to iron deprivation.

The present study indicated that high molecular weight biochemical components of bovine mammary secretions collected during involution and peripartum period have

bacteriocidal or bacteriostatic effect on *Staphylococcus* species and *Corynebacterium bovis*.

REFERENCES

1. Anderson, L., and N.G. Anderson. 1977. High resolution two-dimensional electrophoresis of human plasma proteins. Proc.Natl. Acad. Sci. USA, 74:5421.

2. Eigel, W.N., J.E. Butler, C.A. Ernstrom, H.M. Farrell, Jr., V.R. Harwalker, R. Jenness, and R. McL. Whitney. 1984. Nomenclature of proteins of cow's milk: Fifth revision. J. Dairy Sci. 67:1599.

3. Horst, M.N., and R.M. Roberts, 1979. Analysis of peptide turnover rates in Chinese Hamster ovary cells plasma membranes using two-dimensional electrophoresis. J. Biol. Chem. 254:5000.

4. Hurley, W.L., and J.J. Rejman. 1986. B-lactoglobulin and a-lactalbumin in mammary secretion during the dry period: Parallelism of concentration changes. J. Dairy Sci. 69:1642.

5. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 270:680.

6. Lowry, O.H., J.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193:265.

7. Marcelis, J.H., J.Den Daas-Slagt Hanneke, and Jacomina A.A. Hoogk amp-korstanje. 1978. Iron requirement and chelator production of staphylococci, Streptococcus faecalis and enterobacteriaceae. Antonie van leecuwenhoek. 44:257.

8. O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007.

9. Oliver, S.P., and B.A.Mitchell. 1983. Susceptibility of bovine mammary gland to infections during the dry period. J.Dairy Sci. 65:801.

10. Oliver, S. P., and T. Bushe. 1987. Growth inhibition of Escherichia coli and Klebsiella pneumoniae during involution of the bovine mammary gland:Relation to secretion composition. 48:1669.

11. Nonnecke, B.J., and K.L. Smith. 1984. Biochemical and antibacterial properties of bovine mammary secretions during mammary involution and at parturition.J.Dairy Sci. 67:2863.

12. Schanbacher, F.L., and K.L. Smith. 1975. Formation and role of unusual whey proteins and enzymes:relation to mammary function. J. dairy Sci. 58:1078.

13. Smith, K.L., and D.A. Todhunter. 1982. The physiology of the mammary gland during the dry period and the relationship to infection. Proceedings. 21st Annu Meet NMC. 87.

VITA

The author, Vijay Kumar Juneja, was born in Jammu Tawi, India, on July 1, 1956. He graduated from Central School, New Delhi, India, in June 1972. He joined Rajasthan University, India for pre-medical studies. In July 1974, he entered G. P. Pant University of Agriculture and Technology, Pantnagar, India and graduated in Veterinary Medicine in October, 1978. He worked as a Veterinarian till June 1985 in private and government organizations in India.

In July, 1985 he came to the United States as an immigrant. In January 1986, he began study towards a Master of Science degree at the University of Tennessee at Knoxville. He earned this degree in June, 1988.

The author will begin work on a Doctor of Philosophy degree in Food Technology and Science at the University of Tennessee, Knoxville, in August 1988.