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Changes in Lactoferrin, Immunoglobulin G, Bovine Serum Albumin, and α-Lactalbumin During Acute Experimental and Natural Coliform Mastitis in Cows[1]

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An experimentally induced *Escherichia coli* infection of a bovine mammary gland resulted in a 30-fold increase in lactoferrin (Lf) concentration in the mammary secretion by 90 h postinoculation and a 4-fold increase in total daily production of Lf by 264 h postinoculation in the infected quarter. A simultaneous rise and fall of bovine serum albumin (BSA) and immunoglobulin G (IgG) concentrations occurred during the acute phase of the infection. Peak BSA and IgG levels were reached 36 h before peak Lf levels. BSA concentrations declined rapidly after the acute phase, whereas IgG and Lf levels remained elevated and decreased slowly as the infection subsided. A decline in α-lactalbumin concentration by 48 h postinoculation indicated decreased synthetic capability. The increased Lf production may be a result of a specific response of secretory tissue to inflammatory agents and thus the infectious process. Analogous changes in Lf, IgG, and BSA were observed during a natural coliform infection. Sephadex G-200 chromatography of mastitis skim milk showed that Lf approximated the monomer (molecular weight 77,100) early in infections. As the infection progressed and abated, the apparent molecular weight of Lf increased to approximately that of the trimer and subsequently decreased to about 1.5 times that of the monomer.

Marked changes have been reported in the whey protein composition of mammary secretions during mastitis. However, these studies (3, 7) failed to recognize the presence of lactoferrin (Lf), an iron-binding protein found in milk (11, 12, 21), other external secretions (20, 22), and the granules of polymorphonuclear leukocytes (PMN) (2, 23). Lf has been shown to be bacteriostatic for a variety of microorganisms in vitro (15, 20, 26). This inhibitory activity is believed to be a result of the iron-chelating ability of Lf, making iron unavailable to bacteria. In addition, a combination of Lf and specific *Escherichia coli* antibody has been shown to have greater bacteriostatic capability against *E. coli* than either component tested alone (4). Although the precise biological function of Lf is still in question, it has been suggested that Lf may play a role in the protection of epithelial tissues from infection (21).

Recently, we reported (13) that clinical mastitis in cows resulted in an elevated level of Lf in infected quarters when compared with that of uninfected control quarters. Interpretation of these data suggested that major changes in the concentration of Lf and other whey proteins were occurring during the course of the infection. Thus, the purpose of the present investigation was to determine in greater detail changes in Lf concentration relative to immunoglobulin G (IgG), bovine serum albumin (BSA), and α-lactalbumin (α-LAC) during an acute mammary infection. In addition, changes in the elution behavior of Lf from columns of Sephadex G-200, during the course of acute mastitis, were investigated.

MATERIALS AND METHODS

Animals. Two cows of the Jersey breed in late lactation were used for experimental *E. coli* mastitis studies. Cow 1943 was 6 years old and cow 2127 was 2 years old. Culture of milk samples at the time of *E. coli* inoculation showed an absence of bacterial infection. The milk production of individual quarters and the amount of milk sampled were recorded throughout the entire experiment. The quarter treatment at zero hour was the same for both cows and is shown in Table 1. Samples were obtained at 126, 78, and 30 h prior to inoculation and once imme-

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TABLE 1. *Identification of quarter treatments in experimentally induced mastitis studies*

Quarter	Treatment[a]
RF	Untreated control
RR	Inoculated with 200 CFU[b] of *E. coli* in 1.0 ml of sterile saline
LF	Infused with 1.0 ml of sterile saline
LR	Untreated control

[a] At zero hour.
[b] CFU, Colony-forming units.

diately before the right rear (RR) quarter was inoculated at zero hour. After inoculation, quarter samples were taken every 6 h for the first 2 days, every 12 h for the next 3 days, and then once every 2 or 3 days for the duration of the experiment. Clinical signs of mastitis were recorded throughout the study.

A naturally occurring case of *E. coli* mastitis involving the RR quarter of a 3-year-old lactating Holstein cow (2124) was also studied. Samples were taken from each quarter upon discovery of the mastitis, designated as 24 h, with subsequent sampling at 72, 120, 240, and 360 h. The RR quarter was treated with a commercial antibiotic preparation (penicillin-dihydrostreptomycin ointment; trade name, Pendistrin; Squibb & Sons, Inc., New York) after the day-1 sampling. An uninfected control quarter (left rear, LR) was selected on the basis of a low somatic cell count and culture of milk samples.

Sample preparation. Skim milk samples were prepared by centrifugation of whole milk at 48,000 × *g* for 20 min at 4 C to remove cells and fat. Casein was precipitated from skim milk by lowering the pH to 4.5 with glacial acetic acid and was removed by centrifugation at 48,000 × *g* for 30 min. Skim and whey samples were stored frozen (−15 C) until needed.

E. coli. An *E. coli* culture was isolated from milk of an infected cow from the Ohio Agricultural Research and Development Center (OARDC) dairy herd and was maintained on blood agar slants at 4 C. Before use, the culture was transferred to brain heart infusion broth and incubated for 6 h. Dilutions were made of this culture with sterile saline solution to attain the appropriate number of organisms. The number of colony-forming units per milliliter was determined by plate counts of each dilution.

Culture. The presence and identification of microorganisms in freshly collected milk samples were determined by routine cultures on calf blood agar and eosin methylene blue plates. Hotis tests were also set by placing approximately 9.5 ml of milk into sterile tubes containing 0.5 ml of 0.5% bromocresol purple.

Somatic cell counts. Somatic cell numbers in milk from cow 2124 were determined by the Wisconsin mastitis test according to the method of Thompson and Postle (31), using measuring squares (Z. D. Roundy, Madison, Wis.) calibrated to read directly in somatic cells per milliliter. The direct microscope somatic cell count, using the Newman-Lampert stain, was used to determine cell counts for milk samples from experimental infections.

Antisera. Antiserum to bovine Lf was prepared in this laboratory by intramuscular injection of Lf into rabbits. Rabbit anti-BSA and anti-bovine α-LAC were obtained from Antibodies, Inc., Davis, Calif. Rabbit anti-bovine IgG was obtained from Cappell Laboratories, Inc., Downingtown, Pa.

Protein quantitation. Lf, α-LAC, BSA, and IgG were quantitated in wheys by using electroimmunodiffusion (EID) on cellulose acetate plates (Helena Laboratories, Beaumont, Tex.) as described for Lf by Schanbacher and Smith (28). The following antiserum solutions were used to soak the appropriate EID plates: 1.5 to 2.0% anti-Lf, 2% anti-BSA, 3% anti-IgG, and 2% anti-α-LAC with 4% dextran. Antisera and standards for Lf and BSA were diluted with 0.0125 M sodium phosphate buffer, pH 7.4. A 1:4 dilution (with deionized, distilled water) of Gelman high-resolution buffer [tris(hydroxymethyl)aminomethane (Tris)-barbital-sodium barbital (pH 8.8), 18 g per vial, diluted to 1,000 ml, ionic strength of 0.06; Gelman Instrument Co., Ann Arbor, Mich.] was used to dilute antisera and standards for IgG and α-LAC. EID plates were subjected to electrophoresis for 1 h at 7 mA/plate with 0.1 M sodium phosphate buffer, pH 7.4. α-LAC and IgG EID plates were subjected to electrophoresis for 40 to 45 min at 200 V with Gelman high-resolution buffer, pH 8.8.

Whey protein standards. Bovine Lf was purified in this laboratory from bovine lacteal secretion as described previously (29) and was pure as determined by disc-gel electrophoresis, immunoelectrophoresis, ultracentrifugation, gel filtration, and ion-exchange chromatography. IgG was quantitated using Pentex bovine IgG_1 (Miles Laboratories, Kankakee, Ill.) as the IgG standard. BSA was obtained from Sigma Chemical Co., St. Louis, Mo., and bovine α-LAC was from K. E. Ebner (Oklahoma State Univ., Stillwater). Standards between 0.05 and 0.40 mg/ml were prepared for each protein and run on every plate. Extinction coefficients (absorbancy at 280 nm [A_{280}] per milligram per milliliter) used to determine protein concentration of standard solutions were: α-LAC, 2.01 (30); BSA, 0.66 (30); IgG, 1.37 (6); and Lf, 1.45 (8).

Electrophoresis. Wheys were routinely subjected to electrophoresis in duplicate, using the disc-gel method in standard 7.5% acrylamide gel (pH 8.9) as described by Ornstein and Davis (27) or by a modification of the Ortec system (Ortec, Inc., Oak Ridge, Tenn.) of vertical slab-gel electrophoresis (Ortec Application Note An 32, p. 17-21, 1970). The modified slab-gel system employed a 10%-7%-3.5% polyacrylamide step-gradient gel (pH 9) with a Tris-glycine electrode buffer according to Maurer (24). Proteins identified in whey were β-lactoglobulin, α-LAC, BSA (blood), α- and β-globulin, and γ-globulin as described previously (29).

Gel filtration. A system of two columns (2.5 by 100.0 cm) was packed with Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, N.Y.; bead size, 40 to 120 μm). Flow was in an upward direction with a flow rate of approximately 15 ml/h. The buffer

system was 0.1 M Tris-hydrochloride (pH 8.0) in 0.2 M NaCl and 0.1% sodium azide. Fractions (5 ml each) were collected, and the A_{280} was determined in a Beckman DB-GT grating spectrophotometer.

The elution position of the various whey proteins was determined by EID analysis of the chromatography effluents as described previously (28). The highest peak in the Lf elution profile as determined by EID was designated as the major Lf peak. Minor Lf peaks were any smaller peaks or definite shoulders in the elution profile. The molecular weights of Lf elution peaks were estimated by gel filtration, using the EID profiles for BSA, IgG, and α-LAC in the chromatographic effluent of each sample as molecular weight markers according to the method of Andrews (1).

Some Lf elution profiles were characterized by marked tailing or skewing of the Lf peaks. Merely describing the elution positions of discrete peaks failed to fully describe the elution behavior of Lf. Thus, an attempt was made to determine the entire molecular weight range over which Lf was eluted. For this purpose, the upper and lower limits to the Lf molecular weight distribution were arbitrarily determined at the inflection point of the EID elution profile as it returned to the base line and is indicated as molecular weight range.

RESULTS

Whey protein changes during natural, acute coliform mastitis. The RR quarter of cow 2124 was swollen and sensitive, the rectal temperature was elevated (40 C), feed consumption was decreased, and the secretion had the appearance of blood serum at 24 h, the acute phase of the infection.

Figure 1 shows the changes in Lf, BSA, and IgG concentrations for the infected (RR) and uninfected control (LR) quarters of cow 2124 during the 15-day sampling period. In the RR quarter at 24 h BSA and IgG were elevated to 20.7 and 15.5 mg/ml, respectively. These BSA and IgG concentrations were about 27 and 15 times higher than respective concentrations in the LR quarter at 24 h. BSA levels dropped rapidly on successive sampling days, reaching near normal levels by 360 h. However, IgG levels decreased more gradually, remaining above 5.0 mg/ml through 240 h and dropping to 1.0 mg/ml by 360 h. The Lf level in the RR quarter was the same as that in the uninfected LR quarter at 24 h but increased to 8.2 mg/ml by 72 h. The 120-h Lf level in the RR quarter was still above 7.0 mg/ml but declined to 1.0 mg/ml by 360 h. Peak Lf concentration was reached when BSA and IgG levels were declining. Although BSA levels were higher than IgG levels at 24 h, BSA dropped rapidly to levels below those found for IgG as the infection subsided. A small increase in Lf, IgG, and BSA levels was observed in the uninfected LR

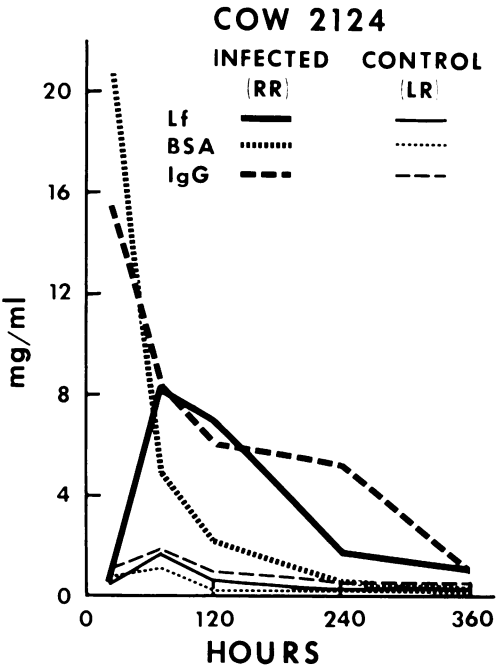

FIG. 1. *Changes in Lf, BSA, and IgG concentrations in the infected (RR) and uninfected control (LR) quarters during a clinical case of coliform mastitis in cow 2124.*

quarter on day 3. IgG and Lf levels were less than 2.0 mg/ml and the BSA level was about 1.0 mg/ml at peak concentration.

Whey protein changes during experimental coliform mastitis. Cow 1943 had a marked response to inoculation. The clinical signs of infection were most severe from about 36 to 90 h postinoculation, the acute phase. In the early hours of the acute phase, the entire right half of the udder was swollen, body temperature was elevated, the cow was depressed and had stopped eating, and the secretion from the injected quarter had the appearance of blood serum. The response of milk production of each quarter is shown in Fig. 2. At 42 h the cow was treated by intramammary (lincocin-neomycin; trade name, Lincocin Forte; Upjohn Co., Kalamazoo, Mich.) and intramuscular (penicillin-streptomycin) administration of commercial antibiotic preparations.

Fig. 3 shows whey protein and cell count changes in the RR quarter of cow 1943 during the first 162 h. Cell counts increased rapidly, going above 25×10^6 cells per ml. BSA and IgG reached peak concentrations of 21.5 and 18.3 mg/ml, respectively, by 54 h postinoculation. Mean blood serum values determined from five tail vein samples taken during the infection were: BSA, 35.6 mg/ml; and IgG, 23.1 mg/ml. α-

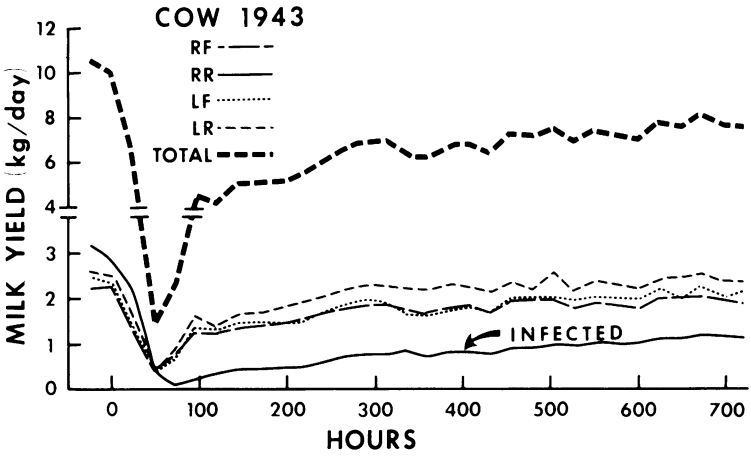

FIG. 2. *Milk yield during experimentally induced mastitis in cow 1943. Total yield per 24 h (day) and yield per 24 h for each quarter is shown.*

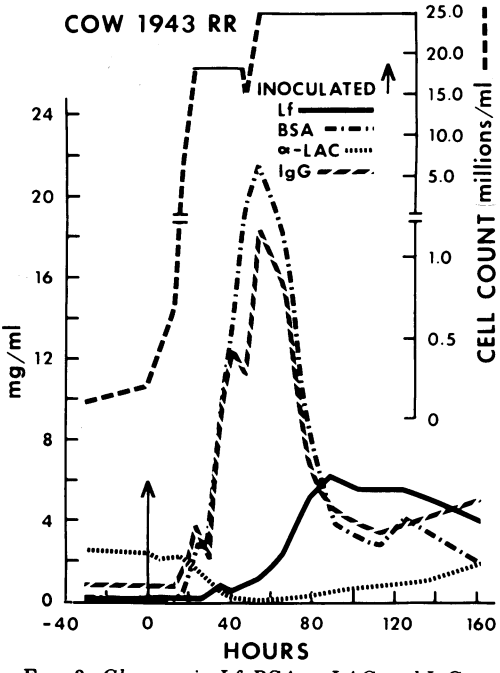

FIG. 3. *Changes in Lf, BSA, α-LAC and IgG concentrations and cell count from 30 h preinoculation to 162 h postinoculation in the infected quarter (RR) of cow 1943.*

LAC began decreasing over the same time period, nearly paralleling the changes in milk yield for the RR quarter (Fig. 2). The changes in α-LAC levels appeared to directly reflect changes in synthetic capability of the quarter. Lf levels did not change as abruptly as BSA and IgG levels. Lf reached its peak concentration of 6.2 mg/ml 36 h after BSA and IgG reached maximum levels. This is comparable to the pattern of change seen for the RR quarter of cow 2124 (Fig. 1), a natural infection. By 90 h postinoculation, both BSA and IgG declined below Lf levels.

Beyond 162 h (Fig. 4) BSA continued to decline, falling below 1.0 mg/ml. However, both IgG and Lf levels remained above 3 mg/ml through 354 h postinoculation, with IgG being the dominant whey protein measured. After this time, IgG levels remained elevated, but Lf decreased to 1.0 mg/ml by 714 h. α-LAC returned to normal levels by 162 h and remained stable thereafter. The slab-gel patterns supported the concentration changes found (Fig. 5).

The left front (LF) quarter of cow 1943, infused with sterile saline, showed a response similar to the RR quarter, although not of the same magnitude (Fig. 6). In contrast to the RR quarter, Lf reached its peak level of 1.85 mg/ml at 66 h postinoculation in the LF quarter. After IgG and BSA had declined in the saline-infused quarter, α-LAC was the major whey protein measured, rather than IgG which was found for the infected quarter (Fig. 3). Although IgG was not quantitated in the two untreated quarters of cow 1943, right front (RF) and LR, the patterns of change for Lf, BSA, and α-LAC are almost identical to those found in the saline-infused LF quarter.

The total daily Lf production during experimental mastitis was calculated. Figure 7 clearly shows that there was an actual increase in the daily Lf production by all quarters of cow 1943, with the most marked and prolonged increase in production in the infected (RR) quarter. These results demonstrate that the observed increases in Lf concentration were not simply a result of decreased milk production and hence a concentrating of the protein.

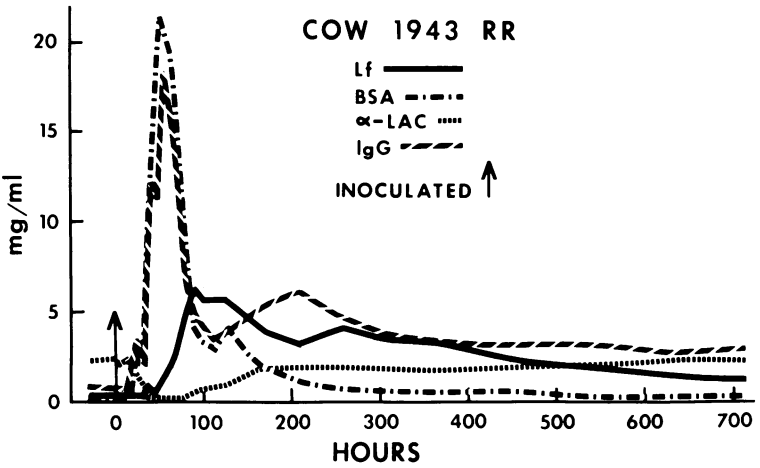

FIG. 4. *Changes in Lf, BSA, α-LAC, and IgG concentrations from 30 h preinoculation to 714 h postinoculation for the infected quarter (RR) of cow 1943.*

Satisfactory acute mastitis did not develop in cow 2127. Protein changes that did occur were modest and transitory.

Molecular weight changes in Lf during acute mastitis. The Sephadex G-200 elution profile for Lf (fractions 70 through 115) from a skim sample from the RR quarter of cow 1943 at 354 h postinoculation is shown in Fig. 8. The elution positions of BSA, α-LAC, IgG, and Lf were determined by the EID assay and are indicated in Fig. 8. The elution profile for Lf frequently showed two adjacent peaks or a large peak with one or more pronounced shoulders. Fraction 90 is the major Lf peak and fraction 80 is a minor Lf peak as explained in Materials and Methods.

Figures 9 and 10 show the molecular weight changes that occurred during the natural coliform infection and during the experimental coliform infection. Lf_1, Lf_2, and Lf_3 indicate the approximate molecular weights expected for the monomer, dimer, and trimer Lf, using 77,100 (8) as the molecular weight of the monomer. These terms are used for descriptive purposes and are not an attempt to assign specific molecular weights to various species of Lf.

Before inoculation of the RR quarter of cow 1943 (Fig. 8), the major Lf peak eluted from the column had an apparent molecular weight of about 135,000. Minor Lf peaks were observed in the chromatographic profile of this normal skim milk sample, suggesting that higher-molecular-weight species were present. Lf in samples taken during the acute phase of infections eluted from the column as monomer Lf. As the infections subsided there was an increase in the apparent molecular weight of Lf. Data from cow 2124 revealed that the apparent molecular weight of Lf increased to approximately that of the trimer by 240 h after the first clinical signs of infection were observed (Fig. 9). A similar trend was evident in samples from cow 1943 (Fig. 10). However, gel filtration was not carried out on skim samples between 90 and 354 h. Thus, it is not known whether the apparent molecular weight would have continued to increase to trimer levels in cow 1943. The data obtained from the two cows suggest that the apparent molecular weight returns to that found in normal skim milk by approximately 400 h postinfection. Secondary peaks of Lf or distinct shoulders were present in the profiles of all samples except for those taken during the acute phase of the infections.

DISCUSSION

This study demonstrated that, during acute mastitis, Lf levels in milk increased to as much as 30 times the normal levels. Lf becomes a major whey protein under such conditions. Although milk production was markedly reduced during acute mastitis, the increased Lf concentration was not due entirely to a concentrating effect since the total daily Lf production increased (Fig. 7), with the greatest total production occurring in the infected quarter. This has not been reported previously.

The importance of the changes in Lf levels observed is related to the origin of the Lf in milk. Since blood is apparently devoid of Lf (22), this protein appears to be produced or released within the mammary gland. Two possible sources can be envisioned for the increased Lf levels. Human neutrophil leukocytes have been shown to contain Lf at approximately 3.0 $\mu g/10^6$ cells (23). Tentative findings

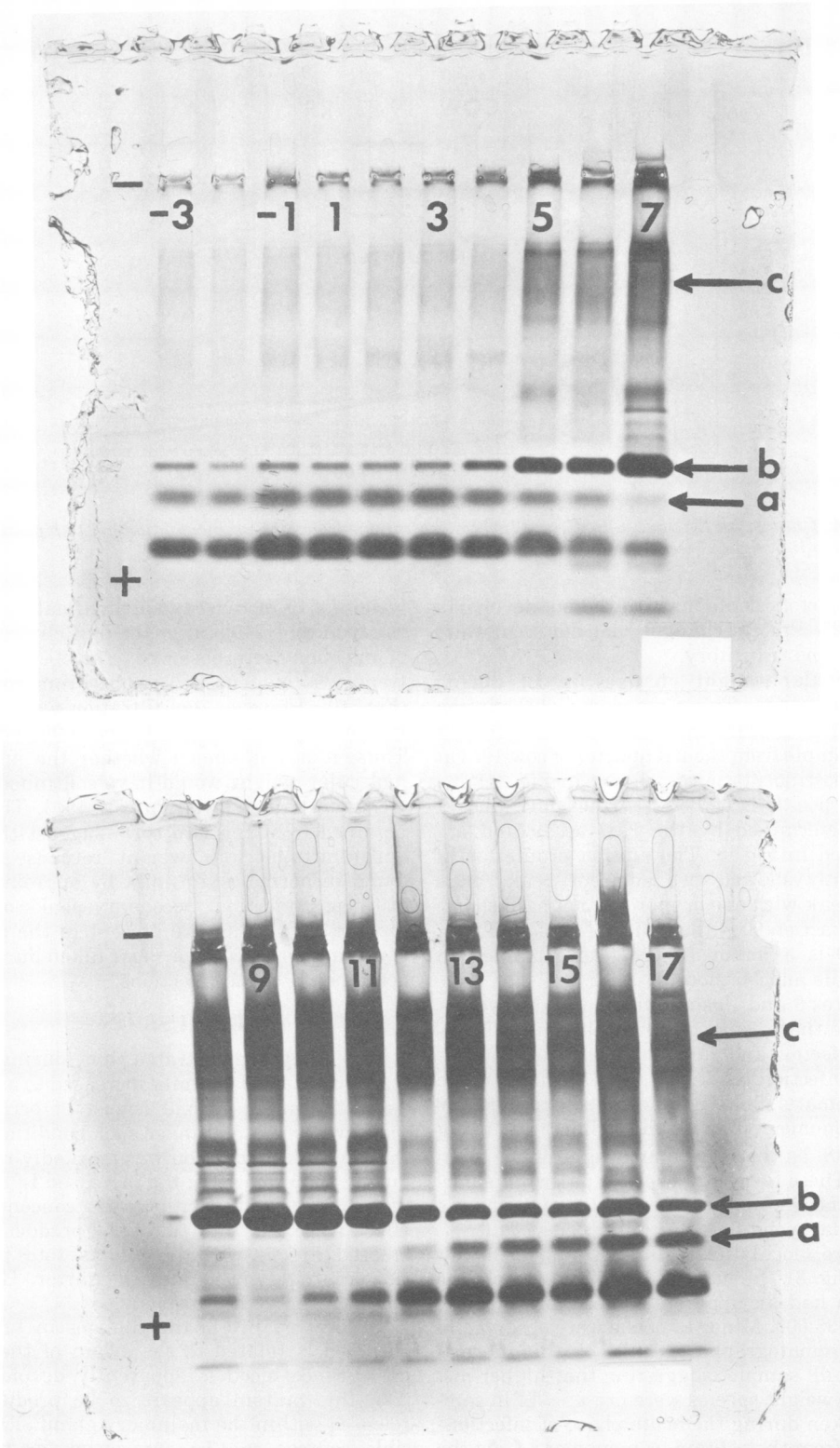

FIG. 5. *Acrylamide slab-gel electrophoresis of wheys from cow 1943, RR quarter, during experimental coliform mastitis. No. −3 to −1,126,78, and 30 h preinoculation, respectively; 1 to 10, 0 to 54 h postinoculation, 6-h intervals; 11 to 16, 66 to 126 h, 12-h intervals; 17 to 21, 162 to 354 h, 48-h intervals; 22 to 26, 426 to 714 h, 72-h intervals. (a) α-LAC; (b) BSA; (c) Lf band is sometimes visible within the IgG region (see gels 12 through 26).*

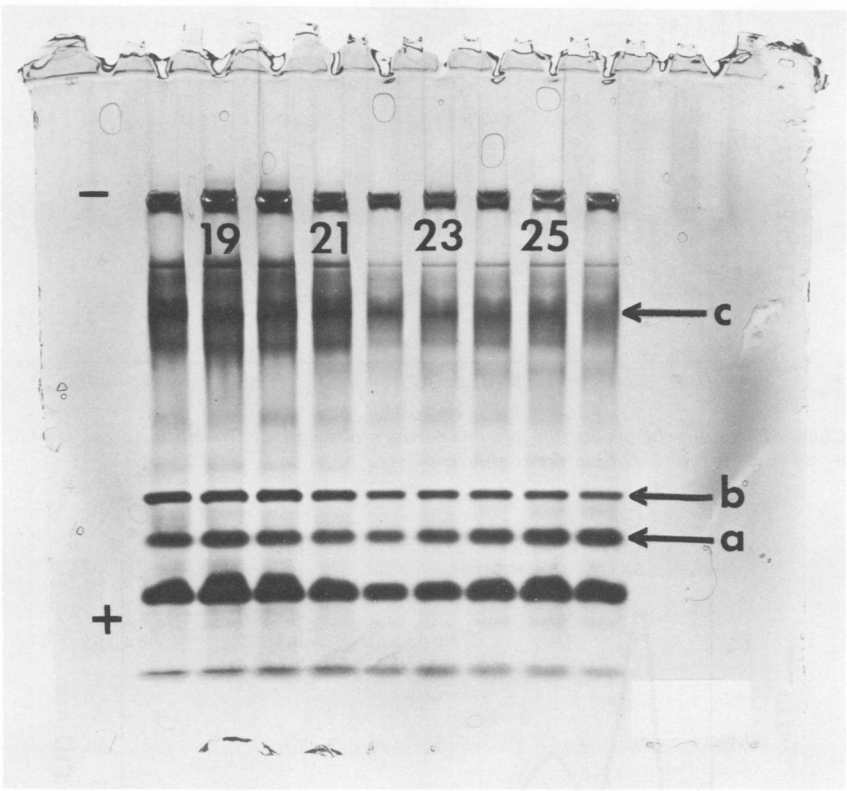

FIG. 5.

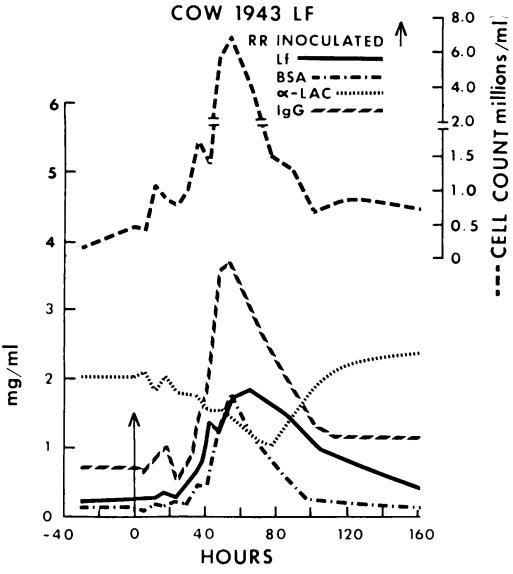

FIG. 6. *Changes in Lf, BSA, α-LAC, and IgG concentrations and cell count from 30 h preinoculation for the saline-infused quarter (LF) of cow 1943.*

from this laboratory indicate that Lf is present in bovine PMNs at similar concentrations (R. J. Harmon, M.S. thesis, The Ohio State Univ., Columbus, 1974). The peak Lf level for the RR quarter of cow 1943 (Fig. 3) was 6.2 mg/ml, and the cell count at this time was greater than 25 $\times$ 10^6 cells per ml. If the number of PMNs present was estimated as high as 200 $\times$ 10^6 cells per ml, 3.0 μg of Lf/10^6 PMNs would still only account for 0.6 mg of Lf/ml, or only 1/10 the amount of Lf that was actually measured in the whey. Hence, it is unlikely that the PMNs are the major source of Lf even though they may contribute to the elevated Lf levels during infection.

The other possible source of Lf is the secretory epithelial cells in the mammary gland. In vitro synthesis of Lf has been demonstrated for guinea pig mammary glands, and it has been noted that Lf is localized in the secretory granules in glandular epithelium (19). The lysosomes in the cell are comparable to the granules in the PMN (10). If Lf is present in the secondary PMN granules (2), Lf may be of lysosomal origin from the secretory epithelial cell. Large lysosomes and PMN granules have been observed among teat sinus epithelial cells from a clinical case of mastitis (9). During infection there is enhanced Lf production (Fig. 7) and, at the same time, there is reduced synthesis of the major milk-specific whey proteins of normal

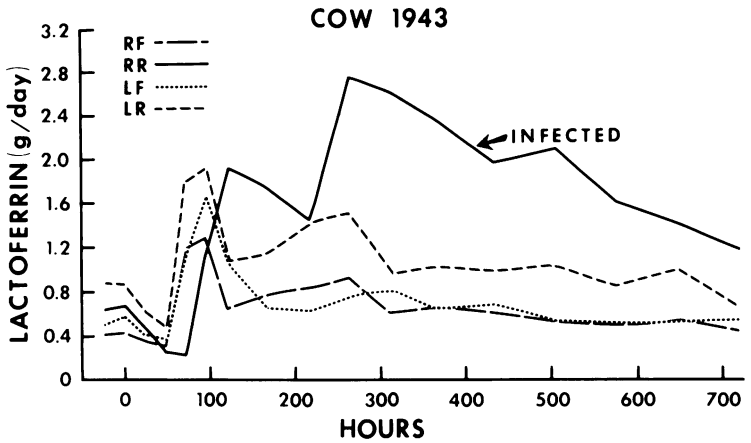

FIG. 7. *Changes in daily Lf production after experimentally induced mastitis in cow 1943. Lf (grams) produced per 24 h (days) for each quarter is shown.*

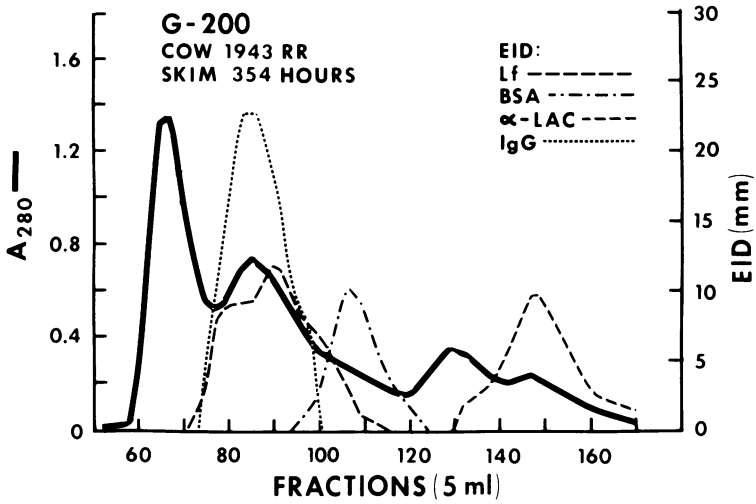

FIG. 8. *Sephadex G-200 chromatogram of skim milk from the RR quarter of cow 1943 at 354 h postinoculation. The elution positions determined by EID are shown for Lf, BSA, α-LAC, and IgG.*

milk, α-LAC (Fig. 3 and 4), and β-lactoglobulin (3). As the gland returns to normal, α-LAC production returns to preinfection levels and the production of Lf decreases. This type of change in Lf and α-LAC concentrations was observed in the four quarters of cow 1943. Thus, it would appear that mammary tissue responds to acute inflammation and/or infection with an increased rate of production of Lf; however, the mechanisms of mammary tissue response are still in question.

Previous workers have suggested that Lf complexes exist in bovine lacteal secretions (12, 26, 29; F. L. Schanbacher, K. L. Smith, and L. C. Ferguson, Fed. Proc. 30:532, 1971). In bovine skim milk, the casein micelle or a component of the micelle has been suggested as a possible moiety with which Lf interacts to form complexes (12, 26; F. K. Welty, M. S. thesis, The Ohio State Univ., Columbus, 1973). If Lf is associating with the casein micelle, Lf should have been detected in the void volume upon Sephadex G-200 chromatography of skim milk samples. Lf was not detected immunologically in the void volume of any chromatographic profiles in the present study. In addition, it has been shown that Lf in bovine whey prepared by acid precipitation of the casein was eluted from columns of Sephadex G-200 as a molecule with an apparent molecular weight of 150,000 to 160,000 (29; Schanbacher et al., Fed. Proc. 30:532, 1971). These data suggest either self-association of the Lf or association with a moiety other than the casein micelle.

Hekman (14) has reported that human Lf interacts strongly with acidic macromolecules

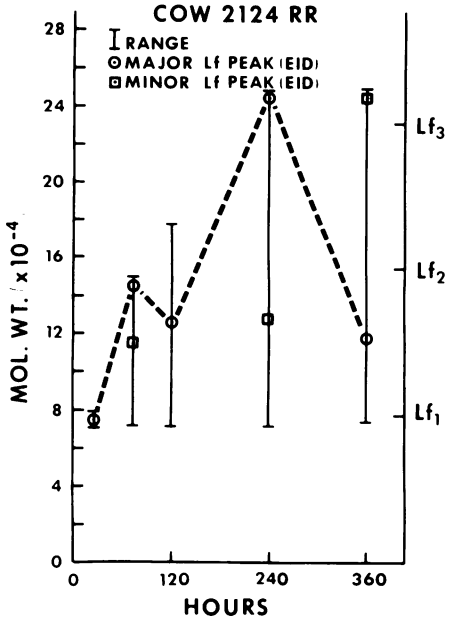

FIG. 9. *Molecular weight changes in Lf during a natural coliform infection in the RR quarter of cow 2124. The determination of major and minor Lf peaks and range are outlined in Materials and Methods. Lf_1, Lf_2, and Lf_3 refer to expected molecular weights of the monomer (77, 100), dimer, and trimer Lf (used for descriptive purposes only).*

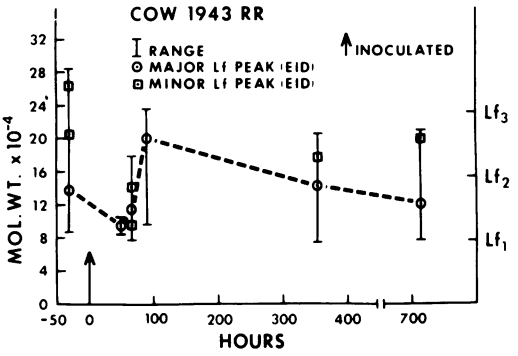

FIG. 10. *Molecular weight changes in Lf during experimentally induced mastitis in the RR quarter of cow 1943. The determination of major and minor Lf peaks and range is outlined in Materials and Methods. Lf_1, Lf_2, and Lf_3 refer to expected molecular weights of the monomer (77,100), dimer, and trimer Lf (used for descriptive purposes only).*

including serum albumin. However, our data show that, during the acute phase of the infection when BSA was greatly elevated, Lf most nearly approximated monomer. In addition, BSA was not detected immunologically in chromatographic fractions containing the higher-molecular-weight species of Lf. From these observations it seems unlikely that a Lf-BSA association explains the apparent molecular weight changes in Lf.

Additional reports in the literature show that Lf can interact and form complexes with various other biological and synthetic polyanionic macromolecules including nucleic acids (16, 18, 23). These observations suggest that Lf may be complexing with other nonprotein molecules in the secretion and may account for the molecular weight form of Lf approximating 1.5 times that of the monomer that frequently occurred in this study. The similarities of the molecular weight changes that occurred during the course of the infections in cows 1943 and 2124 suggest a definite pattern or mechanism of change rather than nonspecific associations.

In the present study, a concurrent rise and fall in BSA and IgG concentration was noted early in the infection. These findings are not in agreement with earlier work (3, 7), which showed an immediate rise and fall in BSA, with IgG increasing more slowly and remaining elevated for several days. These workers (3, 7) reported the relative whey protein changes, determined by electrophoretic patterns on paper or cellulose acetate membranes, and did not recognize the presence of the whey protein Lf. However, the reported change in relative concentrations of an "X protein" and the IgG fraction during an *Aerobacter aerogenes* infection (7) appears to be analogous to Lf and IgG concentration changes observed for the RR quarter of cow 1943 (Fig. 3). The results reported in the present paper and the similarities reported for IgG_2 and Lf with respect to elution behavior from Sephadex G-200, electrophoretic properties, and elution position during ion-exchange chromatography (29) suggest that earlier workers included Lf in the IgG fraction and that the X protein (7) was, in fact, Lf.

The present investigation showed that BSA levels were higher than IgG levels during the acute phase of the infection. During this period the concentrations of these two proteins approached those of blood. These findings support the concept that during acute inflammation there is destruction of the blood-to-milk permeability barriers in the mammary gland, resulting in increased passive transport of IgG and BSA into milk as postulated by Mackenzie and Lascelles (17). However, IgG levels remained elevated after BSA values had returned to normal in the infected quarters, and IgG levels were consistently above BSA levels in uninfected quarters. Such findings cannot be explained on the basis of passive diffusion mechanisms alone. Mackenzie and Lascelles (18) have shown that acute inflammation inhibits the IgG_1 selective transport mechanism. Thus,

after the acute phase of the infection, the selective transport mechanism may recover and operate for a period of time at a rate greater than that of normal lactating tissue. An additional explanation would be that there was an increased local production of IgG (5, 6, 25). These observations are of potential value to the control of bovine mastitis and require further investigation.

Although the precise biological function of Lf is still in question, results suggest that the increased production of Lf may be a result of the specific response of secretory tissue to an infectious process. Therefore, Lf and the response of secretory tissue to infection should be more strongly considered in future investigations of the natural defense mechanisms of the bovine mammary gland.

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