ABSTRACT
Concentrations of α-lactalbumin in blood from cattle in various physiological states were measured as an index of udder development and function. Included were primiparous heifers during gestation and the peripartum period, nonlactating, nonpregnant cows hormonally induced into lactation, and cows milked two or three times daily in early, middle, or late lactation. Concentrations of α-lactalbumin in serum increased in two phases during gestation. Initial values (7.3 ng/ml, up to 120 d prepartum) rose, then leveled at 29.9 ng/ml on d 120 to 30 prepartum. Concentrations subsequently increased, averaging 133.2 ng/ml over the 30 d prior to parturition. During the peripartum period, α-lactalbumin rose from 221.2 ng/ml on d 4 prepartum, peaked at calving (918.8 ng/ml), then declined, stabilizing at approximately 500 ng/ml (1.5 to 3 d postpartum). Concentrations of α-lactalbumin in cows induced to lactate were low on d 1 to 9 of hormone treatment (15.7 ng/ml), rose to a maximum on d 17 (803.4 ng/ml), then fell to a plateau (185.8 ng/ml) on d 21 to 25. α-Lactalbumin concentrations were higher in early (101.9 ng/ml) than in middle or late lactation (81.4 and 79.2 ng/ml, respectively). Concentrations were also greater in twice versus thrice milked cows (101.9 vs. 73.0 ng/ml). Changes in α-lactalbumin concentrations in serum are associated with developmental and functional status of the udder. The measurement provides a noninvasive method to assess mammary gland activity.

INTRODUCTION
α-Lactalbumin (α-lac) is a secretory protein that normally composes about 2.5% of the total protein in milk (5). It is also a component of the lactose synthetase (EC 2.4.1.22) enzyme complex that catalyzes the formation of lactose from glucose and galactose in mammary epithelial cells (3). Because of its unique dual role in both protein and carbohydrate metabolism of the gland, α-lac has been useful as an index of lactogenesis in cultured mammary tissue (8, 25).

Schanbacher and Smith (19) suggested that whey protein and enzymes in milk had been largely overlooked as potential markers of mammary function. Although use of milk components as markers of mammary function is a useful technique, such measurements are obviously limited to periods when secretions are available. A further drawback is that physical removal of secretion may itself alter the physiological process being studied. For example, prepartum milking elicits earlier glandular differentiation and subsequently increased milk yields in cattle (1). Therefore, it is desirable to obtain a noninvasive technique for studying mammary development during nonlactating intervals.

As part of our characterization of a radioimmunoassay for α-lac (2), we determined that α-lac was present in serum of lactating cows and late gestation heifers but was undetected in non-pregnant heifers. This observation suggested that measurement of α-lac concentrations in serum could provide an index of mammary gland development, especially during nonlactating periods. Hartmann et al. (10) recently reported that concentrations of lactose in plasma of pregnant sows provided an earlier temporal measure of lactogenesis than analysis.
of mammary secretion. Others have reported that α-lac is present in serum of humans and that concentrations progressively increased during gestation (15). Recently, Forsyth et al. (7) reported that concentration of α-lac in plasma of pregnant goats was affected by stage of gestation, number of fetuses, and suppression of prolactin secretion by bromocriptine treatment.

The objective of the present study was to evaluate the measurement of α-lac in serum as a potential indicator of lactogenesis and mammary function in cattle. Animals in various physiological states were used to assess the ability of this method to reflect accurately the functional status of the mammary gland.

MATERIALS AND METHODS

Experiment 1

In an initial experiment, Holstein heifers (n = 63) were assigned for blood sampling approximately 50 d postinsemination following rectal palpation to confirm pregnancy. Heifers were maintained on pasture and individuals were sampled every 2 wk until transferred to an adjacent maternity facility about 1 wk prior to expected calving. Sampling was between 2 (n = 3) and 222 d (n = 1) prepertum.

For sampling, heifers were crowded into a chute and blood was collected by puncture of the coccygeal vein or artery. Blood samples were allowed to clot at room temperature for about 4 h, refrigerated overnight, and sera were decanted following centrifugation (2000 x g). Sera were frozen (−20°C) until assayed as described (2). Heifers were sampled between April 25 and December 5, 1984.

For summarization, data from individual animals were segregated into 5-d intervals from parturition to 30 d prepartum and 10-d intervals between 30 and 230 d prepertum. Because sampling began with all available bred heifers, all animals were not sampled throughout gestation. Each individual was sampled from 2 to 15 times, and 6 to 42 animals were sampled at a given period of gestation. At least 14 animals were sampled at any given interval between parturition and 180 d prepertum.

Experiment 2

Blood samples from primiparous Holstein cattle (n = 6) were collected via puncture of the coccygeal vein or artery at 6-h intervals from 4 d prior to until 3 d following the day of parturition. Samples were collected at 0000, 0600, 1200, and 1800 h daily, centrifuged immediately, and plasma stored at −20°C until assayed for α-lac concentrations. Animals calved from April to June 1983.

Experiment 3

Nonpregnant, nonlactating Holstein cows (n = 12) were hormonally induced into lactation. Hormone treatment consisted of twice daily subcutaneous injection of estradiol-17β and progesterone (.1 and .25 mg/kg per d) for 7 d, followed by injections of reserpine (5 mg/d) on d 8, 10, 12, and 14 and single injections of dexamethasone (20 mg/d) on d 18, 19, and 20. Blood samples were obtained (puncture of coccygeal vessels) daily during the treatment period and for 6 d thereafter (26 d total). Sera were harvested and frozen until assayed. This experiment was conducted between January 14 and February 15, 1985.

Experiment 4

Primiparous Holstein cattle were fitted with jugular cannulae at 30 (n = 55), 90 (n = 59), and 200 (n = 63) d postpartum. Cattle were either daughters of commercially available sires (selection group) or second to fourth generation daughters of cows randomly bred to nonartificially inseminated unselected sires originating in the Virginia Tech dairy herd (control group). After parturition, the select and control group animals were randomly assigned to be milked either twice (2×; 0400 and 1530 h) or thrice (3×; 0000, 0600 and 1600 h) daily. Blood samples were collected at 15 min intervals from 1030 to 1300 h. Blood was centrifuged immediately and plasma frozen until assayed for α-lac concentrations. Somatic cell counts (SCC) in milk samples from the afternoon milking collected within 1 wk of cannulation were determined by the Virginia DHI laboratory.

Serum or plasma concentrations of α-lac were measured with a double antibody radioimmunoassay (2). Intra and interassay coefficients of variation on two plasma pools averaged 8.7 and 10.2%, respectively. Characterization of the assay showed that dilution (up to 100 μl) of both plasma and serum produced
inhibition curves parallel with the standard curve and that added α-lac was quantitatively recovered when added to serum or plasma. In no case was more than 100 µl of sample assayed.

Data obtained from Experiments 2 and 4 were subjected to split-plot analysis of variance to test time and treatment effects.

RESULTS

Experiment 1

Mean concentrations of α-lac in serum of early gestation heifers were low, not exceeding 5 ng/ml prior to 160 d prepartum (Figure 1). Between 160 and 120 d prepartum, an initial rise was noted with concentrations increasing to about 25 ng/ml, then remaining fairly constant (23 to 30 ng/ml) from 120 to 60 d prepartum. A second increase in α-lac followed this plateau, as concentrations rose steadily through the remainder of gestation and reached peak levels (308 ng/ml) at the final (0 to 5 d prepartum) sample period. Concentrations of α-lac in serum averaged 7.6, 29.9, and 140.2 ng/ml at >120, 120 to 30, and <30 d prepartum, respectively.

Regression analysis of α-lac concentrations (mean, peak, linear, and quadratic expressions) during gestation on subsequent mature equivalent milk yield (ME7; obtained from DHI records) of heifers did not indicate a significant relationship between the two variables. Thus, concentrations of α-lac in blood during gestation apparently do not accurately predict milk production, although the number of animals in this trial was relatively small.

Experiment 2

Plasma α-lac concentrations in primiparous Holstein cattle from 4 d prior to until 3 d following the day of parturition are depicted in Figure 2. Plasma α-lac concentrations increased as parturition approached, (day -4 < -1 < 0; P<.01) rising from 221.2 ng/ml on d -4 to a peak concentration of 918.8 ng/ml on the day of parturition (P<.01), then declining after calving (462.7 ng/ml, +2 d). Postpartum α-lac concentrations remained greater than concentrations measured 4 d prior to calving (P<.05).

Figure 1. Mean concentrations of α-lactalbumin in sera of pregnant heifers prepartum. Points represent means ± SE from ≥7 heifers. Serum α-lactalbumin concentrations (ng/ml) averaged 7.3 ± 1.2 (n = 146) at >120 d, 29.9 ± 1.8 (n = 305) at d 120 to 30, and 133.2 ± 11.8 (n = 115) at ≤30 d prepartum, respectively. Note the scale of the x-axis changes at 130 and 50 d prepartum.

Figure 2. Mean concentrations of α-lactalbumin in plasma of primiparous Holstein cattle (n = 6) during the periparturient period. Standard error of the means ranged from 16.5 (d 4 prepartum) to 35.5 ng/ml (.5 h postpartum).

Experiment 3

Concentrations of α-lac in sera from dry cows induced to lactate were initially low, averaging 15.4 ng/ml over the first 9 d of treatment (Figure 3). Concentrations began to rise on d 10 and increased rapidly to a maximum of 803.4 ± 156 ng/ml on d 17 after initiation of hormone therapy. α-Lactalbumin levels then declined before stabilizing at 185.8 ng/ml over d 21 to 25.

Experiment 4

No differences (P>.05) in plasma α-lac concentrations were observed between control and selected animals. Therefore, the data presented are not classified according to genetic merit. Plasma α-lac concentrations at 30, 90, and 200 d postpartum in 2× and 3× cattle during the 2.5-h sampling period are presented in Table 1. Overall, concentrations of α-lac in plasma were greater (P<.01) at 30 than at 90 or 200 d postpartum. An effect of milking frequency was also apparent because plasma α-lac concentrations (combined across days postpartum) were elevated in cattle milked 2× compared with those milked 3× (101.9 vs. 73.0 ng/ml; P<.01). No interaction between milking frequency and days postpartum was evident (P>.10).

DISCUSSION

The current conception of lactogenesis suggests that as mammary epithelial cells undergo differentiation, they gradually acquire the necessary complement of enzymes and cellular organelles for synthesis and secretion of milk (4). This process has been described as a two-stage phenomenon (6, 9). In the initial phase (Stage 1 lactogenesis), enzymes needed for biosynthesis of milk components begin to appear in the cell. During a later phase (Stage 2 lactogenesis), changes in hormone concentrations associated with the approach of par-

TABLE 1. Least squares mean concentrations of plasma α-lactalbumin at 30, 90, and 200 d postpartum in cows milked twice or thrice daily.

<table>
<thead>
<tr>
<th>Days postpartum</th>
<th>Frequency of milking</th>
<th>Across frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2×</td>
<td>3×</td>
</tr>
<tr>
<td>30</td>
<td>110.9</td>
<td>92.9</td>
</tr>
<tr>
<td>90</td>
<td>95.5</td>
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<tr>
<td>200</td>
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<td>58.6</td>
</tr>
<tr>
<td>Across DPP</td>
<td>101.9b</td>
<td>73.0</td>
</tr>
</tbody>
</table>

*a Greater (P<.0001) than 90 and 200 DPP.
b Greater (P<.01) than 3×-milked cows.

Values presented are least squares mean concentrations and associated standard errors of the mean.

Days postpartum.
Parturition rapidly elicit the final differentiation required for cells to assume full secretory capacity (17). It is thought that tight junctions (zona occludens) between adjacent epithelial cells become fully functional shortly after parturition, thereby decreasing paracellular movement of milk proteins (16, 17, 24).

The goal of the present study was to take advantage of “leaking” of the secretory protein α-lac into serum as a means to evaluate the functional status of the udder. Our findings suggest that such measurements can provide useful information on mammary development. In particular, as depicted in Figures 1 and 2, α-lac concentrations in blood rise from low levels observed prior to 160 d prepartum, reach a plateau between 50 and 120 d prepartum, then increase rapidly, peaking on the day of parturition. These results are strongly supportive of the two-step theory of lactogenesis (6, 9) and appear to give a relative index of mammary gland differentiation at any time up to parturition. The rapid decline in serum α-lac after reaching peaks (Figures 2 and 3) may be interpreted as evidence of the formation of competent junctional complexes (16, 18), although the data are circumstantial. If this inference is correct, however, blood sampling could be used to study another aspect of glandular differentiation, i.e., the timing of tight-junction formation.

Data in Figure 3 indicate that hormones induce increases in serum α-lac, which presumably reflect overall differentiation of the udder in dry cows receiving treatment. Thus, measurement of α-lac could be applied to evaluation of new regimens for inducing lactation. Only 60 to 70% (22) of treated animals respond well to current induction schemes based on Smith and Schanbacher’s original method (21). Hence, measuring serum α-lac responses could provide an earlier indication of an individual’s sensitivity to hormone treatment than that obtained after completing the regimen and measuring milk production.

Similarly, serum α-lac concentrations are clearly related to the physiological status of the udder. Differences in α-lac concentrations based on days postpartum and milking frequency (Table 1) may be a function of total milk yield, changes in intramammary pressure, aging of cells, or alteration of cellular junctions.

Early reports on the presence of lactose in blood of cows (23) and goats (14) described an association between blood lactose concentrations and milking interval length during established lactation. More recent studies have supported these findings and extended their scope to suggest a relationship between milk constituents in blood and prepartum development of the mammary gland in several species (7, 10, 15). The present results confirm this earlier work and establish a foundation for using these measurements to assess mammary functional status in a variety of experimental approaches.

One aspect of mammary function pertaining to α-lac in blood that remains in question is the route by which α-lac is transferred into circulation. Several prospective routes seem possible: 1) leakage of previously secreted α-lac from the alveolar lumen, through cellular junctions, and into the intercellular space, i.e., a paracellular route; 2) “misdirected” secretion, in which secretory vesicles containing α-lac and lactose could fuse with basolateral plasma membrane (rather than apical plasma membrane) and release their contents into extracellular fluid; 3) reabsorption of secreted α-lac through epithelial cells and into circulation, and 4) leakage through “gaps” left by sloughed or damaged epithelial cells. Our observation that leakage declines just after parturition could represent establishment of tight junctions (11, 16, 18) in support of route 1, or it may be indicative of maturation of cytoskeletal organization (17) and improved vesicle trafficking as in the second hypothesis. Very recent evidence suggests that some α-lac is normally reabsorbed from the lumen into epithelial cells, where it may function to activate lysosomal enzymes (12, 13). A malfunction in such a mechanism could conceivably result in α-lac appearing in blood, as in the third hypothesis. The fourth hypothesis is supported by the fact that plasma α-lac concentrations were correlated with milk SCC (r = .35, P<.01) in cows from the fourth experiment. Because SCC estimate udder health, and are frequently used as an indicator of mastitis (20), they may reflect damage that could allow α-lac to escape into blood.

In summary, concentration of α-lac in serum is related to intramammary pressure, glandular maturity and influx of somatic cells as evidenced by data from experiments on milking frequency.

and days postpartum, gestation, periparturient and induced lactation responses, and SCC, respectively. However, the particular mechanism of leakage remains uncertain and clearly warrants further study.

CONCLUSIONS

Measurement of α-lac concentrations in blood provided a simple, noninvasive technique for monitoring developmental and functional changes in mammary glands of cattle. This method has considerable potential for applications in studies on the physiology of lactogenesis and milk secretion. The potential for use as an index of udder health is currently under investigation.

REFERENCES