Metabolic Adaptations in Mammary Gland During the Declining Phase of Lactation

ABSTRACT

Peak milk yield in the ruminant animal is attained relatively early during lactation, and this is followed by a progressive decline so that at the time of "drying off" yield may be reduced to only 50% of its maximum value. Studies in lactating goats indicate that the fall in milk yield after peak was due ultimately to a decrease in the number of secretory cells and that the remaining cells did not lose their metabolic capacity for milk synthesis to any significant degree. Manipulation of milking frequency or efficiency during declining lactation had both acute and long-term effects on milk yield and mammary cell number and activity. Rapid and reversible stimulation of yield by thrice daily or hourly milking was due to more frequent removal of a whey protein, which modulates milk secretion by negative chemical feedback on the secretory cell. Evidence is presented that the feedback inhibitor may act primarily on the secretory process rather than through inhibition of synthesis of individual milk constituents. Long-term thrice daily milking increased milk yield in the later stages of lactation by preventing, at least in part, the fall in secretory cell number after peak. Secretory cell differentiation was also susceptible to manipulation in declining lactation: inefficient milking for 24 wk after peak, i.e., by increasing the residual milk volume in the gland after milking, caused partial involution (dedifferentiation) of the existing secretory cell population. The role of local intramammary mechanisms in modulating milk yield and mammary function in declining lactation is discussed.

INTRODUCTION

The declining rate of milk secretion that prevails for much of lactation in the dairy cow has long been a source of dismay to the dairy farmer and a challenge to the research scientist. In dairy cows, peak milk yield is reached usually in the 2nd mo of a 10-mo lactation and then decreases by as much as 50% by the time of "drying off". Manipulation of mammary function to prevent or reduce this decline, thereby increasing the "persistency" of lactation, would be a major advance in improving production efficiency and increasing profit margins.

The rate of milk secretion is influenced by many factors such as the nutritional and hormonal status of the animal, but ultimately it is the number and activity of the secretory cells that determine milk yield. Thus, maintenance of milk production during the later stages of lactation depends upon the number of secretory cells lost, the extent of cell replacement (if any), and the retention of synthetic capacity by each cell. We have examined the relationship between cell number, cell activity, and milk yield in lactating goats during declining lactation by serial biopsy of the gland and determination of gland DNA content as a measure of cell number, and the activities of a number of key enzymes involved in the synthesis of milk constituents as markers of the degree of cellular differentiation, i.e., metabolic capacity. In addition, rates of lactose and casein synthesis by explants freshly prepared from biopsy tissue have been measured as an index of metabolic fluxes in secretory cells in vivo.

In this paper we discuss changes in mammary cell number and activity that are responsible for the progressive decrease in milk yield and describe methods by which these changes can be modulated to alter milk production. Recent studies have indicated that both cell number and cell activity are susceptible to manipulation by local control mechanisms operat-
Acetyl-CoA Carboxylase  Fatty Acid Synthetase  Galactosyltransferase

Week of Lactation

Figure 1. Goat mammary enzyme activities in early lactation. Enzyme assays were performed as described in (41). Error bars indicate the SE of the mean. Asterisk denotes the earliest stage at which enzyme activity increased ($P<.05$) over that in late pregnancy (wk 1). Data from Wilde et al. (41).

Discussion

The Lactation Curve

The lactation curve of the goat is qualitatively similar (but quantitatively different) to that of the dairy cow and as such provides a suitable model for studying ruminant lactation. Animals in our Institute herd reach peak milk yield around wk 5 to 7 of lactation, and by the 9th mo (when they are usually dried off) yield has fallen to approximately 60% of the peak value.

The Mammary Gland at Peak Lactation

By the time of peak lactation, the mammary gland of goats has undergone phases of extensive secretory cell proliferation and differentiation. Secretory cell proliferation occurred mainly during pregnancy and continued during the first few weeks postpartum, but cell number showed no significant increase after the 3rd wk of lactation (20). Secretory cell differentiation occurred primarily after parturition: using acetyl-CoA carboxylase, fatty acid synthetase, and galactosyltransferase as markers of differentiation, the synthetic capacity of the secretory cells increased 6- to 10-fold by the time of peak lactation (Figure 1). At the time of peak milk yield the gland normally contains a maximum number of highly differentiated secretory cells.

Enzyme Activities and Metabolic Fluxes During Declining Lactation

The activities of the three marker enzymes and those of other enzymes in the gland did not decrease as milk yield fell after peak lactation. In an initial study, the total activities of acetyl-CoA carboxylase, fatty acid synthetase, and galactosyltransferase increased 3.3-fold, 1.4-fold, and 2.3-fold, respectively, by wk 25, suggesting that, if anything, the metabolic capacity of the cells continued to increase even when milk yield had begun to decline (41). However, because of the limited number of observations at these later stages the increased enzyme activity was significant only for galactosyltransferase ($P<.05$). Rates of lactose and casein synthesis measured in freshly prepared explants also indicated that secretory cell differentiation was
maintained until at least wk 25 when yield had fallen by 38% (41).

The observations were confirmed in a subsequent study (38). Both acetyl-CoA carboxylase and fatty acid synthetase activities were significantly greater on the 23rd and 33rd wk of lactation than at the time of peak milk yield (Figure 2), and galactosyltransferase activity was maintained at its peak value throughout this period (Figure 2). Of the other enzyme activities monitored, glucose-6-phosphate dehydrogenase activity also showed an increase \((P<.01)\) in the declining phase of lactation, an observation consistent with its role in the provision of reduced nucleotides for de novo fatty acid synthesis in the tissue. Isocitrate dehydrogenase, which performs a similar function in the ruminant mammary gland and whose activity increases markedly during early lactation (41) also maintained this high activity throughout declining lactation (results not shown). The activities of these marker enzymes indicate that the degree of cellular differentiation was at least as great in the 33rd wk of lactation when milk yield was on average 52% of its peak value, than at the time of peak milk yield at approximately wk 7 in these goats. The changes occurring in these key enzyme activities are specific: other enzymes such as hexokinase and phosphofructokinase, which are less intimately concerned with milk synthesis and which show less pronounced increases in activity during early lactation (41), are maintained but do not increase further declining lactation. The sustained but relatively modest changes in these enzyme activities appear to represent a background of general cell hypertrophy required for milk synthesis and against which the more strategic changes in key enzyme activities take place. The process is not, however, entirely unselective: aryl esterase, an enzyme involved in drug metabolism, showed no change whatsoever during the lactation cycle.

The metabolic activity of short-term cultures of mammary explants freshly prepared from biopsy tissue, which has been shown previously to provide an index of metabolic fluxes in the tissue in vivo (41), also indicated that cellular differentiation was maintained in declining lactation. Rates of total protein synthesis and casein synthesis that increased markedly between parturition and peak milk yield were maintained until at least wk 33 (Figure 3). The rate of lactose synthesis by freshly prepared ex-
plants was also maintained at its peak lactation value until wk 23 but did show a fall by wk 33 (Figure 3). However, this decrease may reflect the decline in milk lactose concentration in late lactation (28). The rate of DNA synthesis, as measured by incorporation of \( ^{3}H \)thymidine into explants, remained low throughout the declining phase of lactation. Inasmuch as this rate reflects the rate of cell proliferation, it suggests that the decrease in cellular differentiation had occurred in the existing population of cells, rather than through replacement of highly differentiated cells by a new subpopulation of less differentiated ones. However, as DNA synthesis in mammary cells without concomitant cell replication has been reported in mouse mammary explants (32), such data should be treated with caution.

Even in the very late stages of lactation, activities of the key marker enzymes indicated that the potential capacity of the secretory cells for milk synthesis was not in decline. Acetyl-

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Figure 3. Metabolic fluxes in mammary explants prepared during declining lactation (28). Rates of lactose, casein, and DNA synthesis were measured as described by Wilde et al. (41). Mean (± SE) for six goats. *P<.05, **P<.01 compared with wk 5.
CoA carboxylase activity assayed in goats that were milked until the 38th wk of lactation was 49.3 ± 27.5 nmol/min per mg DNA compared with 44.6 ± 14.9 nmol/min per DNA at peak lactation in wk 6; fatty acid synthetase activity was higher compared with peak lactation (1.14 ± .22 versus .34 ± .14 μmol/min mg DNA; P<.05), and galactosyltransferase activity also tended to be higher in late lactation (.61 ± .02 versus .43 ± .16; not significant). These studies indicate that changes in the metabolic capacity of the mammary secretory cells, i.e., their potential for milk synthesis, is not responsible for the decline in milk yield after peak.

Cell Number During Declining Lactation

Cell number during declining lactation was determined as the product of tissue DNA concentration assayed in biopsy samples and udder volume measured by water displacement. The accuracy of the water displacement method was confirmed by comparison of results with gland mass measured postmortem; a significant correlation was obtained (19). The progressive decline in milk yield was associated with a decrease in the total DNA content of the mammary parenchyma, representing a net fall in cell number (Figure 4). A significant decrease between wk 8 (when milk yield was at peak) and wk 23 was followed by a further decline, so that by wk 36 the overall fall in cell number after peak lactation was almost 40%; this accompanied a decrease of 42% in milk yield over the same period.

This evidence suggests strongly that during the declining phase of lactation in the goat, although other factors such as hormone status will modulate secretory rate in an acute manner, it is a net decrease in the number of mammary secretory cells that is responsible ultimately for the fall in milk yield. There is no indication that under normal circumstances the remaining cells lose their capacity for milk synthesis to any significant degree.

The rate of DNA synthesis and, by inference, cell proliferation, falls to a very low rate within a few days of parturition (results not shown) and, as indicated, remains low throughout the declining phase of lactation. With little or no cell replacement, the deduction would be that the decrease in cell number in the later stages of lactation is due simply to the death of a proportion of the secretory cells. Nevertheless, it should be remembered that in such a large mass of mammary tissue, even a low mitotic index could give rise to a significant degree of cell replacement. Moreover, it is clear that the tissue does retain a potential for proliferation during established lactation, for example, in response to hemimastectomy (19) or to manipulation of milking frequency (see later section). Therefore, the net decrease in cell number during declining lactation could be due to a shortfall between cell replacement and an accelerating rate of cell loss.

Declining Lactation and Concurrent Pregnancy

Lactation can be maintained for a considerable period after the usual time of “drying off” simply by continued milking of the animal. In this way, lactation in the goat has been extended to more than 2 yr without an intervening pregnancy (23). However, throughout this period, yield continued a gradual decline; mammary cell number and activity have not been studied during prolonged lactation, but the evidence presented would suggest that the progressive decrease was due to a further loss of secretory epithelial cells.

Milk secretion has also been extended by mating goats at the time of peak milk yield, so
that they kidded out of season and entered a second lactation without an intervening dry period (21). Milk yield initially was similar to control values in the concurrently pregnant animals, but after 8 wk of pregnancy the rate of milk secretion fell more quickly in these animals so that just before parturition yield was 57% of control values. This is not surprising; depression of milk yield in late lactation in concurrently pregnant animals is a well-recognized phenomenon [e.g., (36)]. However, in their second (out of season) lactation, the yield of the test animals was only 12% lower (not significant) than in a normal second lactation.

Both mammary cell number and proliferation rate (as measured by [3H]thymidine incorporation) were higher in the concurrently pregnant and lactating animals than in the controls in wk 23 of the first lactation. Clearly, in this case the higher cell number was not reflected in greater maintenance of milk secretory rate in the first lactation. It may be that new secretory cells did not differentiate efficiently in the presence of increased circulating concentrations of progesterone. Alternatively, the depressed milk yield may have been due to nutritional constraints on lactation during the concurrent pregnancy. However, the greater mammary cell number and proliferation at this stage suggests that at least part of the milk production during the second lactation came not from secretory cells produced during the first pregnancy but from new cells proliferating in the lactating gland in response to the concurrent pregnancy. This is supported by other evidence presented in this paper that the lactating gland retains a potential for growth during established lactations, to allow redevelopment of lobulo-alveolar tissue (27).

**Manipulation of Milk Yield and Mammary Function in Declining Lactation**

The mammary gland does not become refractory to stimuli during declining lactation. It is clear that both mammary cell activity and milk yield remain responsive to endocrine manipulation at this time. For example, in many studies with bovine somatotropin, treatment of dairy cows was not started until d 60, a time when most, if not all, of the animals would normally have reached peak milk yield.

A long-established method for increasing milk yield has been to increase the frequency of milking. This method also remains effective during the later stages of lactation, and a series of studies with lactating goats have demonstrated that the responses occur through local mechanism(s) operating within the mammary gland. The mammary gland shows a sequential response to long-term thrice daily milking (the frequency most often adapted), which after days and, later, weeks is apparent as a stimulation of cellular differentiation and cell proliferation respectively.

**Acute Regulation of Milk Secretion by Manipulation of Local Chemical Feedback**

An increase from the usual twice daily milking to thrice daily (15) or, in experimental situations, hourly milking (5, 6), increased the rate of milk secretion. This response occurred in a matter of hours, and the higher rate of secretion was maintained for as long as the stimulus of more frequent milking was applied. Moreover, the response could be elicited at almost any stage of lactation: introduction of a period of hourly (5) or thrice daily (15) milking in early, peak, or in the declining phase of lactation consistently increased milk yield in the treated glands. The exception to this was in very late lactation (around wk 36) when milk yield did not increase significantly during hourly milking (5).

The acute response occurred not through additional milking-stimulated release of galactopoietic hormones, because it could be elicited unilaterally if more frequent milking was applied to just one gland of the udder (15). It was also not due to alteration of the pattern of physical distension of the gland; if milk removed at an extra third daily milking was immediately replaced by an equal volume of isosmotic sucrose — thereby maintaining the pattern of gland distension and relaxation while still removing milk more often — milk secretory rate was stimulated nevertheless (16).

Our evidence indicates that the acute effect of changing milking frequency is the result of more frequent removal of a milk constituent, which limits milk secretion through negative
feedback on the mammary cell. Using lactose and casein synthesis by mammary explants as a bioassay, we confirmed that goat milk did contain an inhibitor of milk synthesis, and this factor was located in the whey protein fraction (Table 1). Further resolution on the basis of molecular mass indicated that inhibition was exerted by all those fractions containing constituents of >10 kdal, and that the inhibitor was present in a 10 to 30 kdal fraction of whey proteins (Table 1). Inhibition of milk constituent synthesis in vitro was reversible: in three experiments where explants were cultured for 23.5 h in normal medium after 6 h of culture in medium containing the inhibitory protein fraction, lactose and casein synthesis recovered to control values (39). This reversible inhibition was also concentration-dependent: no effect was observed with the 10-to 30-kdal whey fraction present at .5% of its original milk concentration, but increasing degrees of inhibition of lactose and casein synthesis were obtained with whey fraction concentrations of 1, 10, 50, and 100% of that in milk (39).

Fractions prepared from milk during declining lactation were equipotent in inhibition of the synthesis of milk constituents in vitro (Table 2) compared with those obtained in early or at peak lactation. This indicates that the feedback inhibitor is present in milk at this time.

### Table 1. Inhibition of lactose and casein synthesis in rabbit mammary explants by fractions of goat milk.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Molecular mass (kdal)</th>
<th>Lactose synthesis</th>
<th>Casein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>. . .</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Dialyzed milk</td>
<td>. . .</td>
<td>( \bar{X} ) 45.9*</td>
<td>( \bar{X} ) 62.0*</td>
</tr>
<tr>
<td>Whey fraction</td>
<td>. . .</td>
<td>72.8( \times ) 7.1</td>
<td>61.5( \times ) 8.0</td>
</tr>
<tr>
<td>Whey fraction</td>
<td>&lt;10</td>
<td>91.4( \times ) 18.0</td>
<td>98.4( \times ) 12.5</td>
</tr>
<tr>
<td>Whey fraction</td>
<td>10-30</td>
<td>60.7( \times ) 3.2</td>
<td>53.3( \times ) 6.4</td>
</tr>
<tr>
<td>Whey fraction</td>
<td>&gt;30</td>
<td>103.2( \times ) 5.7</td>
<td>106.0( \times ) 13.6</td>
</tr>
</tbody>
</table>

*P<.05 compared with controls.

bP<.01 compared with controls.

Milk fractions were added to the culture medium at their original milk concentration, except for dialyzed milk at one-half milk concentration.

Relative rates of synthesis measured over 6-h periods. Data adapted from Wilde et al. (39).

### Table 2. Inhibition of mammary explant lactose and casein synthesis by goat milk whey proteins obtained at different stages of lactation.

<table>
<thead>
<tr>
<th>Stage of lactation</th>
<th>Lactose synthesis (^1)</th>
<th>Casein synthesis (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(wk)</td>
<td>(%) (n)</td>
<td>(%) (n)</td>
</tr>
<tr>
<td></td>
<td>( \bar{X} ) SE</td>
<td>( \bar{X} ) SE</td>
</tr>
<tr>
<td>1</td>
<td>49.2 2</td>
<td>75.1 2</td>
</tr>
<tr>
<td>10–15</td>
<td>67.2( \times ) 3.0 6</td>
<td>66.8 5.4 6</td>
</tr>
<tr>
<td>25–30</td>
<td>64.2( \times ) 3.0 6</td>
<td>65.9 4.7 6</td>
</tr>
</tbody>
</table>

*P<.05 compared with controls.

bP<.01 compared with controls.

\(^1\)Mean ± SE for relative rates of synthesis in (n) experiments.
and that the effects of more frequent milking are likely to be a consequence of manipulation of its concentration in milk.

The consistent, reversible and concentration-dependent nature of the inhibition observed with these milk fractions in vitro indicate that they were not due to toxic effects on the explants but represented a physiological control mechanism. This was confirmed by testing the 10- to 30-kdal fraction in vivo. When introduced into the gland of lactating goats via the teat duct, it caused a temporary reduction in the rate of milk secretion (Figure 5); the other gland, which received an equal volume of isosmotic sucrose (20 to 25 ml) showed no effect of the intraductal injection. This effect of the whey fraction was dose-dependent: the hourly secretory rate during a 24-h period between successive afternoon milkings following injection was reduced to 89 ± .8% (n = 5) of its pretreatment value with a dose equivalent to 100 ml of milk (designated 100 units), and this decreased progressively so that with 500 units of inhibitor, milk secretory rate was only 54.9 ± 7.4 (n = 5) of that before injection. The effect was also specific to the 10- to 30 kdal fraction of whey proteins; a second fraction containing whey constituents of >30 kdal had no effect on milk secretory rate when tested at doses of 100 to 300 units, and doses of 400 and 500 units of this fraction each reduced the secretion rate in the test gland by only 14%; moreover, the effect of the high doses of >30-kdal fraction was not unilateral and may therefore have resulted from a systemic effect.

Mechanism of Acute Feedback Control of Milk Secretion in Declining Lactation

Milk composition is unaffected by thrice daily milking (16), so local feedback operates on all milk constituents equally. This coordinate regulation may be exerted at the level of synthesis of the individual milk constituents, but an alternative possibility is that the primary action of the inhibitor is at the level of secretion.

The second possibility was investigated in mammary explants by measuring intracellular degradation of newly synthesized casein. This process operates in mammary explants (30) and cell cultures (22) in a manner that appears to regulate net production of the proteins – as it does for other secretory proteins in other tissues (4) – and it appears that the activity of the process depends primarily on the rate at which proteins are translocated from the rough endoplasmic reticulum to the cell surface (26). Thus, in explants from pregnant animals, the proportion of casein degraded intracellularly was high, whereas in explants from lactating tissue there was significantly less degradation (43). At each stage, the degradative process was also sensitive to prolactin, so lactating tissue cultured with prolactin (in addition to insulin and cortisol) showed the least intracellular breakdown of the secretory proteins (Table 3). However, when explants cultured under these conditions (from lactating tissue with prolactin present) were in addition exposed to the 10- to 30-kdal inhibitor fraction, a significant proportion of the casein synthesized was then degraded inside the cell before it could be secreted [ (34) Table
TABLE 3. Degradation of newly synthesized casein in freshly prepared mammary explants.

<table>
<thead>
<tr>
<th>Stage of lactation (wk)</th>
<th>Whey fraction +/-</th>
<th>Casein degradation</th>
<th>Casein degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-Prolactin</td>
<td>+Prolactin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X (SE)</td>
<td>X (SE)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>79.1 (5.5)</td>
<td>ND</td>
</tr>
<tr>
<td>5-8</td>
<td>-</td>
<td>48.0 (19.2)</td>
<td>12.0* (16.2)</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>34.0 (21.8)</td>
<td>5.3 (4.9)</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>ND</td>
<td>5.3 (4.9)</td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>ND</td>
<td>43.4* (8.7)</td>
</tr>
</tbody>
</table>

*P<.05 compared with minus prolactin.

Explants were pulse-labeled with [3]proline, washed, and cultured again in medium containing insulin plus cortisol, with or without prolactin, and the 10- to 30-kdal fraction of goat whey proteins. Casein degradation at 0 to 4 h after the pulse was expressed as a percentage of maximum radiolabel incorporated. Data from Wilde and Knight (43).

Number of animals.

Not determined.

3]. Clearly, this is only circumstantial evidence for an effect exerted on the secretory process rather than at the level of synthesis of milk constituents, and as intracellular casein degradation has not yet been demonstrated in vivo, it is conceivable that the process is an artifact of the culture system. However, the degradative process responds rapidly enough to the feedback inhibitor for this to be potentially a primary site of inhibitor action.

This local control of milk secretion during declining lactation is through feedback by secreted milk constituents, i.e., by an action exerted on the apical surface of the epithelial cell rather than through an intracellular action of a milk protein prior to exocytosis. Furthermore, milk constituents in the alveoli of the gland are responsible for feedback inhibition because if alveolar milk is not removed (for example, by milking the gland through a catheter, thereby removing only cisternal milk), then the gland shows no response to more frequent milking (29). In other words, without milk removal from the alveoli, the gland "sees" no significant change in the degree of feedback inhibition. As the cells lining the alveoli are all of one type, i.e., secretory epithelial cells, the term "autocrine control" seems an appropriate one to describe modulation of secretory rate by an alveolar milk constituent.

The dose-dependent effects of the 10- to 30-kdal whey fraction both in vivo and in vitro suggest that the onset of feedback inhibition with time after the previous milking is achieved by a progressive increase in the concentration of the feedback inhibitor as milk accumulates. How this is achieved and, conversely, how inhibition by residual milk in the gland is relieved after milking is currently under investigation. At present, it seems unlikely that these changes—rising concentrations as milk accumulates, then a rapid reduction in inhibitor concentration after milking—occur through changing rates of inhibitor secretion into milk; this would require invocation of yet another mechanism by which a changing rate of secretion could be achieved. Moreover, if this were the case, reversal of feedback inhibition after milking would depend on dilution of the inhibitor in residual milk by newly synthesized milk containing a low concentration of inhibitor; such a scheme is not consistent with the rapid increase in milk secretion rate obtained with hourly milking (5, 6). An alternative mechanism, and at present a purely speculative one, is that the inhibitor is metabolized to an inactive form in the alveolar lumen, e.g., by proteolysis. If this were the case, the inhibitor in residual milk could be rapidly inactivated after milking, but with the balance between metabolism and a
TABLE 4. Mammary gland weight, DNA content, and cell proliferation rate in glands milked thrice daily (3x) for 37 weeks or twice daily (2x) through lactation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>2x</th>
<th>3x</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>Parenchyma weight, g</td>
<td>462</td>
<td>62</td>
</tr>
<tr>
<td>Parenchyma total DNA, g</td>
<td>1.32</td>
<td>.15</td>
</tr>
<tr>
<td>[3H]thymidine incorporation, DPM/h per mg tissue</td>
<td>580</td>
<td>94</td>
</tr>
</tbody>
</table>

*P<.05 compared with twice-milked gland. Data from Wilde and Knight (42).

7Seven goats.

3Thrice daily milking started in wk 2 to 4 of lactation.

Total DNA calculated as the product of parenchyma weight and DNA concentration.

constant rate of inhibitor secretion favoring its slow accumulation in the alveoli, the onset of a significant degree of feedback inhibition would occur after sustained milk accumulation.

Whatever the intracellular mechanism of local feedback inhibition, this autocrine control mechanism clearly operates during declining lactation to modulate the rate of milk secretion, through rapid, reversible regulation of the metabolic activity of the mammary secretory cell.

Manipulation of Cell Number During Declining Lactation

The evidence presented indicates that a fall in the number of secretory cells is responsible ultimately for the decline in milk yield after peak. Therefore, any treatment that can prevent or reduce cell loss is likely to have long-term effects on the rate and persistency of milk secretion. Little is known about the factors determining cell longevity in the gland, and so it is not possible to predict whether this process is likely to be susceptible to manipulation. However, it is clear from the compensatory mammary growth observed in the remaining gland following hemimastectomy that there is a potential for cell proliferation in the tissue even in established lactation (19).

Long-term, thrice daily milking confirmed that the decline in mammary cell number in declining lactation could be reduced (42). This required thrice daily milking for an extended period constituting virtually the entire lactation, but after 37 wk of milking one gland of the goat three times daily, that gland had a greater parenchymal tissue weight (P<.05) and total DNA content (P<.05) than the contralateral twice daily milked control (Table 4). The greater DNA content, i.e., greater cell number, was associated with a higher rate of DNA synthesis in explants freshly prepared from the thrice-milked gland (Table 4), suggesting that the effect on cell number was at least partly due to a stimulation of secretory cell proliferation. However, at present the possibility cannot be ruled out that cell longevity was increased by more frequent milking.

The mammary gland is the target of a wide variety of growth factors, many of which are found in colostrum and early lactation milk (3, 11, 33). The predominant growth factor in bovine milk was found to be a polypeptide of 30 to 35 kdal (31), and in goat milk a factor related to platelet-derived growth factor has been demonstrated (11). Recent evidence indicates that other factors may also play a role in ruminant mammary growth. Bovine mammary tissue has been demonstrated to contain receptors for epidermal growth factor (EGF; 33), whose number was lower in plasma membranes purified from lactating glands compared with membranes from the glands of pregnant animals. The EGF is a stimulator of rodent mammary cell proliferation both in vivo (35) and in vitro (17), and infusion of this factor was recently demonstrated to stimulate mammary growth in pregnant cows (12). The EGF also stimulated proliferation of ruminant mammary cells in primary culture (24).
Polypeptide growth inhibitors may be of equal importance in regulating mammary growth in lactation. Bohmer et al. (7, 8) demonstrated a 13-kdal polypeptide in bovine mammary tissue that inhibited proliferation of a mammary epithelial cell line in a dose-dependent and reversible manner, the concentration of which is increased dramatically with the onset of lactation (9), and which is secreted in milk associated with the milk fat globule membrane (10). Rat mammary gland has also been found to contain a protein that reversibly inhibits DNA replication in the nucleus, and again, its concentration was low in pregnancy and high in lactating tissue (18).

At present, no evidence implicates any of these factors in the growth response resulting from long-term thrice daily milking. For this to be possible, it is necessary to invoke a mechanism whereby a local response could be elicited in only one gland of the udder. Clearly, this could occur if the growth factor (or inhibitor) were synthesized locally in the mammary gland. However, it is interesting to speculate that a local response by an individual gland to a circulating hormone could be achieved if the sensitivity of each gland to that hormone was modulated independently by a unilateral change in the number of its cell-surface receptors. Preliminary evidence that such a mechanism could operate in mammary gland was obtained in goats subjected to unilateral thrice daily milking; the total number of prolactin receptors increased significantly in the thrice-milked gland compared with the contralateral control (C. J. Wilde, C. H. Knight, and D. J. Flint, unpublished observations). Such a mechanism would allow integration of local (perhaps autocrine) regulation within the gland with the endocrine system to elicit changes in mammary cell number and activity.

Modulation of Secretory Cell Differentiation During Declining Lactation

The degree of secretory cell differentiation is also susceptible to manipulation through an intramammary mechanism. During thrice daily milking this was, in temporal terms, an intermediate response. After 10 d, acetyl-CoA carboxylase, fatty acid synthetase, and galactosyltransferase activities had increased significantly in the thrice-milked gland but not in the twice-milked gland (42). It was also a transient effect, so that at the next time point 10 wk later, the activities of these key enzymes were again similar in the two glands (42). This effect was observed during thrice daily milking before peak lactation, when cellular differentiation was still occurring, and thus could be considered a precocious increase rather than a stimulation of the synthetic capacity of the secretory cells. A local stimulation of secretory cell hypertrophy was also reported in the cow, in this case in response to prepartum milking (1). It is not clear whether the acute feedback inhibitor described is itself responsible for the effects on secretory cell differentiation; however, when the 10- to 30-kdal fraction was injected into the glands of lactating rabbits, a decrease in milk accumulation over the following 24 h was accompanied by a reduction in the activities of the key enzyme markers of cellular differentiation (41).

Secretory cell differentiation also responded to a change in milk demand initiated after peak lactation (38). In this experiment, rather than as before studying the results of relieving feedback inhibition by increasing milk removal, the positive effect of chemical feedback was investigated by increasing the residual volume of milk in the gland after milking. One gland of lactating goats was milked incompletely for 24 wk beginning in the week after peak yield was achieved, such that the residual volume of milk in the gland was calculated to be 100 ml, approximately twice that left by normal milking. In the short term, this had little effect on enzyme activities or metabolic fluxes in the incompletely milked gland (Figure 6), so the treatment was not eliciting an acute response equivalent but opposite to that of thrice daily milking. After 24 wk, however, when the weekly yield of the incompletely milked gland expressed per unit DNA, i.e., on a per cell basis, was 24% lower than that of the control, the total activities of the key enzyme markers — acetyl-CoA carboxylase, fatty acid synthetase and galactosyltransferase — were all significantly lower in that gland (41). Therefore, inefficient milking over a long period caused partial involution or dedifferenti-
Figure 6. Enzyme activities in goat mammary glands milked normally or incompletely for 24 wk. Incomplete milking of one gland (O) was initiated in wk 7 to 10 after peak yield was attained \( *P<0.05, \quad **P<0.001 \) compared with control gland (O) at that stage. Error bars indicate the SE of the mean. Data from Wilde et al. (38).

Thus, the response triggered in the mammary gland by this local mechanism depends not just on the duration of the stimulus, but on the extent to which the normal process of milk removal is perturbed. The low rate of thymidine incorporation into DNA measured in explants from both glands (results not shown) indicated that there was no alteration in the rate of cell turnover during incomplete milking. This suggests that the reduction in cellular differentiation occurred in the existing population of secretory cells rather than through replacement of highly differentiated cells by a new subpopulation of less differentiated ones.

Inefficient milking may become an increasingly important factor in modulating milk yields in successive lactations. The proportion of residual milk in the gland after machine milking increased in the second (2) and succeeding lactations (14). So, although yield generally tends to increase with lactation number, in this respect, milk secretion may become increasingly inefficient. It is notable that omission of “stripping” at the end of milking had a greater effect on milk yield in the second lactation than in the first (13).

CONCLUSION

These experiments with lactating goats indicate that the fall in milk yield after peak lactation is due primarily to a decrease in the number of mammary secretory cells and that the remaining cells do not lose their metabolic capacity for milk synthesis to any significant degree. The studies also show that it is possible to regulate yield during declining lactation, either acutely by manipulating cell metabolic capacity via an autocrine mechanism susceptible to changes in milking frequency or efficiency, or in the long-term by changes in mammary cell number elicited by the same stimulus.

REFERENCES

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