Mammary Growth in Holstein Cows During the Dry Period: Quantification of Nucleic Acids and Histology

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ABSTRACT

Influence of the dry period on mammary growth was studied using multiparous Holstein cows. Sixty days before expected parturition, 13 cows were dried off, and another 13 cows were milked throughout the prepartum period. Lactating cows and dry cows were slaughtered at 53, 35, 20, and 7 d prepartum. Total mammary parenchymal DNA increased twofold from 53 to 7 d prepartum without influence of lactation status. However, overall rate of [^{3}H]thymidine incorporation by mammary tissue was 80% greater in dry cows than in lactating cows, indicating that replacement of mammary cells was greater in dry cows. Of the mammary cells labeled with [^{3}H]thymidine, the percentage of epithelial cells in dry cows was greater than that in lactating cows (96% vs. 86%). By 7 d prepartum, epithelial calls accounted for a greater percentage of total mammary cells in dry cows than in lactating cows (83% vs. 74%). Tissue area occupied by alveolar or ductular lumina decreased by 25 d into the dry period (35 d prepartum) and then increased to a maximum by 7 d prepartum. None of the mammary epithelial cells in dry cows were classified as secretory at 35 d prepartum, but 98% of the epithelial cells of dry cows were classified as secretory at 7 d prepartum. Results indicated that mammary involution did not occur during a typical dry period of dairy cows. Data suggest that a dry period is important for replacing senescent mammary epithelial cells and increasing the epithelial component of the gland prior to the next lactation.

(**Key words**: mammary growth, mammary involution, cell turnover)

Abbreviation key: **[^{3}H]Tdr** = [^{3}H]thymidine, **[^{3}H]Ur** = [^{3}H]uridine.

INTRODUCTION

A nonlactating period prior to parturition in dairy cows, commonly referred to as the dry period, is important for optimal milk production during the subsequent lactation (15). A dry period of <40 d results in reduced milk production during the following lactation (7, 22). If only milk production is considered, optimal duration of the dry period would balance loss of milk production during the current lactation with increased production during the following lactation; this optimal interval is generally 40 to 60 d. The necessity of a dry period appears to be for reasons other than nutritional reasons (19, 20, 22). However, physiological events necessitating a dry period remain unknown.

Despite the importance of a dry period, little is known about the extent of involution, growth, and differentiation that occurs in the bovine mammary gland during this period. Following cessation of milking or weaning of the young, involution of the mammary glands occurs, a process that has been extensively investigated with rodents (11). However, data generated for other species may not be easily extrapolated to the cow. Histological investigations (1, 10) suggest that mammary involution in cattle is slower and far less extensive than that in rodents. In sharp contrast to the situation in most species, dairy cows are typically pregnant when milk removal ceases. Consequently, mammary involution during the dry period is promoted by milk stasis but is opposed by the mammogenic and lactogenic effects of pregnancy.

The importance of concomitant pregnancy during the dry period has been addressed by a limited number of investigators. Swanson et al. (23) assessed mammary gland involution on the basis of udder weight, DNA content, and histology. Involution of mammary glands clearly occurred when nonpregnant cows or cows in early gestation were dried off. Little or no involution was apparent at the end of dry periods that were 15 to 75 d in length and occurring in late pregnancy. However, all comparisons were

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relative to lactating, nonpregnant controls or were between lactating and involuting udder halves. Because involution is retarded in dry glands when milk is removed from the other mammary glands of the animal (2, 24) and because compensatory growth occurs in the lactating gland (5), the appropriateness of such comparisons between udder halves is questionable.

The dry period is critical for maximizing milk secretion and could be a critical time to concentrate on management strategies to enhance the succeeding lactation. However, knowledge of intramammary events that occur during the dry period of the bovine lactation cycle is incomplete. In lieu of recommendations for a 40- to 60-d dry period, the objective of this investigation was to determine the influence of lactation status (lactating vs. dry) on mammary involution, growth, and differentiation during a 60-d prepartum interval in Holstein cows.

MATERIALS AND METHODS

Cows and Experimental Design

Twenty-six multiparous Holstein cows were paired by stage of gestation and milk production. Within a pair, expected calving dates were within a 40-d range, with the exception of one pair that differed by 163 d; the milk production of both cows was within 20% of each other. Paired cows were randomly assigned to be dried off 60 d before expected calving or to be milked twice daily throughout the preparturient period. The week before cows were assigned to treatment, foremilk samples were collected for determination of SCC (13) and bacteriology (12). Cows were free of intramammary infection and remained free of clinical mastitis throughout the study. Cows were not pregnant for the initial 3 to 5 mo of the current lactation. Beginning approximately 4 wk prior to the experimental period (90 d prepartum), supplemental lighting provided 16 h of light from 0600 to 2200 h.

Three lactating cows and 3 dry cows were killed at 53, 35, and 20 d prepartum, and 4 lactating cows and 4 dry cows were killed at 7 d prepartum (corresponding to 7, 25, 40, and 53 d into the dry period). Cows were killed at the USDA abattoir (Beltsville, MD) by exsanguination after a stun to the head with a captive bolt pistol. Those cows in the lactation group were milked 1 to 2 h before slaughter. Because cows were slaughtered before parturition, we could not confirm concordance between expected calving date and actual parturition.

Tissue Samples

At slaughter, the udder was removed, trimmed of extraparenchymal tissue, and separated into right and left halves. Each udder half was weighed. The right udder half provided tissue for quantification of total parenchymal DNA, RNA, and hydroxyproline. Parenchyma was ground using a commercial meat grinder (Hobart Corp., Troy, OH), and aliquots were stored at –20°C until compositional analyses. The left udder half provided tissue for morphometric and metabolic studies. Samples of mammary parenchyma were obtained from the midregion of the left rear quarter for nucleotide incorporation studies. Samples of mammary parenchyma were obtained from three regions within the left front quarter for autoradiographic analysis of incorporation of [^{3}H]thymidine (**[^{3}H]Tdr**) and for morphometric analysis. Parenchyma was sampled at the base of the gland adjacent to the gland cistern (zone 1), midway between the gland cistern and dorsal boundary of the mammary parenchyma (zone 2), and near the dorsal parenchymal border (zone 3). All zones were approximately oriented along an axis through the center of the gland in line with the teat. Tissue was transported to the laboratory in ice-cold Tris-sucrose buffer (25 m*M* Tris, 0.3 *M* sucrose, and 1 m*M* EDTA; pH 7.4).

Tissue Incubations

Mammary tissue was sliced with a Stadie-Riggs hand microtome (Arthur H. Thomas Co., Philadelphia, PA). Quadruplicate tissue slices (approximately 150 mg each) from the midregion of the left rear quarter were incubated in Medium 199 (GIBCO Laboratories, Grand Island, NY) at a concentration of 1 μCi of [^{3}H]Tdr/ml (25 Ci/mmol; Amersham, Arlington Heights, IL), and quadruplicate slices were incubated in Medium 199 containing 1 μCi of [^{3}H]uridine (**[^{3}H]Ur**)/ml (5 Ci/mmol; Amersham). Similarly, duplicate slices from each zone of the left front quarter were incubated with [^{3}H]Tdr. Incubations were for 2 h at 37°C under an atmosphere of 5% CO_2 and 95% O_2. Following incubation, tissues were rinsed in 0.9% saline. Slices from each zone of the left front quarter were fixed and processed for autoradiography (described subsequently), and slices from the left rear quarter were stored at –20°C for later quantification of incorporation of [^{3}H]Tdr and [^{3}H]Ur.

Incorporation of [^{3}H]Tdr and [^{3}H]Ur

To determine the quantity of [^{3}H]Tdr and [^{3}H]Ur that had been incorporated, tissue slices were first homogenized in 5 ml of homogenization buffer (0.05

M sodium phosphate, 2 *M* NaCl, and 2 m*M* Na_2-EDTA; pH 7.4). Duplicate 0.5-ml aliquots of each homogenate were then precipitated with 0.5 ml of ice-cold 10% trichloroacetic acid. The pellet was washed three times with 2 ml of ice-cold 5% trichloroacetic acid, and the final pellet was solubilized and counted in ACS II scintillation fluid (Amersham).

Analysis of the Composition of the Udder Half

To quantify total nucleic acids in mammary parenchyma from the right udder half, homogenates of ground tissue were precipitated and washed three times with 0.3*N* perchloric acid and then hydrolyzed in 0.5*N* perchloric acid at 70°C as previously described (5). Quantities of DNA and RNA were determined by the diphenylamine and orcinol methods on perchloric acid hydrolysates (5). Hydroxyproline content of mammary parenchyma was determined according to the methods of Woessner (26).

Autoradiography and Morphometric Analysis

Tissues were fixed in modified Karnovsky's fixative and processed for autoradiography as described previously (17). For each zone per cow, labeled epithelial cells were enumerated within the boundaries of six random microscopic fields defined by the boundary of an ocular grid, 100 × 100 μm. Labeled cells were overlaid with 12 or more grains, but background labeling was <1 grain per nucleus. Labeled cells were classified by cell type (epithelial, fibroblast, endothelial, or leukocyte). Epithelial cells were further classified by morphological appearance of their secretory state. The percentage of tissue area occupied by epithelia, stroma, and lumina was determined by a modification of the quantitative morphological analysis described by Chalkley (6). A 33-point ocular reference grid was superimposed over six randomly selected fields, which yielded an evaluation of 198 contact points per zone for each cow. Determinations were made with coded microscope slides to prevent observer bias.

Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (16). Cows were grouped according to calving date in incomplete blocks of 2 to 11 cows. Except for 1 cow, it was possible to construct blocks with a difference in calving date of <75 d. The residuals for that cow were not unusual, and, therefore, even though one block had a difference of 163 d, the cow was included in all analyses.

For BW, udder composition, and [^{3}H]Tdr and [^{3}H]Ur incorporation data, the model included group, day, and the group by day interaction as fixed sources of variation; block (calving date) and residual cow variation were defined as random sources of variation. Variables from histological and autoradiographic analyses resulted in multiple values for the same variable on mammary tissue from different areas (zones) within the udder of each cow. Because data recorded from different zones on the same cow may be correlated, the repeated measures features of the SAS mixed model procedure were used to compute both the zone variances and the covariances between zones. Examination of Akaike's information criterion was used to identify a variance-covariance structure that adequately described the variance covariance relationship among zones. The variance-covariance structures used were as follows: 1) percentage of tissue area occupied by epithelia, stroma, and lumina (first-order autoregressive); 2) percentage classification of mammary cells according to cell type (compound symmetry); and 3) percentage classification of cells labeled with [^{3}H]Tdr (independent). In addition, examination of residuals for these (percentage) variables indicated the typical binomial pattern of heterogeneous variances and were therefore transformed using the arcsine transformation. For these analyses, the model included group, day, and the group by day interaction as fixed sources of variation; block, cow within group and day, and the residual variation were defined as random sources of variation. For presentation of data, the inverse transformation (sine) of arcsin-transformed means was used to reconvert to mean percentages and standard errors. The positive and negative standard errors that were obtained by inverse transformation were not equal. When these percentage data were summarized in tabular form, only the larger standard errors were presented.

Main effects and interactions were declared significant at $P \leq 0.05$ and nonsignificant at $P > 0.15$. Trends were considered at $P > 0.05$ to $P = 0.15$.

RESULTS

Parameters of mammary mass and BW did not differ between dry and lactating cows (Table 1; $P > 0.15$). During the prepartum period, live BW tended to increase for both groups ($P = 0.1$), but carcass weight was unaffected by day of gestation or lactation status ($P > 0.15$). Mammary growth proceeded at similar rates for both groups. Parenchymal mass ($P = 0.1$) and total parenchymal DNA ($P = 0.03$) in-

creased during the prepartum period for both groups but did not differ between groups ($P > 0.15$). The ratio of RNA:DNA in mammary parenchyma was not influenced by day of gestation ($P > 0.15$). Despite considerable variability in total mammary RNA, the ratio of RNA:DNA tended to be greater ($P = 0.12$) for lactating cows than for dry cows throughout the experimental period. The mass of the extraparenchymal mammary fat pad tended ($P = 0.14$) to increase as gestation advanced without the influence of lactation status ($P > 0.15$). Hydroxyproline content of mammary parenchyma, used as an index of collagen content, suggested that the total content of parenchymal collagen increased during the prepartum period ($P = 0.05$, effect of day; $P = 0.09$, –53 vs. –7 d). Hydroxyproline content tended to be greater in the mammary tissue of dry cows than that in the mammary tissue of lactating cows ($P = 0.06$). In dry cows, hydroxyproline content increased by 35 d prepartum to the extent that a group difference was evident on that day ($P = 0.005$), and there was a tendency toward a group by day interaction ($P = 0.08$).

Although patterns of mammary growth of dry and lactating cows did not differ on the basis of parenchymal weight or DNA content (Table 1), [^{3}H]Tdr was incorporated at a greater rate by prepartum mammary tissue from dry cows than by prepartum mammary tissue from lactating cows ($P = 0.02$; Figure 1). At 53 d prepartum (7 d into the dry period), incorporation of [^{3}H]Tdr was identical for mammary tissue of both groups. Thereafter, incorporation rate of [^{3}H]Tdr appeared to increase more rapidly in dry cows than in lactating cows. However, this group difference was not statistically demonstrable because there was no group by day interaction ($P > 0.15$). The same pattern of incorporation was evident when the percentage of mammary epithelial cells that were labeled with [^{3}H]Tdr was quantified autoradiographically (Figure 2). At 35 d prepartum, the mammary epithelia of cows that had been dry for 25 d had a greater [^{3}H]Tdr labeling index than did the mammary epithelia of cows that had been lactating ($P < 0.05$). This group difference tended to persist through the remainder of the prepartum period ($P = 0.15$), and the labeling index increased for both groups as gestation advanced ($P = 0.001$, –53 vs. –7 d). The percentage of epithelial cells that were labeled tended ($P = 0.15$) to be greatest in the basal region of the gland, averaging 2.1, 1.7, and 1.1% for zones 1, 2, and 3, respectively (data not shown). Although a 1% difference in labeling index between zones 1 and 3 appears to be minor, these values (2.1% vs. 1.1%) indicate that nearly twice as many cells were in the S-phase (DNA synthetic phase) of the cell cycle in the lower region of the mammary gland (zone 1) than in the upper region (zone 3). In con-

TABLE 1. Udder weight, BW, and udder composition.[1]

	Time before parturition											
	–53 d		–35 d		–20 d		–7 d			Contrast[2]		
	Lac	Dry	Lac	Dry	Lac	Dry	Lac	Dry	SE	G	D	G × D
										P		
Cows, no.	3	3	3	3	3	3	4	4				
Live BW, kg	678	706	684	705	742	761	762	738	26	NS[3]	0.10	NS
Carcass, kg	325	334	337	343	338	360	347	337	18	NS	NS	NS
Milk,[4] kg/d	11.1		10.0		8.4		7.0		4	. . .	. . .	. . .
Udder half												
Parenchyma, kg	6.7	6.7	5.8	7.6	7.9	7.2	11.0	10.2	1.8	NS	0.10	NS
Fat, kg	1.7	1.4	1.4	1.8	2.0	2.3	2.0	1.9	0.3	NS	0.14	NS
DNA, g	27.7	21.1	23.0	37.9	38.0	34.0	52.3	51.1	9.5	NS	0.03	NS
RNA, g	45.8	31.7	40.6	50.1	49.3	45.5	71.1	61.8	15.1	NS	NS	NS
RNA:DNA	1.6	1.5	1.8	1.1*	1.3	1.3	1.4	1.2	0.2	0.12	NS	NS
OH-Pro,[5] mg	14.9	17.1	10.8	52.8*	42.9	54.8	34.5	38.5	9.2	0.06	0.05	0.08

[1]Cows were dried off 60 d before expected parturition (Dry) or milked throughout the prepartum period (Lac). Least squares means and pooled standard errors (n = 3) are presented.

[2]Probabilities are summarized for the effects of group (G), day (D), and group by day interactions (G × D).

[3]$P > 0.15$.

[4]Milk production within 2 d of slaughter is presented for comparison. Milk production of cows in the dry group at dry-off averaged 10.2 ± 3 kg/d.

[5]Hydroxyproline.

*Differs ($P < 0.05$) from lactating cows at the same stage of gestation.

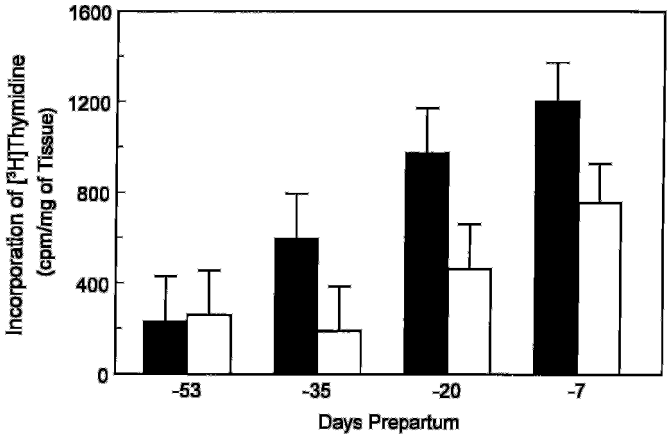

Figure 1. Incorporation of [3H]thymidine by mammary tissue from dry (solid bars) and lactating (open bars) cows. Each bar indicates the mean (±SE) for 3 or 4 cows. Probabilities for the effects of group (G; dry or lactating), day (D), and group by day interactions (G × D) were G = 0.02, D = 0.004, and G × D > 0.15.

trast to epithelial labeling, the percentage of nonepithelial cells that were autoradiographically labeled did not differ between treatment groups and was not influenced by region within the mammary gland ($P > 0.15$).

Data in Table 2 focus on classification of the small proportion of total cells that were labeled with [3H]Tdr. In all cases, the majority of cells labeled with [3H]Tdr were alveolar epithelial cells; however, epithelial cells accounted for a greater percentage of the labeled cells in dry glands than in lactating glands ($P = 0.05$). Overall, 96 and 86% of those cells labeled with [3H]Tdr were epithelial in dry and lactating glands, respectively. A substantial proportion of cells labeled with [3H]Tdr were fibroblasts. The greatest proportion was found in lactating glands (10% vs. 1%; $P < 0.05$). In lactating glands, the percentage of labeled epithelial cells decreased from lower to upper regions, and the reverse was true for labeled fibroblasts ($P < 0.05$). The contribution of various cell types to the population of labeled cells did not differ by region within dry glands ($P > 0.15$). On average, 2.4% of labeled cells were endothelial cells, and this percentage was not influenced by lactation status, day of gestation, or region within the gland ($P > 0.15$). Leukocytes accounted for an insignificant proportion (0.01%) of labeled cells that was not influenced by group, day, or zone ($P > 0.15$; data not shown).

Morphological changes in mammary tissue during the prepartum period were quantitatively evaluated, and results of area classifications are summarized in Figure 3. The percentage of tissue area occupied by mammary epithelia was not influenced by day of gestation ($P > 0.15$). Although the epithelial area across all days tended to be greater ($P = 0.14$, effect of group) for dry cows than for lactating cows, no differences ($P > 0.15$) were evident on any individual day. Luminal area in mammary tissue from dry cows decreased ($P = 0.01$) to a minimum of 9.5% on d 35 prepartum (d 25 of the dry period), which was lower ($P = 0.04$) than the equivalent value (21.5%) for lactating cows on that day. Thereafter, the luminal area in mammary tissue from dry cows increased and tended to be greater than that in lactating tissue by 7 d prepartum ($P = 0.07$). The luminal area in mammary tissue from lactating cows did not differ ($P > 0.15$) with day of gestation but averaged 21% of the total tissue area. These data were consistent with an initial absorption of luminal secretions during the dry period, followed by the accumulation of mammary secretions during final phases of lactogenesis. Furthermore, stromal area appeared to be inversely related to luminal area, was influenced by day of gestation ($P = 0.02$), and tended ($P = 0.11$) to be influenced by a group by day interaction. Stromal elements in mammary tissue of lactating cows accounted for 42% of total tissue area and did not vary ($P > 0.15$) with stage of gestation. However, stromal area in mammary tissue from dry cows increased to a maximum at 35 d prepartum ($P = 0.02$) and then decreased to a minimum at 7 d prepartum ($P = 0.0002$), at which time stromal area was less for dry cows than that for lactating cows ($P = 0.04$). Region

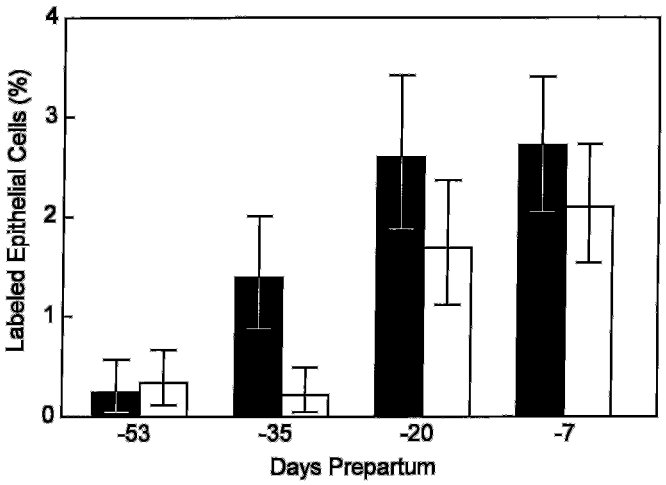

Figure 2. Epithelial cells labeled with [3H]thymidine in mammary parenchyma from dry (solid bars) and lactating (open bars) cows. The percentage of epithelial cells that were labeled was determined autoradiographically. Each bar indicates the mean (±SE) across regions within the gland for 3 or 4 cows. Probabilities for the effects of group (G; dry or lactating), day (D), and group by day interactions (G × D) were G = 0.12, D = 0.001, and G × D > 0.15.

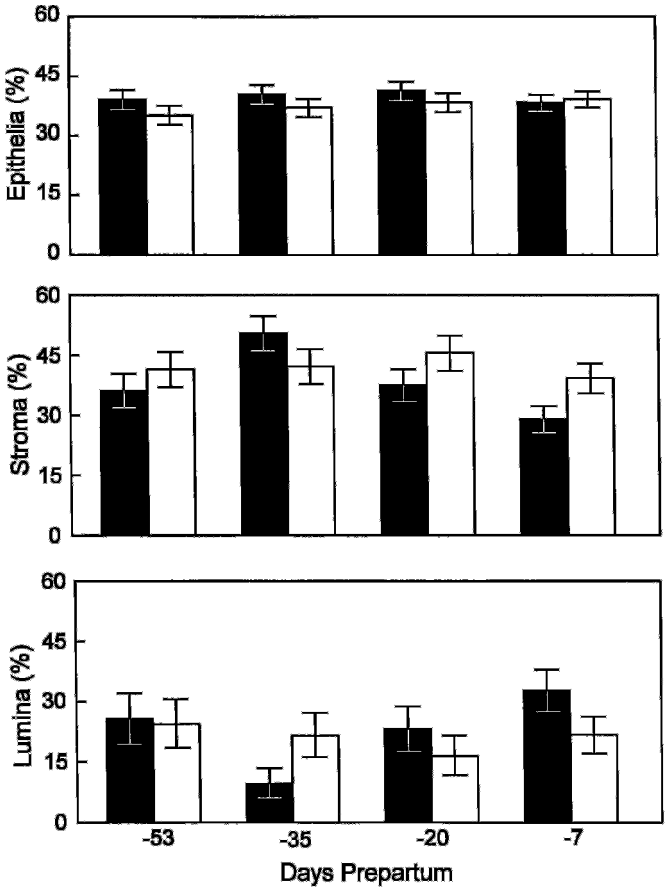

Figure 3. Morphometric analysis of mammary parenchyma from dry (solid bars) and lactating (open bars) cows. Percentage of tissue area occupied by alveolar epithelia (upper panel), stroma (middle panel), and lumina (lower panel) are presented. Each bar indicates the mean (±SE) across regions within the gland for 3 or 4 cows. Probabilities for the effects of group (G; dry or lactating), day (D), and group by day interactions (G × D) were 1) epithelia: G = 0.14, D > 0.15, and G × D > 0.15; 2) stroma, G > 0.15, D = 0.02, and G × D = 0.11; and 3) lumina: G > 0.15, D = 0.03, and G × D = 0.04.

within the gland had no influence on the percentage of tissue area occupied by epithelia ($P > 0.15$). However, stromal area progressively decreased ($P < 0.05$) from 44% in the lower region of the gland to 40 and 37% in the upper regions of the gland. Conversely, luminal area increased ($P < 0.05$) from 18% in the lower region to 22 and 25% in the upper regions of the gland.

Previously summarized data were related to tissue area and thus reflected the relative number of the different cell types evaluated as well as changes in cellular volume and the volume occupied by noncellular elements (e.g., luminal secretions and extracellular matrix). Consequently, these percentages provided an ambiguous picture of the proportion of different cell types.

The relative percentages of different cells, based on classification of 35,087 cells in random fields, are summarized in Table 3. At 7 d prepartum, the percentage of epithelial cells was greater ($P < 0.02$) in dry glands (83%) than in lactating glands (74%). Although myoepithelial cells were not enumerated and, thus, accounted for an unknown proportion of parenchymal cells, observation of sections suggests that myoepithelial cells constituted a small (≈5%) and relatively consistent proportion of the epithelial fraction. Conversely, the percentage of fibroblasts tended ($P = 0.06$) to be lower in dry glands than in lactating glands at 7 d prepartum, averaging 9.3 and 14.3% for dry and lactating glands, respectively. The endothelial component of mammary parenchyma was not influenced by lactation status or day of gestation ($P > 0.15$). Region within the mammary gland did not exert an effect ($P > 0.15$) on the proportion of cells that were epithelial or endothelial. However, percentage of fibroblasts tended ($P = 0.15$) to be greater in the lower region (14.3%) than in the upper regions (13.0 and 12.6%) of the gland, and the proportion of leukocytes tended ($P = 0.15$) to be lower (4.5%) in the lower region than in the upper regions (5.4 and 5.2%) of the gland. Furthermore, there tended ($P = 0.09$) to be an interaction of group and day on the leukocyte content of mammary tissue. The percentage of leukocytes in mammary tissue from dry cows was greatest at 35 d prepartum (25 d of the dry period; $P < 0.05$), averaging 5.1, 7.7, 4.6, and 3.2% at 53, 35, 20, and 7 d prepartum, respectively; however, the leukocyte content of mammary tissue from lactating cows was not affected by day of gestation ($P > 0.15$).

Changes in the secretory appearance of mammary epithelial cells were evident during the preparturient period (Figure 4). In lactating cows, the proportion of epithelial cells that morphologically appeared to be engaged in secretion averaged 62% and did not change ($P > 0.15$) with day of gestation. In contrast, influence of day of the dry period was striking. At 53 d prepartum, 25% of the mammary epithelial cells in cows that had been dry for 7 d contained secretory vesicles and lipid droplets and were conservatively classified as secretory. However, by 35 d prepartum, when cows had been dry for 25 d, none of the epithelial cells that were evaluated contained secretory vesicles or lipid droplets. After 35 d prepartum, the percentage of cells that demonstrated secretory activity increased to 78% at 20 d prepartum ($P = 0.01$) and to 98% at 7 d prepartum ($P = 0.0008$). Region within the mammary gland did not affect secretory appearance of epithelial cells ($P > 0.15$).

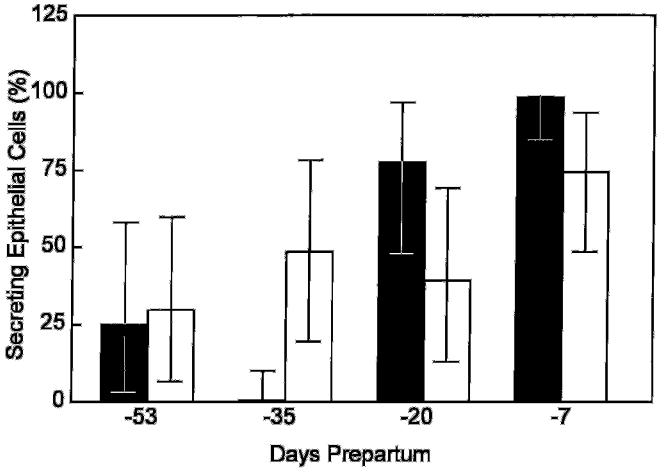

Figure 4. Influence of lactation status and stage of gestation on secretory state of mammary epithelial cells. Mammary epithelial cells were classified as secreting or nonsecreting based on morphological criteria. Percentage of secreting epithelial cells from dry (solid bars) and lactating (open bars) cows are presented. Each bar indicates the mean (±SE) across regions within the gland for 3 or 4 cows. Probabilities for the effects of group (G; dry or lactating), day (D), and group by day interactions (G × D) were G > 0.15, D = 0.03, and G × D = 0.14.

Incorporation of [^{3}H]Ur by mammary tissue of lactating and dry cows during a 2-h incubation is summarized in Figure 5. Incorporation of [^{3}H]Ur by mammary tissue of lactating cows averaged 250 cpm/mg of tissue across the prepartum period and was not influenced by stage of gestation ($P > 0.15$). However, incorporation of [^{3}H]Ur by mammary tissue from dry cows tended to increase ($P = 0.06$) from 221 cpm/mg of tissue at 53 d prepartum to peak incorporation rates of 689 and 685 cpm/mg of tissue at 35 and 20 d prepartum, respectively, and declined ($P = 0.07$) thereafter.

DISCUSSION

Others have suggested that little loss of mammary tissue occurs during the dry period. Swanson et al. (23) used udder weight, DNA content, and histology to investigate mammary involution. Data suggested that very little involution occurred with dry periods up to 75 d in length in late pregnancy. However, all comparisons in that study were relative to lactating, nonpregnant cows or were between udder halves that had been dried off at different times. Because involution is retarded in dry glands when milk is removed from the other glands of an animal (2, 24), the appropriateness of this interpretation has been questioned. More recently, others (9, 21) characterized the sequence of cytological changes in mammary tissue for a period of weeks following cessation of milking. Because those researchers (9, 21) did not observe sloughing of epithelial cells into the alveolar lumen or detachment of epithelial cells from the basement membrane, they concluded that little or no loss of epithelia occurs during the dry period. However, in the absence of accompanying DNA measurements, this conclusion appears largely speculative. For example, Akers et al. (1) demonstrated that mammary alveolar structure was maintained for at least 42 d after suckling of nonpregnant beef cows was terminated, despite a 60% decrease in total mammary DNA.

In the present investigation, biochemical measures of the composition of udder halves were combined with histological measures to examine the processes of mammary involution and growth during the dry period. During the 60-d prepartum period, there was no evidence that a net loss of mammary cells (involution or regression) had occurred in dry cows. At no time did mammary glands from dry cows contain less DNA or parenchymal mass than those from lactating cows. Furthermore, morphometric analysis demonstrated that tissue area occupied by mammary epithelia did not decline and that alveolar structures remained intact during the dry period. Both dry and lactating cows entered the final days of gestation with an equivalent number of mammary cells (DNA) and equivalent ratios of RNA:DNA. However, the proportion of mammary cells that were epithelial cells was greater in dry cows than in lactating cows at 7 d prepartum.

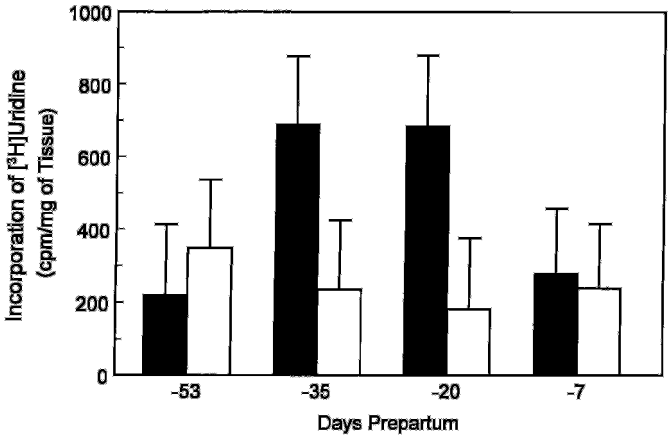

Figure 5. Incorporation of [^{3}H]uridine by mammary tissue from dry (solid bars) and lactating (open bars) cows. Each bar indicates the mean (±SE) for 3 or 4 cows. Probabilities for the effects of group (G; dry or lactating), day (D), and group by day interactions (G × D) were G = 0.06, D > 0.15, and G × D > 0.15.

Although the total number of mammary cells was not affected by lactation status during the 60-d prepartum period, rate of DNA synthesis, as assessed by incorporation of [^{3}H]Tdr, was 80% greater in mammary tissue from dry cows than in mammary tissue from lactating cows. Autoradiographs indicated that increased incorporation of [^{3}H]Tdr was due to an increase in the percentage of mammary epithelial cells incorporating the tracer, which most likely indicated increased division of epithelial cells within dry glands. Because the number of mammary cells in dry cows did not exceed that in lactating cows, we concluded that increased division was for the purpose of cell renewal or turnover. Furthermore, this process involved a greater number of epithelial cells in dry glands than in lactating glands, which appeared to result in a greater proportion of epithelial cells in dry glands than in lactating glands during the final week of gestation. Pitkow et al. (14) reported that approximately 75% of mammary cells were carried over into a second lactation of rats when no dry period was permitted. Considerably fewer cells were carried into the ensuing lactation following a dry period of 7 to 8 d. Although the possibility cannot be ruled out that at least a portion of the [^{3}H]Tdr was incorporated for purposes of DNA repair in the present study, the density of autoradiographic labeling suggests that incorporation of [^{3}H]Tdr was primarily for DNA replication (4). In either event, these data suggest that the value of the dry period might be to repair or replace damaged or senescent mammary epithelial cells prior to the next lactation.

During the initial period of milk stasis following cessation of milking, engorgement of alveolar lumina and secretory epithelial cells occurs (9). Milk composition then changes as milk secretion rate drops and resorption of accumulated milk components occurs (3, 10, 18). Accumulation of a feedback inhibitor of lacta-

TABLE 2. Classification of mammary cells labeled with [^{3}H]thymidine.[1]

Cell type	Epithelial		Fibroblasts		Endothelial	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Dry						
–53 d	89.6	16.8	0.0	3.8	10.4	8.2
–35 d	94.7	9.9	3.1	5.7	2.0	3.1
–20 d	98.5	7.9	1.1	5.0	0.4	2.0
–7 d	97.9	5.5	0.9	3.2	1.2	2.1
Lactating						
–53 d	83.7	9.9	15.4	12.7	0.8	2.6
–35 d	78.7	11.8	21.3	13.7	0	0.9
–20 d	89.2	15.4	2.2	5.5	8.5	5.2
–7 d	88.7	15.9	7.8	8.9	3.4	5.5
Dry						
Zone 1[2]	94.8	9.0	3.5	5.9	1.5	2.4
Zone 2[3]	98.4	7.1	0.6	3.3	1.0	2.0
Zone 3[4]	93.2	10.4	0.0	2.7	6.7	5.2
Lactating						
Zone 1	95.9	8.4	1.1	4.3	3.0	3.2
Zone 2	80.3	12.7	15.1	9.5	4.6	3.5
Zone 3	77.6	13.0	22.1	11.6	0.2	1.4
	P					
Source						
Group (G)	0.05		0.01		NS[5]	
Day (D)	NS		NS		NS	
Zone (Z)	NS		NS		NS	
G × D	NS		NS		NS	
G × Z	NS		0.03		NS	
G × D × Z	NS		NS		NS	

[1]Mammary cells labeled with [^{3}H]thymidine were classified according to cell type, and data are expressed as a percentage of labeled cells. A total of 417 labeled cells from dry cows and 385 labeled cells from lactating cows were evaluated.

[2]Base of the gland adjacent to the gland cistern.

[3]Midway between the gland cistern and dorsal boundary of the mammary parenchyma.

[4]Near the dorsal parenchymal border in line with the teat.

[5]$P > 0.15$.

tion (25) and increased alveolar pressure (8) appear to cause the decline in milk secretion at drying off. Subsequently, by 25 d into the dry period (35 d prepartum), a nonsecretory state was achieved: none of the epithelial cells at that time contained secretory vesicles or fat droplets, and mammary luminal area had declined to the minimum. This nonsecretory state coincided with the period of maximum fluid resorption (10). As luminal area decreased to its minimum, stromal area increased to a maximum. Although this change in stromal area might simply reflect the elasticity of this tissue component under reduced compression by luminal contents, our observation that hydroxyproline content of mammary tissue increased parallel to increases in stromal area suggested that increased synthesis of stromal elements occurred at this time. By 7 d prepartum, luminal area was at its maximum, and stromal area was at its minimum, reflecting the initial accumulation of colostral secretions prior to calving. These data concur with those of Sordillo and Nickerson (21).

The time at which secretory activity and luminal volume appeared to be at a minimum might be a pivotal time for mammary development during the dry period. At that time, incorporation of [^{3}H]Ur appeared to increase, suggesting an increase in RNA synthesis. Also, the temporary increase in hydroxyproline content at that time might suggest that the extracellular matrix is being remodeled or transiently increased, which might lay the foundation for epithelial turnover and mammary growth; both appear to be augmented at this time.

A degree of heterogeneity was evident across regions within the mammary gland. Autoradiographic labeling indicated that [^{3}H]Tdr labeling was greatest in the lower regions of the gland and indicated that nearly twice as many cells were dividing in the basal region of the gland as in the upper region. This result was consistent with the greater incorporation of [^{3}H]Tdr in lower regions of the mammary gland during a compensatory response to cessation of milk removal in adjacent glands (5). In dry glands, the percentage of labeled cells that were epithelial or fibroblastic was not influenced by region of the gland. In lactating glands, epithelial cells contributed more, and fibroblasts contributed less, to the population of

TABLE 3. Classification of mammary cells according to cell type.[1]

Cell type	Epithelial		Fibroblasts		Endothelial		Leukocytes	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Dry								
–53 d	74.3	3.0	13.7	2.3	6.9	1.0	5.1	0.9
–35 d	70.8	2.9	15.5	2.3	6.2	1.0	7.5	1.0
–20 d	77.7	2.8	11.0	2.1	6.6	0.9	4.6	0.8
–7 d	82.5	2.2	9.2	1.7	5.2	0.8	3.1	0.7
Lactating								
–53 d	73.8	3.0	13.3	2.0	6.8	1.0	5.5	0.8
–35 d	72.9	2.4	13.8	2.3	7.5	1.0	5.3	0.8
–20 d	74.3	2.9	15.2	2.3	5.6	0.7	4.7	0.9
–7 d	74.2	2.9	14.3	1.9	6.0	0.8	5.2	0.9
Zone[2]								
1	74.7	1.2	14.3	0.9	6.3	0.5	4.5	0.4
2	75.0	1.2	13.0	0.9	6.4	0.4	5.4	0.4
3	75.7	1.2	12.6	0.9	6.4	0.6	5.2	0.4
	P							
Source								
Group (G)	0.10		0.11		NS[3]		NS	
Day (D)	0.09		NS		NS		0.06	
Zone (Z)	NS		0.15		NS		0.15	
G × D	NS		NS		NS		0.09	
G × Z	NS		NS		NS		NS	
G × D × Z	NS		NS		NS		NS	

[1]Mammary cells were classified according to cell type and the number of a given cell type expressed as a percentage of total cells evaluated. A total of 18,142 cells from dry cows and 16,945 cells from lactating cows were evaluated.

[2]1 = Base of the gland adjacent to the gland cistern, 2 = midway between the gland cistern and dorsal boundary of the mammary parenchyma, and 3 = near the dorsal parenchymal border in line with the teat.

[3]$P > 0.15$.

dividing cells in lower regions of the gland than in upper regions of the gland. In accordance with previous findings (1), the lower region of the mammary gland appeared to be the most sensitive to stimuli promoting turnover of epithelial cells.

In an informative review of the literature dealing with changes in the mammary gland and its secretions during the nonlactating period, Smith and Todhunter (18) suggested that bovine mammary glands progressed through three functional states during the dry period: 1) a period of active involution, which begins with cessation of milking and is associated with decreased synthesis of milk constituents and regression of synthetic tissue; 2) a period of steady-state involution during which the mammary gland is fully involuted and is in a nonsecretory state; and 3) a period of lactogenesis during which synthetic tissue is regenerated and differentiation and colostrogenesis is initiated. These stages were based on evaluation of data concerning volume and composition of mammary secretions during the dry period as well as extrapolation of histological data from rodents. Although this three-stage concept has merit with regard to secretory activity, data from the current study suggest that these stages are inappropriate and misleading in reference to changes in the number of mammary cells.

Because our data indicate that loss of mammary cells is not significant during a typical 60-d dry period, involution and regression are inappropriate terms in this context. Furthermore, in the present study, mammary growth appeared to have been initiated by 25 d into the dry period so that the concept of a steady-state period prior to mammary growth and differentiation seems questionable in a 60-d dry period. A degree of cell loss and achievement of an involuted steady state might occur if cows were dried off earlier in gestation. Indeed, Holst et al. (9) observed more extensive mammary regression than that in the present study. Those researchers (9) used two nonpregnant cows and two pregnant cows; stage of gestation was not specified. "Alveolar buds" might have been observed because of a complete or relative lack of gestational effects on the mammary gland.

CONCLUSIONS

Data from the current study indicate that net loss of mammary cells does not occur during the dry period in dairy cows. Because there is a change only in the stage of lactation, mammary involution and regression are inappropriate terms to refer to changes in the bovine mammary gland during a typical 60-d dry period. Enhanced turnover of mammary epithelial cells during the dry period suggests that the importance of the dry period is to permit replacement of damaged or senescent epithelial cells prior to the ensuing lactation. Furthermore, although the total number of mammary cells did not differ between dry and lactating mammary glands during the preparturient period, processes of proliferation and cell turnover seemingly increased the percentage of epithelial cells in mammary glands of dry cows prior to parturition. Approximately 35 d prepartum appears to be a pivotal time for these events.

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