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PHYSIOLOGY AND MANAGEMENT

A Model to Describe Growth Patterns of the Mammary Gland During Pregnancy and Lactation

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ABSTRACT

Extensive proliferation and death of cells in the mammary gland occur during pregnancy and lactation. In this study, a mechanistic model was developed that yielded a single equation to describe the pattern of mammary growth of mammals throughout pregnancy and lactation. The model contains a single pool, which is the cell population of the mammary gland; one influx, representing cell proliferation; and one efflux, representing cell death. The parameters of the equation lend themselves to direct physiological interpretation. The model fitted data on mammary gland DNA adequately and can be related to current knowledge on factors and inhibitors of mammary gland growth. A unique definition of the parameters of the model can be difficult because of the high degree of variation among animals, an improper number of observations, or timing, as indicated by analyses of simulated data. The model can also be applied to the study of the entire lactation curve. The widely applied gamma equation and the equation that was developed in this study were compared using weekly production data from dairy cows. The new model performed well, particularly when a sharp peak in milk production occurred. The model has the advantage of providing, for the first time, a simple biological description of the lactation curve that can be used to discriminate changes in lactational performance that are associated with experimental treatments.

(**Key words**: modeling, mammary gland, lactation)

Abbreviation key: **DFFT** = dry fat-free tissue, **DW** = Durbin-Watson statistic.

INTRODUCTION

Endocrine changes associated with pregnancy stimulate extensive cell proliferation in the mammary gland that continues into early lactation in many species. The cell population then declines markedly until the cessation of involution (18, 22). The pattern of growth of the gland during pregnancy and lactation has been studied quantitatively for many years using descriptors such as wet weight, dry fat-free tissue (**DFFT**), and DNA content (45). Because the size of the cell population is a crucial determinant of milk production (19, 21, 44), hyperplastic growth of mammary tissue is of particular interest.

Mammary growth patterns in pregnancy and lactation have been described empirically until now using segmented regression relationships that contain linear, quadratic, cubic, exponential, and logarithmic terms (3, 4, 5, 6, 26, 42). These equations are often unwieldy, and ascribing physiological meaning to their parameters is usually difficult. A mechanistic model of the lactation curve was developed by Neal and Thornley (27). In that model, the mammary gland was represented by undifferentiated cells, differentiated cells that were produced by cell division from undifferentiated cells and had a finite lifetime, and a storage compartment that represented the alveoli, ducts, and gland cistern. Neal and Thornley (27) obtained reasonable agreement when fitting the model, but the practical use of the model is limited because the inputs that are required are not generally

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available. In the current study, a mechanistic model was proposed that yields a single equation to describe the pattern of growth throughout pregnancy and lactation in several species. The parameters of this new equation lend themselves to direct physiological interpretation, providing a simple means of measurement of cell proliferation and, for the first time, cell death.

This new equation considers the relationship between gland size or cell numbers and milk production (13, 21, 44, 45) and offers an alternative method to describe lactation production data. In contrast to the proposed mechanistic model, none of the empirical models of the lactation curve (11, 15, 25, 30, 36), including the widely used gamma equation that was proposed by Wood (50), lend themselves to direct physiological interpretation. In this study, the proposed model was applied to the entire lactation curve of dairy cows and was compared with extant models of the milk production curve.

MATERIALS AND METHODS

The Model

The scheme that was assumed is shown in Figure 1. The model comprises a single pool, one influx, and one efflux. The pool is the cell population of mammary tissue (N; milligrams), represented by DNA accumulation during pregnancy and subsequent lactation, and the fluxes are cell proliferation and cell death. In the pregnant animal, marked physiological changes take place at parturition (T; days since conception), and, therefore, two phases are distinguished: pregnancy and lactation. Usually, rapid cell proliferation is observed during pregnancy. In rodents, the rate of proliferation, as measured using the incorporation of tritiated thymidine, is relatively low during early pregnancy and high during midpregnancy and near parturition (20, 41, 43). With the assumption that the death rate of the undifferentiated mammary cells during pregnancy is negligible, the rate:state equation is

$$dN/dt = \mu N, \; t < T \qquad [1]$$

with

$$\mu = \mu_T \exp[-k_1(T - t)], \; t < T \qquad [2]$$

where t = time since conception (days), μ = specific rate of cell proliferation (per day), μ_T = the value of μ at parturition, and k_1 = decay parameter (per day). The kinetic assumptions underlying the model after parturition are that the specific rate of cell proliferation declines exponentially with time and that the specific rate of cell death, λ (per day), is constant. The rate:state equation for the lactation phase is

$$dN/dt = \mu N - \lambda N, \; t \geq T \qquad [3]$$

with

$$\mu = \mu_T \exp[-k_2(t - T)], \; t \geq T \qquad [4]$$

where the constant k_2 (per day) = decay parameter. Substitution for μ in Equations [1] and [3] using Equations [2] and [4], respectively, and then integrating yields the following:

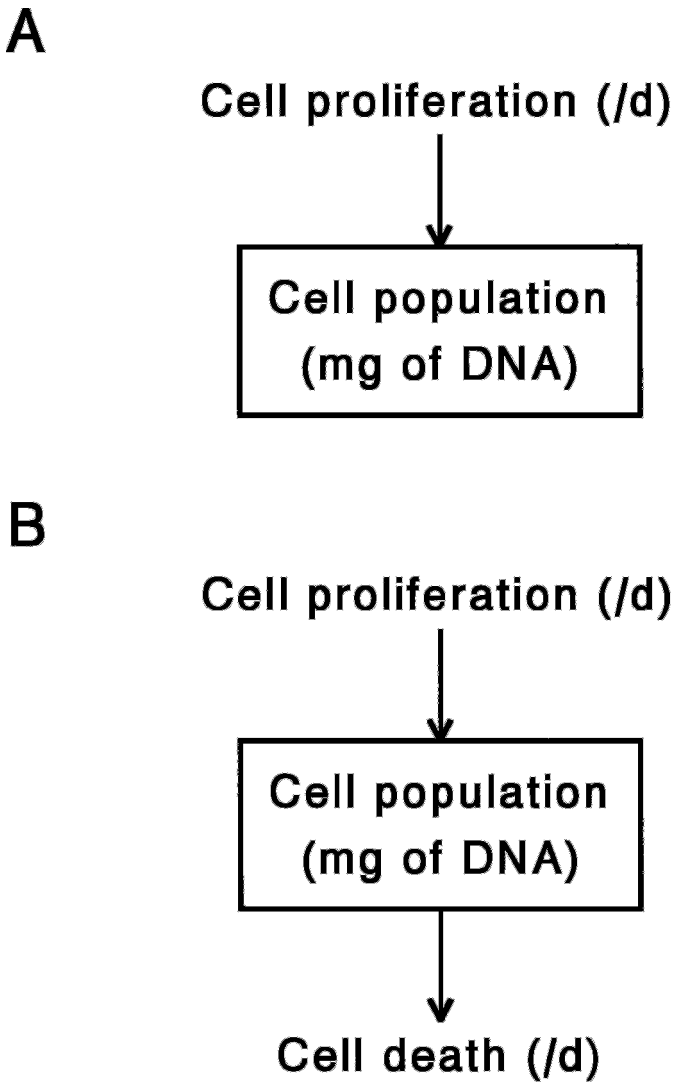

Figure 1. Schematic representation of the model of mammary gland growth patterns in pregnancy (A) and lactation (B).

$$N = N_T \exp\{-\mu_T[1 - \exp(-k_1(T - t))]/k_1\},\ t < T \quad [5a]$$

and

$$N = N_T \exp\{\mu_T[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\},\ t \geq T \quad [5b]$$

where N_T (milligrams) = cell population at parturition. Equations [5a] and [5b] provide a simple model, and the parameters of the model, N_T, μ_T, k_1, k_2, and λ, support physiological interpretation to fit experimental data on growth patterns of the mammary gland during pregnancy and lactation.

Because the specific rate of cell proliferation varies over time, the value of the mean rate of cell proliferation over an interval of pregnancy and lactation (t_1 to t_2) is of interest. This mean is given by

$$\bar{\mu}_{t_2 - t_1} = t_1 \int^{t2} \mu dt/(t_2 - t_1). \quad [6]$$

Substitution for μ in Equation [6] using Equations [2] and [4] and then integrating yields the mean specific rate of cell proliferation (per day) in pregnancy and in lactation:

$$\bar{\mu}_{t_2 - t_1} = \mu_T\{\exp[-k_1(T - t_2)] - \exp[-k_1(T - t_1)]\}/[k_1(t_2 - t_1)],\ t_2 < T; \quad [7a]$$

$$\bar{\mu}_{t_2 - t_1} = \mu_T\{[1 - \exp(-k_1(T - t_1))]/k_1 + [1 - \exp(-k_2(t_2 - T))]/k_2\}/(t_2 - t_1),\ t_1 < T \text{ and } t_2 \geq T; \quad [7b]$$

and

$$\bar{\mu}_{t_2 - t_1} = \mu_T\{\exp[-k_2(t_1 - T)] - \exp[-k_2(t_2 - T)]\}/[k_2(t_2 - t_1)],\ t_1 \geq T. \quad [7c]$$

The time to peak cell population, t_p (days), is

$$t_p = T,\ [\ln(\mu_T/\lambda)]/k_2 \leq 0 \quad [8a]$$

and

$$t_p = [\ln(\mu_T/\lambda)]/k_2 + T,\ [\ln(\mu_T/\lambda)]/k_2 > 0. \quad [8b]$$

The cell populatin at parturition (N_T) can be calculated relative to peak cell proliferation (N_p) as

$$N_T/N_p = 1/\exp\{\mu_T[1 - \exp(-k_2(t_p - T))]/k_2 - \lambda(t_p - T)\}. \quad [9]$$

The model is equally applicable to the description of mammary growth patterns other than those of the cell population. All that is necessary is renomination of the pool and fluxes. To describe the pattern of growth of DFFT, for example, N now denotes the quantity of DFFT, μ denotes its fractional synthesis rate, and λ represents its fractional degradation rate. Application of the model to changes in the cell population of the mammary gland during pregnancy and lactation as well as to other indices of mammary gland growth are provided subsequently.

The Model as a Lactation Curve

To utilize the model (Figure 1) in the study of the entire lactation curve, some minor redefinition is again needed. The symbol N now denotes the number of secretory cells, t denotes the time since parturition (hence, T = 0 d), and μ and λ represent the specific rates of secretory cell proliferation and death, respectively. Let Y (constant) be defined as mean milk production per secretory cell per day; then, daily milk production by the mammary gland, M (kilograms of milk per day), is given by

$$M = YN. \quad [10]$$

Substitution for N in Equation [10] using Equation [5b] yields

$$M = M_0 \exp\{\mu_T[1 - \exp(-k_2 t)]/k_2 - \lambda t\} \quad [11]$$

where M_0 (or YN_T) is the theoretical initial milk production (kilograms of milk per day) at parturition (t = 0 d). Note that Y and N_T cannot both be defined uniquely from lactation data. Equation [11] can be fitted to standard data for milk production, which permits representation of the lactation curve as the combined result of a cell growth and death process. Application of this new model to describe the lactation curve of dairy cows is presented subsequently.

Formulas for commonly used summary statistics on lactational performance by dairy cows are as follows. Time to peak production, t_p (days), and peak production, M_p (kilograms per day), are given by

$$t_p = [\ln(\mu_T/\lambda)]/k_2 \quad [12]$$

and

$$M_p = M_0\ (\lambda/\mu_T)^{\lambda/k_2} \exp[(\mu_T - \lambda)/k_2]. \quad [13]$$

Total milk production over the lactation, M_{tot} (kilograms), is

$$M_{tot} = M_0 \int_0^{t_f} \exp\{\mu_T[1 - \exp(-k_2 t)]/k_2 - \lambda t\}dt \quad [14]$$

where t_f (days) = length of lactation. This integral is nonanalytical, but many software packages are available with procedures that easily yield numerical solutions.

The relative rate of decline midway between peak lactation and the end of lactation, R (per day), is

$$R = \mu_T \exp[-k_2(t_p + t_f)/2] - \lambda. \quad [15]$$

RESULTS

Mammary Growth

To apply the new model, data on the total DNA contents of the mammary gland during pregnancy and lactation were used. At least three observations on different days in pregnancy and three observations on different days in lactation were considered to be the minimum number of data points necessary to fit the model. Data on mammary DNA of mice (8, 20, 26), rats (7, 26, 46, 47), golden hamsters (40), guinea pigs (5, 28), and goats (3) were used. The number of observations per data file ranged from 7 to 33. Each data file was fitted by Equations [5a] and [5b] using NONLIN (39) (Figures 2 and 3). The STEP function of NONLIN was used to represent the pregnancy and lactation phases. Estimates of the parameters and relevant statistics are presented in Table 1.

Mammary gland development, as characterized by DNA content, was generally well described by the model. The variation accounted for by the model fluctuated between 84 and 99%. Obviously, the cell population of the mammary gland at parturition increases as the mature weight of the species increases, and the reversed tendency is clear for the specific growth rate at parturition. Mammary gland development during pregnancy varied between 55.8 and 100% of total mammary gland development, indicating that, for most of these animal species, cell proliferation in the mammary gland continued into lactation. The decay parameter, k_1, was positive in all cases, indicating an increased rate of cell growth in the mammary gland as pregnancy proceeded. The decay parameter for growth in lactation, k_2, ranged from 0.002 to 0.533/d; the highest values were observed for guinea pigs, and

TABLE 1. Estimated parameter values, preparturient cell proliferation relative to maximum cell proliferation (N_T/N_p), residual sums of squares (RSS), R^2, and the Durbin-Watson statistic (DW) obtained by fitting Equations [5a] and [5b][1] to DNA contents (milligrams) in the mammary gland of various animal species.

Data file and reference	Observations	N_T		μ_T		k_1		k_2		λ		N_T/N_p	RSS	R^2	DW
	(no.)	(mg)		(/d)								(%)		(%)	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD				
Mice (8)	8	8.7	0.4	0.108	0.029	0.034	0.033	0.073	0.076	0.056	0.048	81.5	0.64	99.1	3.45
Mice (26)	14	2.1	0.4	0.163	0.088	0.115	0.107	0.077	0.183	0.065	0.162	61.1	1.0	94.5	2.44
Mice (20)	7	4.5	0.8	0.334	0.092	0.550	0.380	0.118	0.170	0.144	0.174	55.8	1.2	96.1	2.66
Rats (26)	13	6.9	0.7	0.137	0.058	0.094	0.075	0.078	0.139	0.058	0.105	69.1	2.5	97.9	2.36
Rats (46, 47)	16	19.4	0.8	0.088	0.014	0.016	0.024	0.082	0.086	0.031	0.033	74.2	16.8	98.3	2.00
Rats (7)	12	13.2	0.6	0.334	0.049	0.238	0.063	0.401	0.090	0.036	0.008	58.2	6.0	98.9	2.80
Hamsters (40)	8	21.9	1.1	0.149	0.045	0.025	0.058	0.002	0.020	0.165	0.055	100.0	4.8	98.7	3.55
Guinea pigs (28)	33	32.5	3.0	0.389	0.110	0.299	0.151	0.533	0.197	0.041	0.010	70.2	1.8×10^3	84.1	1.98
Guinea pigs (5)	10	56.6	3.2	0.105	0.031	0.044	0.024	0.467	0.456	0.021	0.012	89.9	1.2×10^2	97.8	2.62
Goats (3)	9	3312	161	0.030	0.013	0.009	0.012	0.141	0.431	0.004	0.021	88.3	4.0×10^5	99.2	2.66

[1]Equation [5a]: $N = N_T \exp[-\mu_T[1 - \exp(-k_1(T - t))]/k_1]$, $t < T$; Equation [5b]: $N = N_T \exp\{\mu_T[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milligrams) = cell population of mammary tissue, N_T (milligrams) = cell population at parturition, μ_T (per day) = specific rate of cell proliferation at parturition, k_1 (per day) = decay parameter in pregnancy, k_2 (per day) = decay parameter in lactation, λ (per day) = specific rate of cell death, t (days) = time since conception, and T (days) = the time of parturition.

the lowest values were observed for mice and golden hamsters. The specific death rate, λ, varied between 0.004 and 0.165/d. However, decay parameters and the specific rate of cell death were rather poorly estimated using the variance of the estimates of the parameters. For these parameters, the likelihood that the actual value differed from 0 was significant ($P < 0.10$) only for rats (7), golden hamsters (40), and guinea pigs (28). From the plots of the residuals (Figures 3), no obvious problems regarding fit or systematic deviations were apparent. The Durbin-Watson statistic (**DW**), the ratio of the sum of squares of residual first-order differences to the residual sum of squares, was used to test the presence of first-order serial correlation. In most cases, DW was >2 (Table 1), which indicated the possible presence of negative first-order correlations; the difference (4 − DW) had to be used as the test statistic. The DW was not significant ($P > 0.10$) for any of the cases, indicating that the residuals were distributed satisfactorily around the abscissa.

The generally poor estimation of the decay parameters and the specific rate of cell death might have been the result of the large variation between the individual animals, the low number of observations, or the inadequate distribution of data points within the pregnancy and lactation phases. A small simulation study was performed to examine the effects of variation between animals and the effects of the distribution of observations. Ten replicate sets of 12 observations of total DNA in the mammary gland, distributed as in the experiment of Baldwin and Milligan (7), were simulated using the model (Equations [5a] and [5b]) and the estimated parameter values of Baldwin and Milligan (7). The simulated observations were multiplied by (1 + 0.1e) and (1 + 0.3e), where e is a pseudorandom deviate from the normal distribution, N(0, 1). This calculation reflects a coefficient of variation of 10 and 30%, which is typical for the data on mammary gland DNA of several species. Equations [5a] and [5b] were then fitted to the simulated data using NONLIN as described previously. The number of data files (of the 10 files simulated) in which a parameter was significant at $P < 0.05$ or at $P < 0.10$ is presented in Table 2. Applying the lower coefficient of variation (10%), cell population at parturition and the specific rate of cell proliferation at parturition were significant ($P < 0.05$) in all cases, and the decay parameters and the specific rate of cell death reached significance in more than one-half of the replicate data files. However, increasing the coefficient of variation to 30% resulted in a marked increase of asymptotic standard errors of the parameter estimates, and the decay parameters and the specific rate of cell death were not significant ($P > 0.10$) in any of the simulated data files. Given the low number of observations and the high variation among animals from the data files of Table 1, these simulations help to explain the poor estimation of the parameters and indicate the need to reduce variation among animals if reliable estimates are to be secured.

TABLE 2. Number of simulated data files (out of 10 possible) in which a parameter of the model (Equations [5a] and [5b][1]) was different ($P < 0.05$ or $P < 0.10$) from 0.[2]

	CV			
	10%		30%	
Parameter	$P < 0.05$	$P < 0.10$	$P < 0.05$	$P < 0.10$
N_T	10	10	10	10
μ_T	9	10	1	3
k_1	5	7	0	0
k_2	6	6	0	0
λ	6	7	0	0

[1]Equation [5a]: $N = N_T \exp\{-\mu_T[1 - \exp(-k_1(T - t))]/k_1\}$, $t < T$; Equation [5b]: $N = N_T \exp\{\mu_T[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milligrams) = cell population of mammary tissue, N_T (milligrams) = cell population at parturition, μ_T (per day) = specific rate of cell proliferation at parturition, k_1 (per day) = decay parameter in pregnancy, k_2 (per day) = decay parameter in lactation, λ (per day) = specific rate of cell death, t (days) = time since conception, and T (days) = the time of parturition.

[2]Twelve observations per data file were simulated using the model and parameter values N_T = 13.2 mg, μ_T = 0.33/d, k_1 = 0.24/d, k_2 = 0.40/d, and λ = 0.04/d and applying a CV of 10 or 30%.

The effects of distribution of observations on the reliability of estimated parameters was also examined in a simulation study. Again, 10 replicate data files were simulated as described previously, applying a coefficient of variation of 20%; each file contained 22 observations that were distributed as follows: A) observations at equal time intervals (beginning on the day of conception and continuing, every 2 d until d 42), B) emphasis on observations in the pregnancy period (beginning on the day of conception and continuing every 1.5 d until d 18, then from d 18 every 2.5 d until d 40.5), C) emphasis on observations in the lactation period (beginning on the day of conception and continuing every 2.5 d until d 22.5, then from d 22.5 every 1.5 d until d 40.5), and D) emphasis on observations shortly before and shortly after parturition when large changes in cell numbers occurred (beginning on the day of conception, then from d 4 every 3 d until d 16, from d 16 every 1 d until d 21, from d 21 every 0.5 d until d 23, from d 23 every 1 d until d 25, from d 25 every 3 d until d 37, and on d 41). Results are presented in Table 3. Compared with observations at regular time intervals, frequent observations during the pregnancy period improved the number of data files in which the decay parameters and the specific rate of cell death reached significance

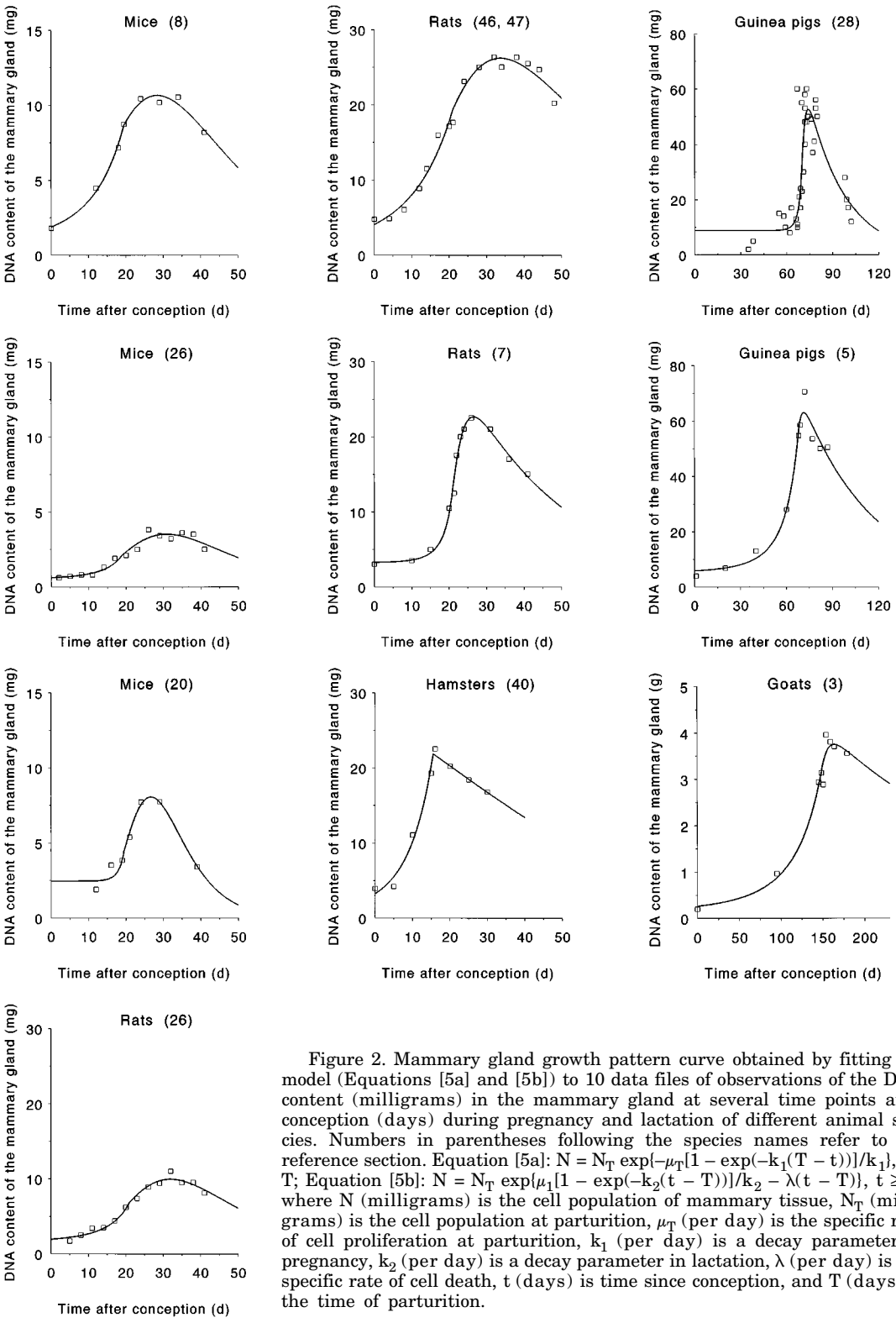

Figure 2. Mammary gland growth pattern curve obtained by fitting the model (Equations [5a] and [5b]) to 10 data files of observations of the DNA content (milligrams) in the mammary gland at several time points after conception (days) during pregnancy and lactation of different animal species. Numbers in parentheses following the species names refer to the reference section. Equation [5a]: $N = N_T \exp\{-\mu_T[1 - \exp(-k_1(T - t))]/k_1\}$, $t < T$; Equation [5b]: $N = N_T \exp\{\mu_1[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milligrams) is the cell population of mammary tissue, N_T (milligrams) is the cell population at parturition, μ_T (per day) is the specific rate of cell proliferation at parturition, k_1 (per day) is a decay parameter in pregnancy, k_2 (per day) is a decay parameter in lactation, λ (per day) is the specific rate of cell death, t (days) is time since conception, and T (days) is the time of parturition.

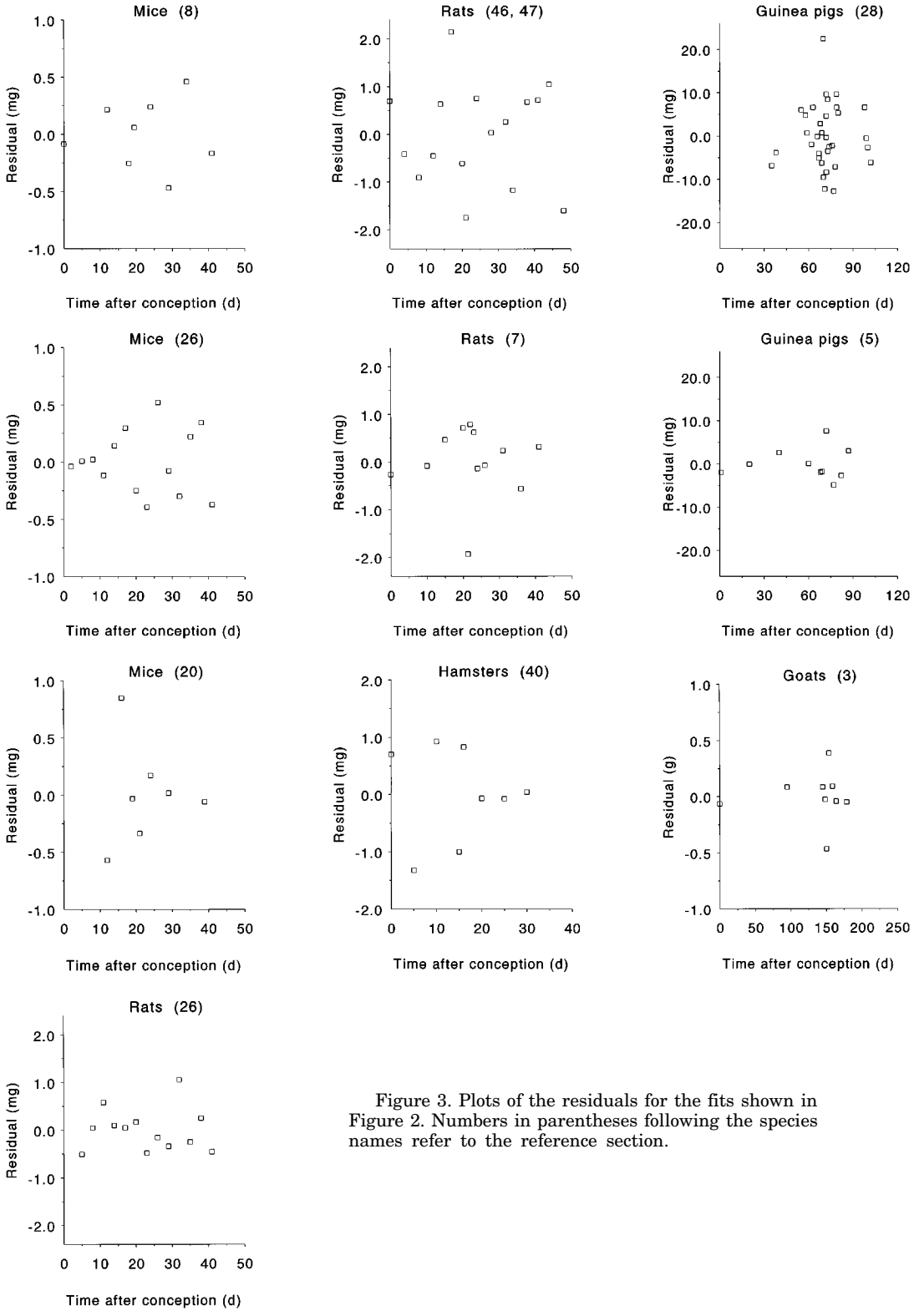

Figure 3. Plots of the residuals for the fits shown in Figure 2. Numbers in parentheses following the species names refer to the reference section.

TABLE 3. Number of simulated data files (out of 10 possible) in which a parameter of the model (Equations [5a] and [5b][1]) was different ($P < 0.05$ or $P < 0.10$) from 0.[2]

	Distribution of observations							
	A		B		C		D	
Parameter	$P < 0.05$	$P < 0.10$	$P < 0.05$	$P < 0.10$	$P < 0.05$	$P < 0.10$	$P < 0.05$	$P < 0.10$
N_T	10	10	10	10	9	9	10	10
μ_T	8	10	7	8	5	7	10	10
k_1	1	4	4	6	0	0	3	8
k_2	2	2	3	5	0	2	2	7
λ	3	5	4	6	4	5	1	3

[1]Equation [5a]: $N = N_T \exp\{-\mu_T[1 - \exp(-k_1(T - t))]/k_1\}$, $t < T$; Equation [5b]: $N = N_T \exp\{\mu_T[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milligrams) = cell population of mammary tissue, N_T (milligrams) = cell population at parturition, μ_T (per day) = specific rate of cell proliferation at parturition, k_1 (per day) = decay parameter in pregnancy, k_2 (per day) = decay parameter in lactation, λ (per day) = specific rate of cell death, t (days) = time since conception, and T (days) = the time of parturition.

[2]Twenty-two observations per data file were simulated using the model and parameter values N_T = 13.2 mg, μ_T = 0.33/d, k_1 = 0.24/d, k_2 = 0.40/d, and λ = 0.04/d. A CV of 20% was applied, and observations were distributed at equal time intervals (A), with an increased number of observations during pregnancy (B), with an increased number of observations during lactation (C), and with an increased number of observations shortly before and after parturition (D).

but reduced the number of data files in which the specific rate of cell growth at parturition reached significance. Frequent observations during the lactation period yielded poorer estimates of the specific rate of cell proliferation at parturition and of the decay parameters but better estimates of the specific rate of cell death. When the emphasis was on observations around parturition, the decay parameters and the specific rate of cell growth at parturition reached significance in more cases, but the reverse was noted for the specific rate of cell death. Thus, frequent measurements in a particular period allowed the shape of part of the curve to be described with satisfactory accuracy.

To illustrate the use of the model to describe patterns of mammary growth other than those of the cell population, data on the development of the parenchymal volume (milliliters) in goats were used (13). The volume of parenchyma in goats was measured using magnetic resonance imaging, and 12 observations (7 in pregnancy and 5 in lactation) were available (Figure 4). The model accurately described the development of parenchymal volume in pregnant and first lactation goats (R^2 = 99%; DW = 2.80). All parameters were significant ($P < 0.05$), except for the decay parameter for pregnancy, which was the only case in which a slightly negative estimate of the decay parameter was obtained. It is interesting to compare the model rate parameters of parenchymal volume with those of cell population in goats. The latter was estimated from data presented by Anderson et al. (3). The growth rate at parturition was slightly lower for parenchymal volume (0.022 vs. 0.030/d), which indicated that the parenchymal volume per mammary gland cell became smaller. The growth decay parameter in lactation was also lower (0.048 vs. 0.141/d), but the specific death rate hardly differed (0.005 vs. 0.004/d), suggesting an increase in parenchymal volume per cell during lactation. Relative to maximum cell proliferation, the estimated cell proliferation prior to parturition was higher (88.3%) than the corresponding value for parenchymal volume (80.8%). However, because of the poor estimation of parameters that describe cell population of mammary glands of goats, these comparisons were not significant ($P > 0.10$).

Development of the cell population in the mammary gland can be affected by a variety of factors, including hormonal treatments, milking frequency, and nutrition. Tucker (44) examined the effect of suckling intensity on the development of the mammary gland of rats. From the first day of lactation, the total DNA content of mammary gland was measured every 3 d up to d 24, and the suckling intensity was varied by allowing two, four, or six pups to suckle per six glands. Equation [5b] was fitted to these observations, using t as time after parturition (days) and allowing k_2 and λ to differ between suckling intensities. The N_T and μ_T variables were kept at the same estimated value for all three suckling intensities. This balance was achieved by using the STEP function of NONLIN (39). Thus, a total of eight parameters (N_T, μ_T, and three sets of k_2 and λ) were fitted to 21 observations (Figure 5; Table 4). The fit of the curve to each of the treatments was generally satisfactory, except for the suckling intensity at two pups per six glands. At that intensity, the aberration at d 8 caused relatively large residuals. The DW was not significant for any of the three suckling intensities. The growth decay parameter increased as the number of pups per

TABLE 4. Estimated parameter values, residual sum of squares (RSS), R^2, and the Durbin-Watson statistic (DW) obtained by fitting Equation [5b][1] to observed mammary gland DNA contents (milligrams) of rats suckling two, four, or six pups per six glands [data from Tucker (44)].

Treatment	N_T		μ_T		k_2		λ		RSS	R^2	DW
(pups per six glands)	(mg)		(/d)							(%)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD			
2	16.7	0.5	0.318	0.221	0.011	0.009	0.278	0.225	8.6	65.7	3.08
4	16.7	0.5	0.318	0.221	0.025	0.024	0.233	0.223	2.1	97.8	2.11
6	16.7	0.5	0.318	0.221	0.035	0.034	0.207	0.235	2.3	98.9	2.97

[1]$N = N_T \exp\{\mu_T[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milligrams) = cell population of mammary tissue, N_T (milligrams) = cell population at parturition, μ_T (per day) = specific rate of cell proliferation at parturition, k_2 (per day) = decay parameter in lactation, λ (per day) = specific rate of cell death, t (days) = time since parturition, and T (days) = the time of parturition.

six glands was increased (from 0.011/d with two pups to 0.035/d with six pups per six glands). The parameter for specific death rate showed the reverse trend (0.278, 0.233, and 0.207/d with two, four, and six pups per six glands, respectively). These results suggested that more intense suckling in rats decreased the specific growth rate of mammary cells but increased the survival of the cells during lactation. However, the standard errors were relatively large, and parameter values at various suckling intensities did not significantly deviate from one another.

Milk Production

The entire lactation curve, which is based on the growth and death of mammary gland cells, Equation [11], was compared with the gamma model proposed by Wood (50), which is the most widely used model of the entire lactation curve. In the gamma model,

$$M = At^b \exp(-ct) \qquad [16]$$

where M is milk production (kilograms of milk per day) at time t of lactation (weeks), and A, b, and c are parameters that determine the shape and scale of the curve. Data for the entire lactation (mean daily milk production for each week of lactation) of 23 animals fed a variety of diets (36) were used to fit both models. Length of individual lactation periods ranged from 30 to 51 wk; peak milk production ranged from 14.1 to 36.8 kg/d. Residual mean squares within each lactation, R^2, and DW for both models were compared (Table 5). The gamma model yielded a convergence in all cases, but the new model failed to converge on one occasion. In general, the residual mean squares were lower for the new model (17 of 23 animals). Residuals obtained with the model of Wood (50) generally were positively autocorrelated (DW varied between 0.31 and 2.13), but this autocorrelation was less pronounced using the new equation (DW between 0.59 and 2.50), indicating that the new equation more successfully described the underlying trend. For example, good fit and bad fit of the new model were compared with the gamma model (Figure 6). The fit of the gamma model was less satisfactory when the lactation curve was characterized by a sharp production peak (Figure 6a). Conversely, the new model was less suitable for fitting whole lactation data when the approach to peak production and the subsequent decline were smoother (Figure 6b). With smooth patterns, the likely reason for the inability of the model to yield a better fit than the gamma

TABLE 5. Mean, minimum (min), and maximum (max) residual mean squares (RMS), R^2, and the Durbin-Watson statistic (DW) of the gamma model (Equation [16][1]) and the new model (Equation [11][2]) fitted to whole lactation data of 23 dairy cows [data from Rook et al. (36)].

	Gamma equation	New equation
RMS		
Mean	2.95	2.00
Min	0.64	0.34
Max	13.45	4.06
R^2		
Mean	90.2	93.6
Min	72.7	86.4
Max	97.3	97.7
DW		
Mean	1.11	1.40
Min	0.31	0.59
Max	2.13	2.50

[1]$M = At^b \exp(-ct)$, where M (kilograms of milk per day) = milk production, t (days) = time of lactation, and A, b, and c = parameters that determine the shape and scale of the curve.

[2]$M = M_0 \exp\{\mu_T[1 - \exp(-k_2 t)]/k_2 - \lambda t\}$, where M (kilograms of milk per day) = milk production, t (days) = time of lactation, M_0 (kilograms of milk per day) = theoretical initial milk production, μ_T (per day) = specific rate of secretory cell proliferation at parturition, k_2 (per day) = decay parameter, and λ (per day) = specific rate of secretory cell death.

model was that the number of cells and the enzymatic activity per cell cannot both be defined uniquely from lactation data as is discussed subsequently. The initial milk production estimated using the new model ranged from 0.1 to 32.1 kg/d. The estimated growth rate showed large variation between individual cows (0.02 to 4.76/d). The highest values were obviously nonphysiological and occurred particularly when a low number of observations prior to the peak were available, causing parameters to be highly correlated. In case of unique estimation of parameters, the specific rate of cell growth at parturition had reasonable values (mean, 0.08/d). For most animals (17 of

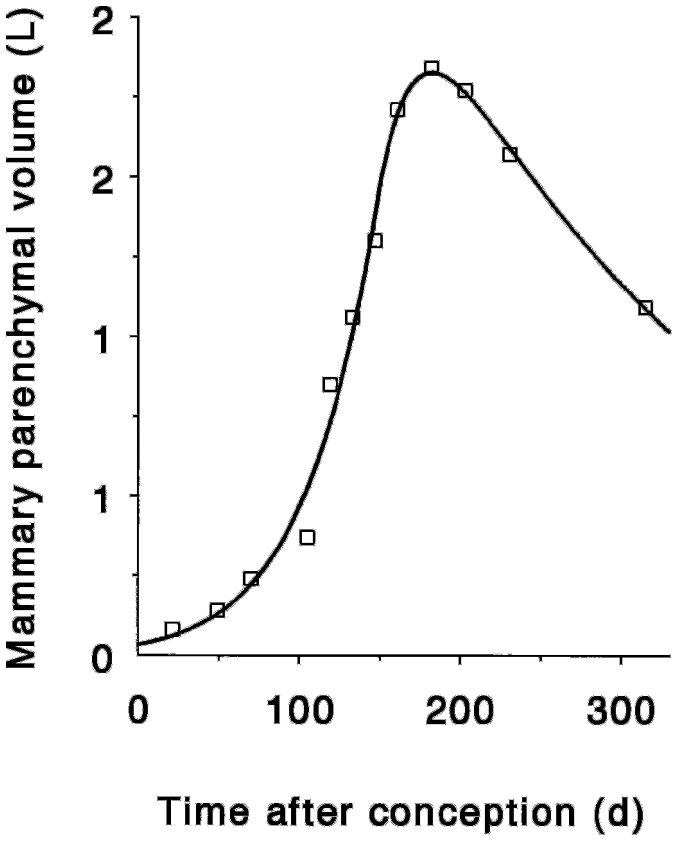

Figure 4. Mammary gland growth pattern curve obtained by fitting the model (Equations [5a] and [5b]) to mammary parenchymal volume (milliliters) at several time points after conception (days) during pregnancy and first lactation of goats [data from Fowler et al. (13)]. Parameter estimates (±asymptotic SD) were N_T = 1475 (±51) ml, μ_T = 0.022 (±0.003)/d, k_1 = –0.002 (±0.005)/d; k_2 = 0.048 (±0.017)/d, and λ = 0.005 (±0.001)/d. Residual sum of squares = 4.9×10^4, R^2 = 99.0%, and Durbin-Watson statistic = 2.80. Equation [5a]: $N = N_T \exp\{-\mu_T[1 - \exp(-k_1(T - t))]/k_1\}$, $t < T$; Equation [5b]: $N = N_T \exp\{\mu_1[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milliliters) is the parenchymal volume, N_T (milliliters) is the parenchymal volume at parturition, μ_T (per day) is a decay parameter in pregnancy, k_2 (per day) is a decay parameter in lactation, λ (per day) is the specific rate of cell death, t (days) is the time since conception, and T (days) is the time of parturition.

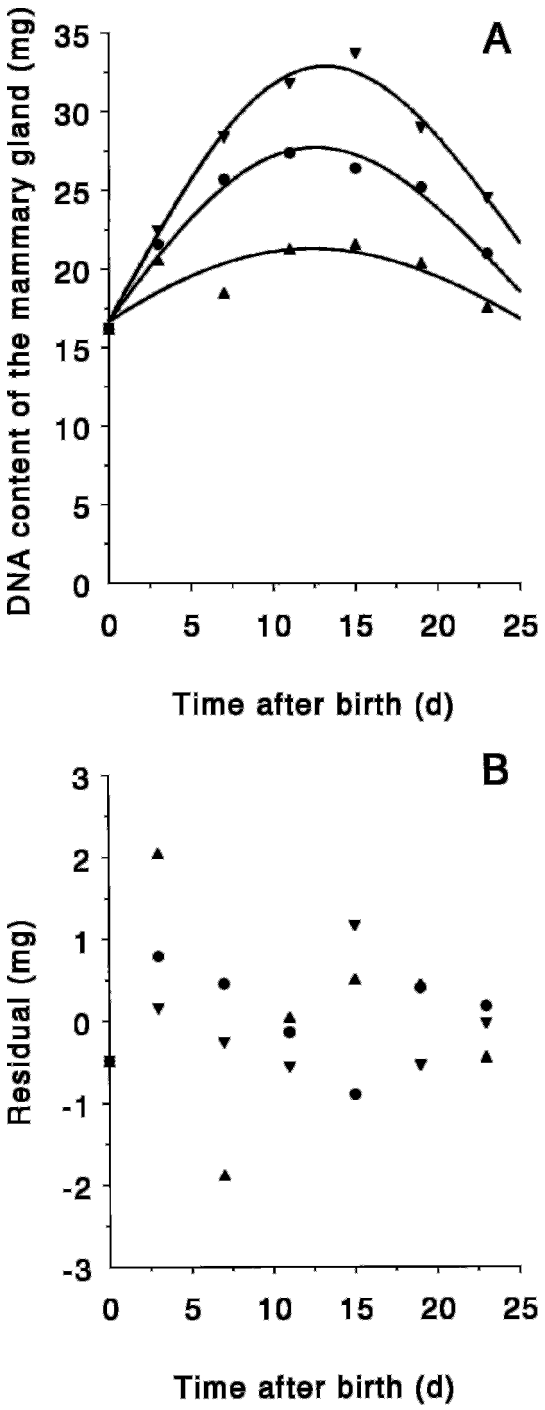

Figure 5. A) Mammary gland growth pattern obtained by fitting the model (Equation [5b]) to DNA contents of the mammary gland (milligrams) of rats suckling two (▲), four (●), or six (▼) pups per six glands [data from Tucker (44)]. Equation [5b]: $N = N_T \exp\{\mu_T[1 - \exp(-k_2(t - T))]/k_2 - \lambda(t - T)\}$, $t \geq T$, where N (milligrams) is the cell population of mammary tissue, N_T (milligrams) is the cell population at parturition, μ_T (per day) is the specific rate of cell proliferation at parturition, k_2 (per day) is a decay parameter in lactation, λ (per day) is the specific rate of cell death, t (days) is time since parturition, and T (days) is the time of parturition. B) Plots of the residuals for the fits shown in Figure 5A.

23), the decay parameter and the specific rate of cell death were significant ($P < 0.01$). In these animals, the decay parameter varied between 0.01 and 0.16/d. High values of the decay parameter coincided with rapidly occurring, sharp peaks in milk production. The specific rate of cell death ranged from 0.002 to 0.008/d. From Equation [15], it is obvious that higher values of the specific rate of cell death generally yield more rapid rates of relative decline at the point midway between the peak and end of lactation (R = –0.002 to –0.007/d). The lactation curves for which the decay parameter and the specific rate of cell death did not reach significance were characterized by a relatively low number of observations prior to the peak, causing high correlation between the parameters and unreliable estimates of the parameters.

DISCUSSION

Mammary Growth

The model presented in this paper is based on knowledge of the growth and death processes of mammary cells, yielding a single equation to describe mammary growth patterns throughout pregnancy and lactation. The parameters of the model support direct physiological interpretation. The mammary gland is the target of several growth factors including mitogens, such as IGF-I and epidermal growth factor (31, 38), as well as polypeptide growth inhibitors, such as MDGI-1 (33). One of the assumptions of this model is that the mammary cells are subject to death during lactation but not during pregnancy. Epithelial cells that differentiate to perform secretory functions have a limited lifespan (20). Plasmin has been implicated in the degradation of the secretory machinery of the cell and the subsequent death of cells in rodents (29) and dairy cows (34). In bovine milk, protease inhibitors that were derived from plasma decreased dramatically during the first 3 d postpartum, and it was suggested that such changes in protease inhibitors were related to mammary gland development and regression (9). Exponential growth of mammary cells during pregnancy has been reported in many species (3, 5, 7, 8, 20, 26, 28, 40, 46). In our analysis of the DNA contents of the mammary gland, the decay parameter k_1 was always positive (Table 1), indicating accelerated growth in pregnancy (Equation [2]). In contrast, the growth rate rapidly declined during lactation (Equation [4]), as was indicated by the positive values of decay parameter k_2 (Table 1). Rapid differentiation of mammary cells occurs around parturition, during the initial stage of lactation, and up to peak lactation. Thereafter, the differentiated state of the tissue is maintained throughout lactation (7, 19, 21, 49). A role for MDGI-1 as a differentiation

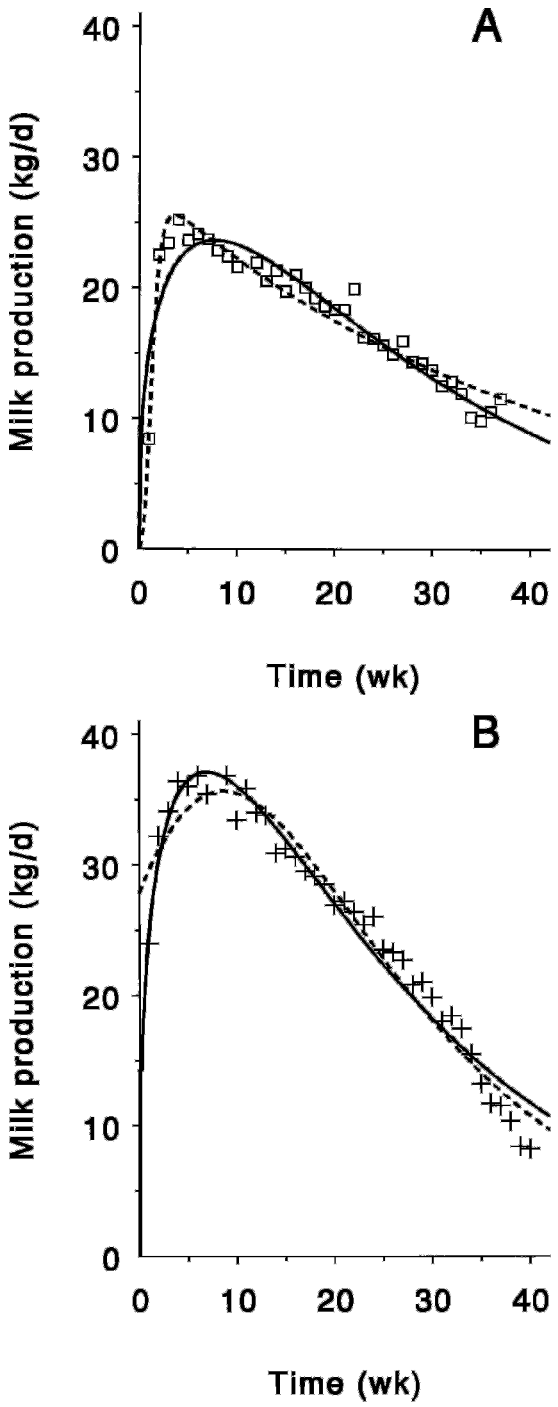

Figure 6. Fitted lactation curves for A) animal 2 and B) animal 6 using the gamma model (Equation [16]) (———) and the new model (Equation [11]) (– – – – –). Equation [16]: $At^b \exp(-ct)$, where M (kilograms of milk per day) is milk production, t (days) is time of lactation, and A, b, and c are parameters that determine the shape and scale of the curve. Equation [11]: $M = M_0 \exp\{\mu_T[1 - \exp(-k_2 t)]/k_2 - \lambda t\}$, where M (kilograms of milk per day) is the theoretical initial milk production, μ_T (per day) is the specific rate of secretory cell proliferation at parturition, k_2 (per day) is a decay parameter, and λ (per day) is the specific rate of secretory cell death.

factor, rather than as an antiproliferative factor, has been suggested (32). Although actively secreting cells are apparently able to divide (14), the rate of division is far less than that for undifferentiated cells (22). Therefore, the decline of the specific growth rate during lactation in the present analysis was likely related to the increased ratio of differentiated to undifferentiated cells and the lower division rate of differentiated cells.

The differences in patterns of mammary growth (Figure 1) and values for the specific rates of cell growth and death (Table 1) apparently reflect the differences between the lactational physiology of rodents and ruminants. The role of hormones in the initiation and subsequent maintenance of lactation is different between species (18). Prolactin is necessary for both initiation and subsequent maintenance of lactation in rodents, but, in ruminants, prolactin is likely not required once lactation is established (12). Preparturient treatment of cows with a prolactin blocker caused a reduction in milk production and a reduction in the ratio of RNA to DNA, but the total amount of mammary DNA remained constant (1). Reduced milk production was associated with reduced secretory activity per cell rather than with a reduction in secretory cells. Prolactin receptors in the mammary tissue of rabbits, rats, and mice are low during pregnancy but increase dramatically with the onset of milk secretion. Thus, hormonal control involves not only circulating hormones but also receptor numbers and the mechanisms that control these receptors in mammary tissue. The morphology of mammary in rodents is also quite different from that in ruminants. Mammary tissue in rodents has adipocytes in close proximity to epithelial cells. The developed rumen generally prevents large quantities of unsaturated fatty acids from reaching the mammary gland. However, mammary adipocytes of rodents have been shown to store large quantities of unsaturated fatty acids (18). To supply unsaturated fatty acids that would not be hydrogenated in the rumen, lambs were fed diets that contained unsaturated fats that had been ruminally protected; these diets stimulated growth of mammary parenchyma and increased prolactin receptors in parenchyma (23, 24). In the current study, the specific rate of cell death was always lower for goats than for rodents (0.004 and 0.005/d, respectively; Table 1 and Figure 4), which is consistent with the length of lactation and with differences in the involution process of mammary tissue in bovines and rodents. Rapid sloughing of epithelial cells during involution was observed in murine mammary tissue but not in bovine mammary tissue (17). Although bovine epithelial cells appeared markedly different during involution than during lactation, they were nonetheless functioning, and the involuted bovine gland had the potential to develop ductal and alveolar structures quickly. Overall, it appears that complete extrapolation of data from rodents to explain mammary development in ruminants is inappropriate.

The stimulatory and inhibitory effects of mitogens, hormones, and growth inhibitors on the proliferation and death of mammary cells have largely been determined in vitro. Attempts to extend these observations to the in vivo state could be evaluated using the present model if estimates of udder mass and DNA content were obtained. However, the proper number of observations and precise timing would be required for the model to define uniquely the parameters of interest. The results from the simulation study indicated that, because of the typically high variation among animals, relatively large numbers of observations are necessary (Table 2). The inadequate number of observations in the data files on which the model was fitted resulted in an unsatisfactory degree of uncertainty of the rate parameters. The proper timing of observations helps to define uniquely the model parameters as well (Table 3). The need for proper timing was very clear in the study of Baldwin and Milligan (7). In that study, frequent measurements were made shortly after parturition, and a relatively large number of observations prior to peak lactation were available. Thus, the utility of the model is limited when used on inadequate data.

A disadvantage of the use of DNA as an index of mammary gland development is that it gives a measure of all cells in the gland, i.e., stroma and parenchyma, although the parenchyma (secretory cells) are often those of interest. In goats, parenchymal growth rates increased much faster than stromal growth rates during pregnancy, and the rate of involution of parenchyma in lactation was higher than that of stroma (6). Morphological analysis of bovine mammary sections collected from udders during lactation and the nonsuckling periods indicated that secretory cells declined in number following cessation of milking (2). Therefore, growth associated with increases in tissue mass during late gestation and early lactation are most likely confined to secretory cells. One might assume that the specific rate of cell proliferation would represent secretory cell growth and that the specific rate of cell death would represent secretory cell death.

Milk Production

Changes in secretory cell numbers should be associated with changes in secretory potential.

However, as stated previously, the enzymatic activity per secretory cell varied, not reaching a maximum until several days or weeks after parturition, after which the activity remained constant. In contrast, cell proliferation was >50% completed at parturition, and, after peak lactation, cell numbers declined (22). The combination of enzymatic capacity per cell and cell numbers appeared to explain the changes in milk production throughout lactation. Using the model, the number of cells and the enzymatic or secretory activity per cell cannot both be uniquely defined from lactation data (Equations [10] and [11]). The increase in the activity per cell is reflected in the high, unphysiological values of the specific rate of cell growth (up to 4.76/d). This phenomenon might explain the reduced ability of the model to fit lactation data without a sharp peak in milk production compared with the well-known gamma equation (Equation [16]). Around peak production, the activity per cell might still increase even though the cell number is already in the declining phase (or the reverse). This combination causes little change in milk production but decreases the accuracy of the fit of the model. Although the specific rate of cell growth is significantly affected by changes in activity per cell, the decay parameter and the rate of cell death are less affected. Indeed, the values of these rate parameters in goats (0.141 and 0.004/d, respectively, for observations on total mammary gland DNA and 0.048 and 0.005/d, respectively, for observations on parenchymal volume) are well within the range of corresponding rate parameters estimated from lactation data for dairy cows.

Application of the proposed model to milk production might not necessarily interpret data clearly in relation to secretory cell development because of the complexities of cell metabolism in the udder. For example, bST treatment apparently alters the capacity of the udder to metabolize several amino acids (16). However, Prosser et al. (35) observed an increase in milk production within 6 h of initiating an IGF-I infusion and a return to basal milk production within 6 h of termination of the infusion. Such temporal changes are consistent with changes in secretory cell activity as opposed to changes in cell numbers. Therefore, if such a treatment were initiated after peak lactation, these apparent enzymatic changes could not be discerned from changes in the rate of cell death. Treatments such as more frequent milking (three or four times daily versus twice daily) are known to increase milk production (10). The discovery of a regulatory factor in the whey fraction of milk (48) appears to be involved in this phenomenon. Whether such regulation results in a change in cell death rates or whether it simply acts at the enzymatic level has been discussed, and several explanations have been offered (22).

However, one must not overlook the utility of the model to discriminate changes in the lactational performance that are associated with experimental treatments. The gamma model of Wood (50) has limited utility when applied to individual cows (25, 36), particularly if the changes in milk production around the peak are pronounced (37). Obviously, if fits are biased, the use of any statistical comparison of the parameters is reduced. The model reported here appears to have greater utility when fitted to individual cow data and, thus, may lend itself to use for statistical analyses of lactation curves. Having identified such changes, it is incumbent on the investigator to determine whether the changes are due to changes in cell number or in cell activity. Wood (51) attempted to integrate the rate parameters of the gamma model with the processes of growth and death of mammary gland cells, but practical interpretations of the rate parameters in the gamma model were not provided. Rook et al. (36) evaluated a variety of functions to describe the lactation curve and found the performance of the combination of Mitscherlich and exponential function to be better than the gamma model or other functions that were considered. The superior performance of the Mitscherlich and exponential model and the gamma model over the models in which a sigmoid function was used for the increasing phase of the lactation curve suggested that the growth in number of secretory cells at parturition was already in the decelerating phase. As described previously, this result is consistent with the assumptions in the present model that the decline of the specific rate of cell growth after parturition is likely related to the increased ratio of differentiated to undifferentiated cells and the lower division rate of differentiated cells. Multiphasic equations of the lactation curve can provide a very good fit (15), which is not unusual, given the high number of parameters in the multiphasic analysis. However, a convincing justification for modeling lactation by a multiphasic process has yet to be proposed. In their mechanistic model of the mammary gland, Neal and Thornley (27) assumed that the division rate of undifferentiated cells was subject to hormone concentration, which decreases during lactation. However, the practical use of that model is limited because the inputs that are required are not generally available. Thus, the mechanistic model described in the present study provides a satisfactory description of the lactation curve that includes simple expressions for summary statistics, including time to peak production, maximum

lactation production, and relative rate of decline midway through the declining phase, and that is based on the physiological processes of growth and death of mammary cells.

CONCLUSIONS

The mechanistic model developed to describe the pattern of mammary growth of mammals throughout pregnancy and lactation provides parameters that lend themselves to direct physiological interpretation. The model fitted data on mammary gland DNA and parenchymal volume satisfactorily and can be related to current knowledge on growth factors and inhibitors of the mammary gland, but unique definition of its parameters can be difficult because of the high variation among animals, few observations, and improper timing of observations. As an alternative to the widely used gamma model to describe the entire lactation curve, the model performed well, particularly when a sharp peak in milk production occurred. This model has the advantage of providing, for the first time, a simple, biologically based description of the lactation curve, that can be utilized to discriminate changes in lactational performance that are associated with experimental treatments.

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