

Thyroxine and Triiodothyronine in Milk of Cows, Goats, Sheep, and Guinea Pigs¹ (41957)

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Abstract. Milk was collected for the first 21 days of lactation twice daily from dairy cows and once daily from goats, sheep, and guinea pigs. Thyroxine (T_4) and triiodothyronine (T_3) were extracted from 100 μ l of milk using acidified ethanol. T_4 and T_3 were reconstituted in 100 μ l buffer and measured by radioimmunoassay. Concentrations (ng/ml) of T_4 and T_3 for milk of cows, goats, sheep, and guinea pigs, respectively, were: 0.97 and 0.94, 1.24 and 0.52, 0.99 and 0.79, and 1.41 and 0.53. T_4 concentration for guinea pig milk was significantly higher than for cow and sheep milk, but not for goat milk ($P < 0.05$). T_3 was found in higher concentration in milk of cows and sheep than in milk of goats and guinea pigs ($P < 0.05$). Species differences in conversion of T_4 to T_3 in mammary gland cells are suggested. Summations of T_4 and T_3 concentrations in milk indicated no differences among the four species. Regression analyses of changes in milk production, T_4 and T_3 concentrations, total T_4 and T_3 in milk per day, and ratios of T_4 to T_3 revealed variations in patterns. Concentrations of T_4 or T_3 tended to decrease as lactation progressed over 21 days. Total T_3 tended to increase, and the ratio of T_4 to T_3 tended to decrease. Amounts of T_4 and T_3 available to offspring from milk were calculated to be minor sources (4 to 7%) of total requirements for maintenance of metabolic functions. © 1984 Society for Experimental Biology and Medicine.

The thyroid gland normally secretes an iodinated amino acid derivative, thyroxine (T_4), together with much smaller quantities of other iodinated tyrosine derivatives. Among these is 3,5,3'-triiodothyronine (T_3). In the peripheral tissues T_4 is converted to a variety of metabolic products, including T_3 (1). Thyroxine (T_4) and its active derivatives influence the maturation, growth, metabolism, and function of virtually all tissues. Thyroid hormones are calorigenic and play a vital role in the regulation of general body metabolism in all species. Thyroid hormones are also galactopoietic and may play an important role in the regulation of lactation (2). These hormones affect growth and maturation during early fetal life. The thyroid in many species begins to function during fetal life and may be fully functional at birth (3).

Milk not only provides a vital nutrient

source to the offspring, but may provide a supplemental source of thyroid hormones during the critical neonatal period (4). The importance of thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), in milk is not known. Measurements are now possible in the whole milk of different species by radioimmunoassay procedures. However, there is no available information on the level of thyroid hormones in the milk of farm animals. Thus, the purpose of this investigation is to measure the level of the thyroid hormones in whole milk of different species during different stages of lactation, and to determine if it represents a significant amount of T_4 and T_3 to the offspring.

Materials and Methods. Animals used in this investigation were cows ($N = 6$), goats ($N = 5$), sheep ($N = 6$), and guinea pigs ($N = 7$). Water and feed were provided *ad libitum*. Daily milk production was recorded. Milk samples were collected twice daily from cows and once daily from goats, sheep, and guinea pigs during 21 days of lactation. Each sample was collected in a Whirl-Pak (Nasco, Fort Atkinson, Wisc.) bag and frozen (-15°C)

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until extracted for T₄ and T₃. One hundred microliters of milk was extracted by using 200 μ l of acidified 95% ethanol. The procedure for milk extraction was similar to that reported by Strbak *et al.* (5) and modified by Magdub (6). The measurement of T₄ and T₃ in milk by radioimmunoassay (RIA) was

similar to that described by Chopra (7) and Endocrine Sciences (Tarzana, Calif., 1972, 1973). Thyroxine (T₄) and triiodothyronine (T₃) standards (Endocrine Sciences) were used for the preparation of the standard curve in T₄ and T₃ RIAs. The radiolabeled antigens [I^{125}]T₄ and [I^{125}]T₃ with specific activity of

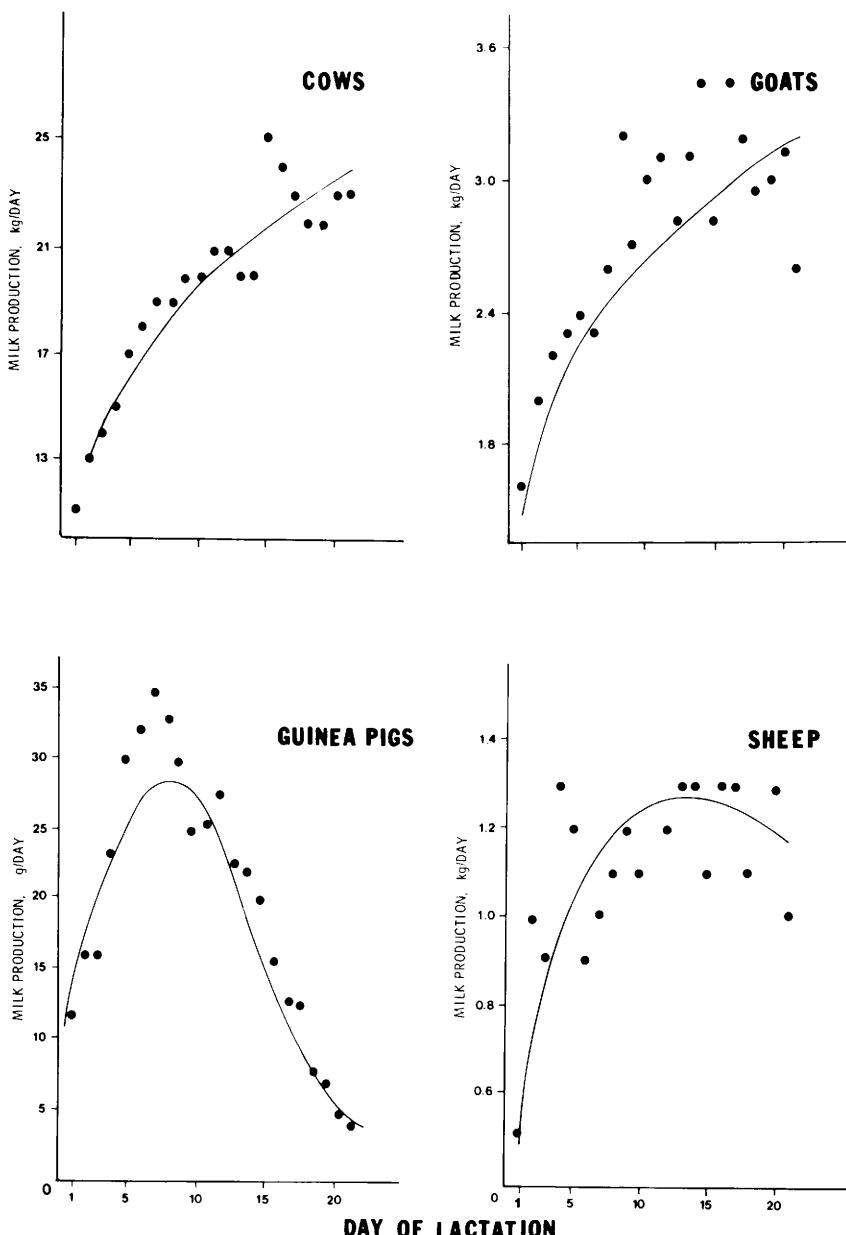


FIG. 1. Milk production (kg/day) for the first 21 days of lactation in cows, goats, sheep, and guinea pigs.

100 mCi/mg were obtained from Industrial Nuclear, St. Louis, Missouri. The buffer used was barbital buffer (0.25 M barbital, pH 8.6), 8-anilino-1-naphthalin sulfonic acid (ANS) and polyethylene glycol (PEG, 600, Sigma Chemical P2139). γ -Globulin solution was 16.5% human immune serum (UMC Medical Center Pharmacy). Ethyl alcohol (95% in 10 N H₂SO₄) was used as an extraction solvent for milk fat. The thyroxine and triiodothyronine antisera (T₄-15 and T₃-38) were obtained from Endocrine Sciences, Tarzana, California. Within assay variation was 2.1% for 10 assays, while interassay variation was 19.7% for T₄. Intraassay variation for T₃ was 8.2% for 10 assays, with interassay variation of 11.9%. The recovery of T₄ from milk samples was 82%, while T₃ recovery from milk was 86%. Total observations for T₄ or T₃ of milk for cows were 126, for goats 105, for sheep 126, and for guinea pigs 147. Statistical analysis within a species was by regression analysis.

Comparisons among species were by Tukey's *w* procedure (8). Statistical differences between means were tested at $\alpha = 0.01$ ($P < 0.01$). In the former case, the differences were described as being significant and in the latter case as being highly significant. Regression equations relating daily measurements of various components (milk production, concentrations of T₄ to T₃, daily total amounts of T₄ or T₃, and the ratio of T₄ to T₃) were generated for the following models: linear, quadratic, cubic, exponential to a linear power, exponential to a quadratic power, exponential to a cubic power, and a γ function. All components of the equations were tested including the intercept and coefficients at $\alpha = 0.05$. Only those meeting the statistical tests were chosen for presentation in this study.

Results. Milk production of the animals used in this study averaged 20.03 kg/day in cows, 2.77 kg/day in goats, 1.20 kg/day in

TABLE I. EQUATIONS RELATING VARIABLES TO DAY OF LACTATION IN FOUR SPECIES
USING LEAST-SQUARES REGRESSION

Species	Variable	Equation	R ₁ ²	R ₂ ²
Cow	Total milk prod.	$10.4X^{0.28}$	0.37	0.95
	T ₄ concn in milk	NS	—	—
	Total T ₄ in milk	$10.4X^{0.25}$	0.14	0.73
	T ₃ concn in milk	NS	—	—
	Total T ₃ in milk	$8.1X^{0.32}$	0.14	0.69
	Ratio of T ₄ :T ₃	NS	—	—
Goat	Total milk prod.	$1.44X^{0.25}$	0.16	0.84
	T ₄ concn in milk	$2.1 - 0.13X + 0.0034X^2$	0.25	0.73
	Total T ₄ in milk	NS	—	—
	T ₃ concn in milk	$0.77 - 0.065X + 0.0053X^2 - 0.00014X^3$	0.21	0.59
	Total T ₃ in milk	$0.99 \pm 0.084X - 0.0032X^2$	0.06	0.43
	Ratio of T ₄ :T ₃	$3.1 - 0.061X$	0.10	0.44
Sheep	Total milk prod.	$0.50e^{0.045}X^{0.58}$	0.23	0.67
	T ₄ concn in milk	$2.5 - 0.39X + 0.028X^2 - 0.00063X^3$	0.51	0.91
	Total T ₄ in milk	$1.2X^{-0.12}$	0.03	0.14
	T ₃ concn in milk	$1.0 - 0.049X + 0.0019X^2$	0.06	0.29
	Total T ₃ in milk	$0.50e^{-0.023}X^{0.33}$	0.05	0.23
	Ratio of T ₄ :T ₃	$2.2X - 0.29$	0.14	0.52
Guinea pig	Total milk prod.	$1.2e^{-0.23}X^{1.4}$	0.59	0.89
	T ₄ concn in milk	$2.0 - 0.058X$	0.28	0.76
	Total T ₄ in milk	$20e^{-0.31}X^{1.6}$	0.63	0.90
	T ₃ concn in milk	$0.79 - 0.040X + 0.0011X^2$	0.14	0.58
	Total T ₃ in milk	$9.3e^{-0.23}X^{1.6}$	0.44	0.79
	Ratio of T ₄ :T ₃	NS	—	—

Note. NS = not significant; R₁² = coefficient of determination using all values (significance level is $\alpha = 0.05$); R₂² = coefficient of determination using the mean of all animals for each day of lactation (significance level is $\alpha = 0.05$); T₄ = thyroxine; T₃ = triiodothyronine.

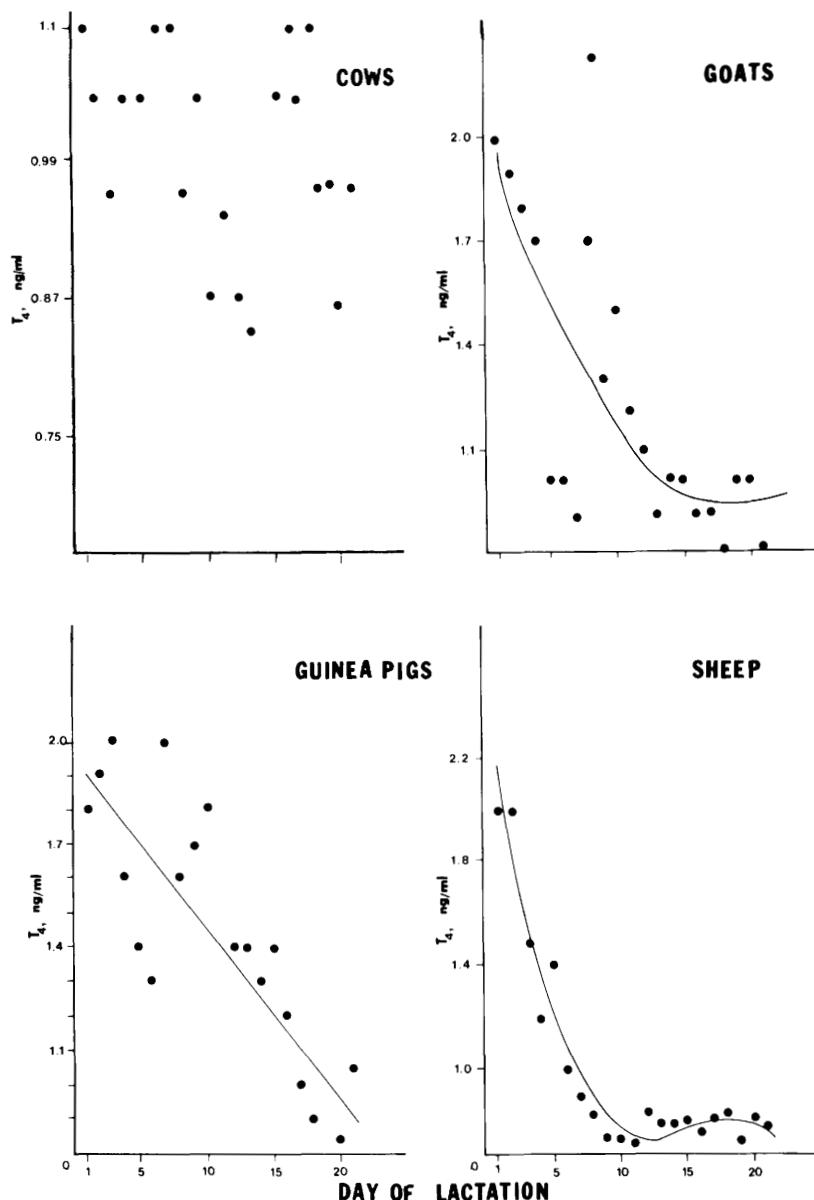


FIG. 2. Concentration of tetraiodothyronine (T₄, ng/ml) in the milk of four species for the first 21 days of lactation.

sheep, and 0.02 kg/day in guinea pigs. The lactation curves of dairy cows and goats usually peak between Days 30 and 60. For this reason, the milk production rose continuously during the sampling period, the first 21 days of lactation in these two species (Fig. 1). On the other hand, the sheep in this study

were not bred for high milk production and as a consequence the milk production began to decline prior to the end of the 21-day lactation period in this study. Milk production in guinea pigs rose sharply to peak on Day 7 of lactation and then dropped rapidly to Day 21. In the cases of cows and goats a

TABLE II. CONCENTRATIONS OF T₄, T₃, AND T₄ PLUS T₃ (ng/ml) IN FOUR SPECIES

Species	N ^c	T ₄ (ng/ml)	T ₃ (ng/ml)	T ₄ plus T ₃ (ng/ml)
Cow	6	0.97 ± 0.03 ^a	0.94 ± 0.05 ^a	1.96 ± 0.05 ^a
Goat	5	1.24 ± 0.06 ^{a,b}	0.52 ± 0.01 ^b	1.77 ± 0.07 ^a
Sheep	6	0.99 ± 0.04 ^a	0.79 ± 0.03 ^a	1.79 ± 0.06 ^a
Guinea pig	7	1.41 ± 0.05 ^b	0.53 ± 0.02 ^b	1.94 ± 0.07 ^a

Note. Values are means ± SE.

^{a,b} Means within a column followed by the same letter are not significantly different ($P > 0.5$).

^c Number of animals with each representing 21 daily values.

rectilinear regression equation expressed the milk production data very well (Table I). Both regression coefficients were highly significant. Since milk production peaked and began to decline within the 21-day sampling period, sheep and guinea pigs had regression equations for milk production which have been described as γ -type functions (Table I, Fig. 1). Both their regression coefficients were highly significant.

Thyroxine in milk of cows varied from a low of 0.85 ng/ml on Day 13 of lactation to 1.23 ng/ml on Day 17. No trend in the pattern of T₄ concentration in milk was observed or found by regression analysis (Fig. 2). However, significant changes in concentrations of T₄ in milk of three other species tested (goats, sheep, and guinea pigs) were found. In goats the T₄ concentration of milk was 2.11 ng/ml on the 1st day of lactation and fell to 0.82 ng/ml on the 21st day. The regression equation developed for T₄ concentration was: Y (T₄, ng/ml) equals $2.1 - 0.13X + 0.034X^2$, where X was day of lactation. The intercept and coefficients of this quadratic equation were highly significant (Table I). In sheep, T₄ concentration in milk varied from 2.1 ng/ml on Day 2 of lactation to 0.60 ng/ml on Day 11, with a concentration of 0.75 ng/ml on Day 21, the last day of sampling. Components of a cubic regression equation were found to be highly significant and the best mathematical expression for the data. Thyroxine concentration in guinea pig milk had a mean of 1.4 ng/ml (Table II). It reached 2.1 ng/ml on Day 3 and fell to 0.67 ng/ml on Day 20. The linear regression equation ($Y = 2.0 - .058X$) was highly significant (Fig. 2).

Triiodothyronine in milk of cows averaged 0.94 ng/ml and ranged from 1.35 ng/ml on Day 19 to 0.76 ng/ml on Day 11. No trends in the data were found when subjected to regression analysis. On the other hand, all other species showed a significant regression coefficient in the T₃ concentration of their milk. In goats, the mean T₃ concentration was 0.52 ng/ml. It was highest on Day 1 at 0.68 ng/ml and lowest on Day 16 at 0.44 ng/ml. The regression equation best describing the data was a cubic equation (Table II, Fig. 3) which was significant. Triiodothyronine in sheep milk averaged 0.79 ng/ml over the first 21 days. This value was significantly higher than in goat milk and guinea pig milk, but it was not different from the concentration of T₃ in cow milk (Table I). The mathematical expression for T₃ concentration in sheep milk was a quadratic equation and was significant. Guinea pig milk had a T₃ concentration of 0.53 ng/ml, similar to that of goat milk. The regression model for guinea pig milk T₃ concentration was a quadratic equation (Table I, Fig. 3) with highly significant intercept and coefficients.

Total T₄ and total T₃ in milk were calculated by multiplying concentration times volume of milk. In cow milk, the total T₄ and total T₃ showed patterns characterized best by exponential regression curves (Table I, Figs. 4 and 5), which were highly significant. Total T₄ in milk was lowest at 12.6 μ g/day on Day 1 of lactation and highest at 26.4 on Day 15. The mean was 20.3 μ g/day (Table III). Total T₃ was lowest on Day 1 and highest on Day 15 also, having been 9.34 μ g/day and 31.1 μ g/day, respectively. The mean total T₃ was 19.7 μ g/day, almost equal to the

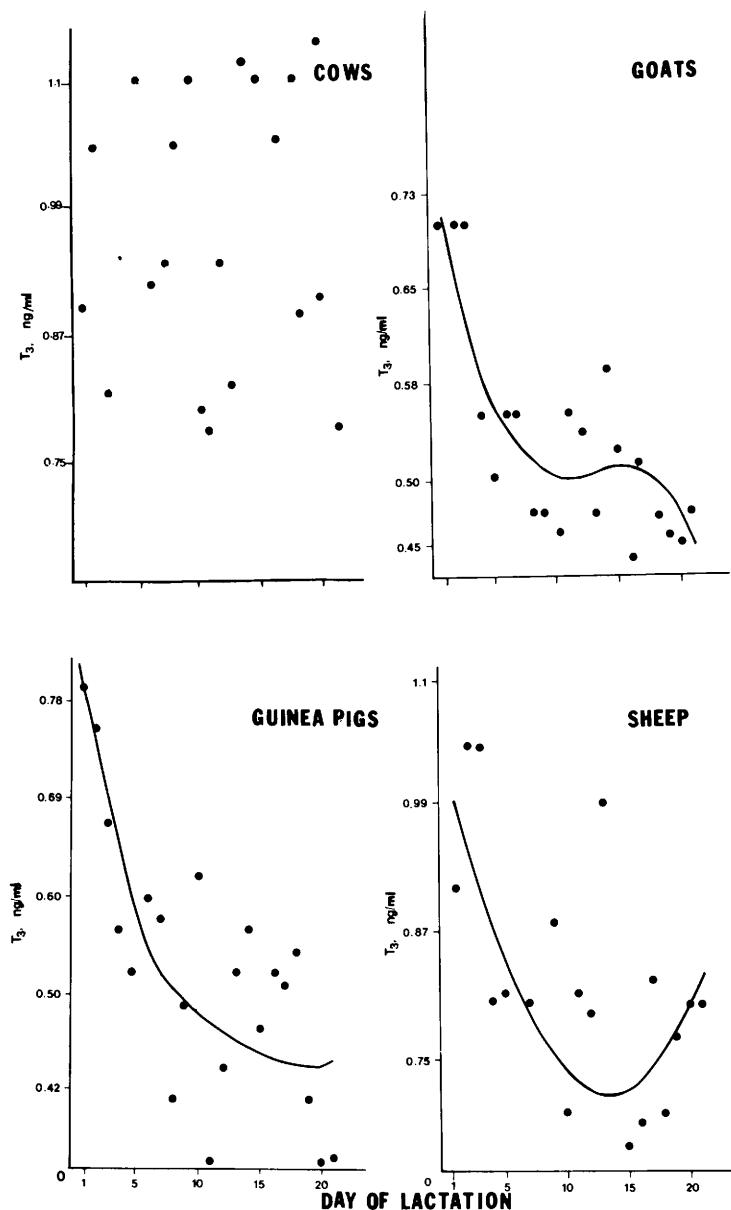


FIG. 3. Concentration of triiodothyronine (T₃, ng/ml) in the milk of four species for the first 21 days of lactation.

amount of T₄ lost in milk. In goat milk, the total T₄ averaged 3.20 μ g/day (Table III), showing no pattern of change through the first 21 days of lactation (Table I). On the other hand, total T₃ in goat milk showed a significant exponential regression pattern (Table I, Fig. 5), while the average total T₃

was 1.42 μ g/day (Table III), significantly lower than total T₄. Total T₄ and total T₃ in sheep milk were 1.09 and 0.96 μ g/day, respectively (Table III), not significantly different from each other. Total T₄ regression showed a significant exponential decrease, while total T₃ regression increased according to a γ

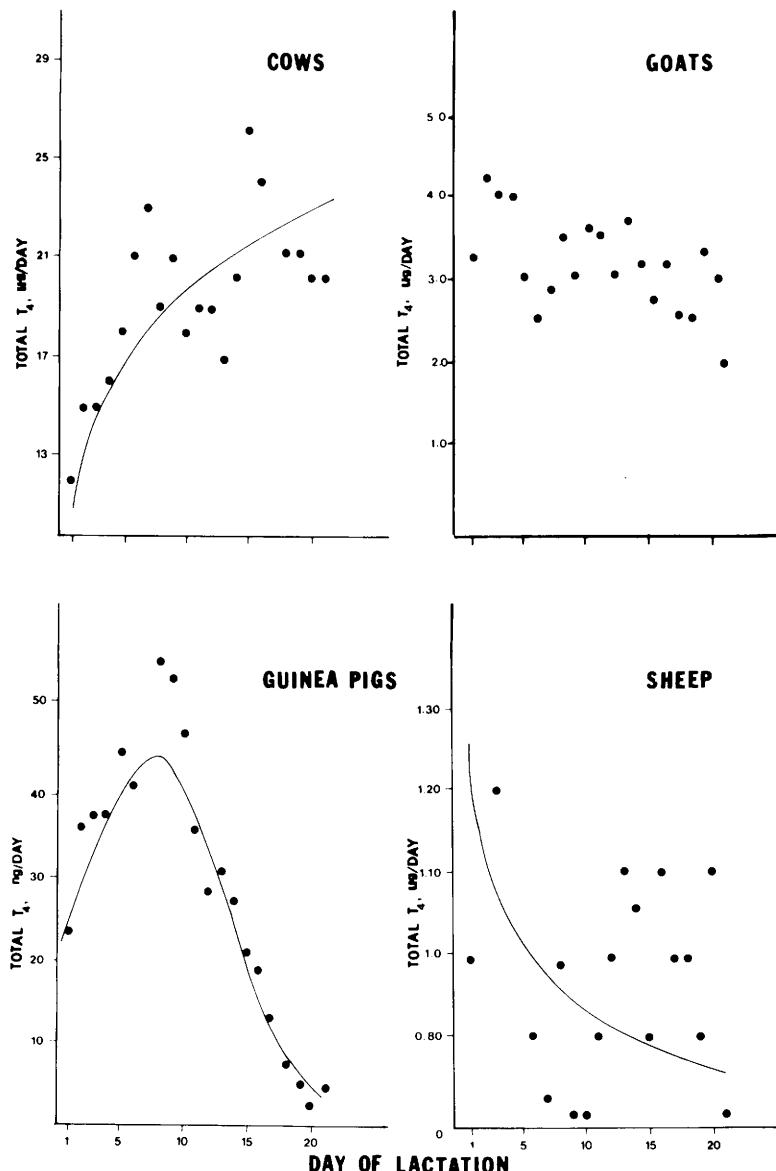


FIG. 4. Total tetraiodothyronine (TT₄, μg) in the milk of four species for the first 21 days of lactation.

function (Table I, Figs. 4 and 5). Total T₄ (0.030 $\mu\text{g}/\text{day}$) and total T₃ (0.011 $\mu\text{g}/\text{day}$) in milk of guinea pigs were significantly different from each other, and the regression equations were γ -type curves, similar to the regression model for milk production in this species.

The ratios of T₄ to T₃ were not different throughout lactation in the milk of cows and

guinea pigs. However, a rectilinear decrease in the ratio of T₄ to T₃ in goats milk was shown by regression analysis, while the ratio in sheep milk decreased according to an exponential function (Table I, Fig. 6).

Comparisons of concentrations of T₄ and T₃ in milk among the four species studied, showed that T₄ was significantly higher in guinea pig milk than in cow milk or sheep

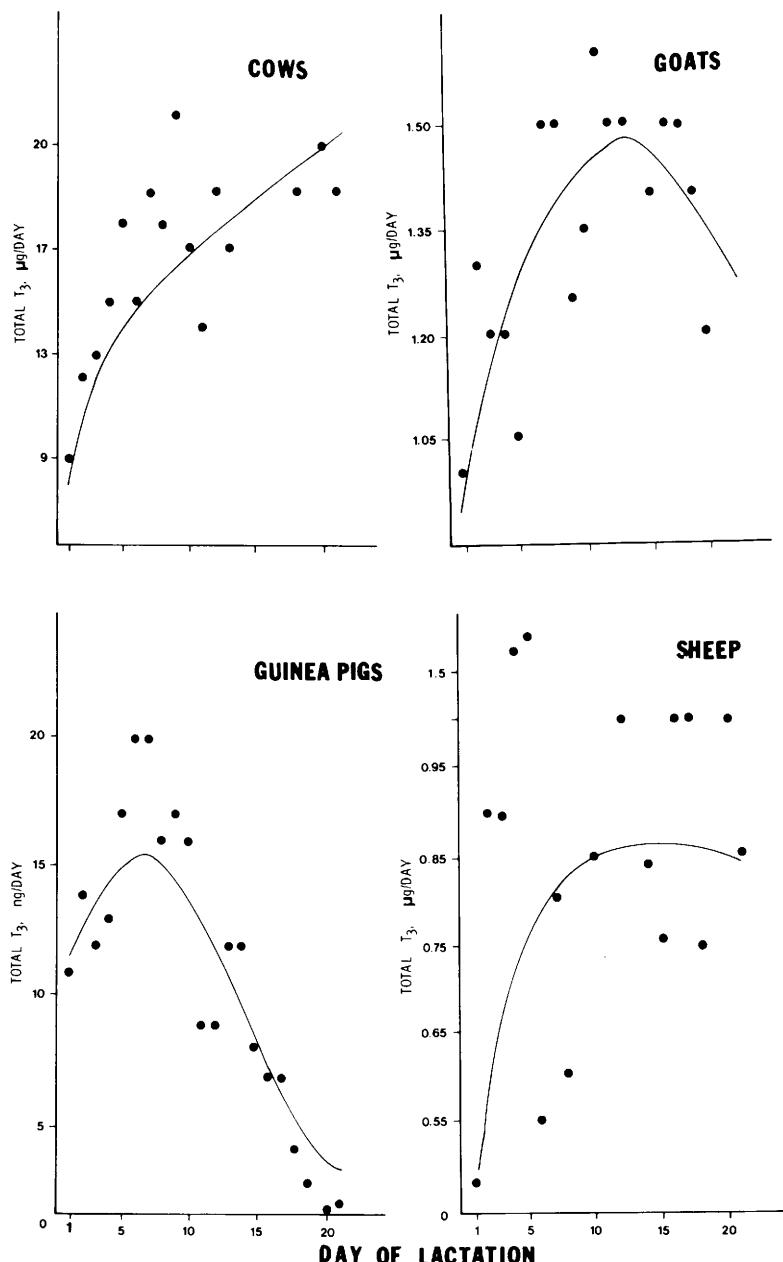


FIG. 5. Total triiodothyronine (TT_3 , μ g) in the milk of four species for the first 21 days of lactation.

milk. T_3 concentration was lower in goat milk and guinea pig milk than in cow milk or sheep milk (Table II). When the concentrations of T_4 and T_3 were summed, no differences were found among these species.

Milk production varied among the four species and total amounts of thyroid hormones, T_4 and T_3 , did likewise (Table III). However, total amounts of T_4 and T_3 were different in two of the four species. Milk

TABLE III. MILK PRODUCTION, TOTAL MILK T₄ AND T₃ (μg/DAY) IN FOUR SPECIES

Species	N ^a	Milk production (kg/day)	Total T ₄ (μg/day)	Total T ₃ (μg/day)
Cow	6	20.03 ± 0.61	20.30 ± 0.95	19.70 ± 1.08
Goat	5	2.77 ± 0.11	3.20 ± 0.17	1.42 ± 0.05
Sheep	6	1.20 ± 0.04	1.09 ± 0.05	0.96 ± 0.05
Guinea pig	7	0.02 ± 0.01	0.030 ± 0.003	0.011 ± 0.002

Note. Values are means ± SE.

^a Number of animals with each representing 21 daily values.

samples from goats had significantly higher concentrations of T₄ to T₃ and so did guinea pig milk, while cow milk and sheep milk showed no significant differences in total amounts of T₄ and T₃ passing into milk each day.

Body weights of the species in this study varied from 500 kg to 800 g, a difference of over 600-fold. One method to equalize these differences was to calculate milk production on a unit body weight basis. Milk production as a percentage of body weight was significantly higher in the goat than in the cow, which, in turn, was significantly higher than the sheep or the guinea pig (Table IV). An alternative method was to express milk production per unit of body weight to the three-quarter power. This has been designated as more meaningful because milk production has been suggested to be a variant of metabolic rate and the metabolic rate among species varies according to the three-quarter power of body weight (9). When these calculations were made, the cow was shown to be most efficient, followed by the goat, the sheep a distant third, and the guinea pig last (Table IV).

T₄ and T₃ concentrations in milk were multiplied by the volume of milk consumed per day by offspring for each species. Dairy calves consume whole milk at the rate of 10% of body weight per day. Consumption of 4.5 kg milk by a 45-kg calf would result in an intake of 4.5 μg T₄/day and a similar amount of T₃/day. If T₃ has at least three times the potency of T₄, the T₄ equivalent intake per/day via the milk equals 18 μg. T₄ secretion rates for calves from birth to 3 months of age have been found to be 1 mg/100 kg body wt or 450 g/45 kg (10). There-

fore, the milk may provide only 4% of the thyroid hormone needs in cattle. In goats, sheep, and guinea pigs, quantities of milk consumed per day by newborn offspring are near 10% of body weight and secretion rates of thyroid hormones are 0.5 μg/100 g body wt. At best, no more than 7% of the animal's thyroid hormone needs may be provided by the milk it consumes (Table V).

Discussion. Thyroid hormones, T₄ and T₃, have been found to be present in milk of cows, goats, sheep, and guinea pigs in low concentrations. These levels of thyroid hormones in milk agree reasonably well with several other reports (11-14). In contrast to these concentrations, iodide has been found in concentrations of 80 ng/ml or more when iodine intake is normal. Most iodine in milk is thought to be carried by phenyl rings of tyrosine residues incorporated in casein of milk. T₄ and T₃ concentrations in milk of goats, sheep, and guinea pigs were high during the first few days compared to later days of lactation. This milk is referred to as colostrum because it is high in concentrations of γ globulins. Since globulins bind T₄ and T₃ better than other proteins, the higher concentration of these proteins during the first few days of lactation accounts for the higher levels of T₄ and T₃ at this time. Similar observations of high T₄ in milk early in lactation have been reported by Strbak *et al.* (5) who studied human milk.

Thyroid hormone concentrations in blood of the species in this study have been found to be 30 to 60 ng/ml for T₄ and 1 to 2 ng/ml for T₃ (14). Concentrations of T₄ in milk were found to be 1 to 1.4 ng/ml in this study. Concentrations of T₃ in milk were 0.5 to 1.0 ng/ml, approximately half as concentrated as

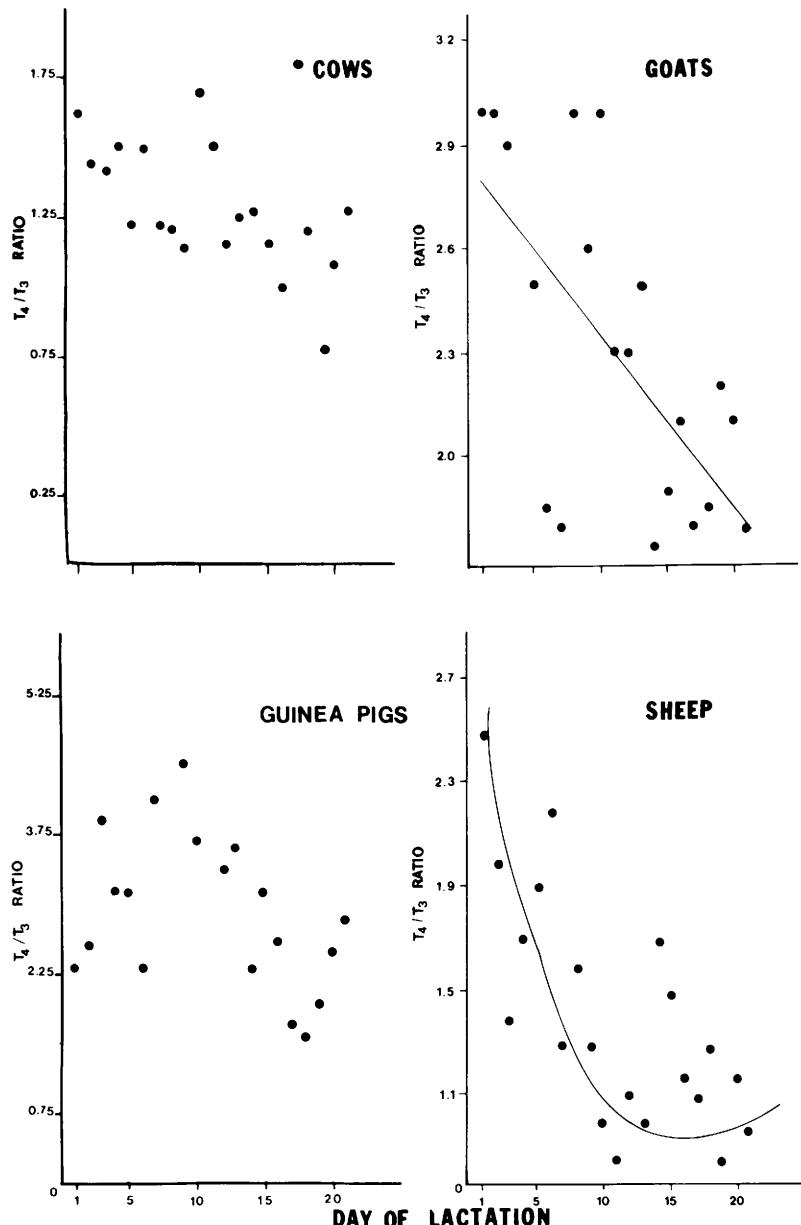


FIG. 6. Ratio of tetraiodothyronine to triiodothyronine (T_4/T_3) in the milk of four species for the first 21 days of lactation.

blood. One explanation for this could be comparably lower levels of T_4 and T_3 binding proteins in milk relative to blood.

Decreases in concentrations of T_4 and T_3 in milk as lactation progressed were noted in goats, sheep, and guinea pigs. Since the decreasing trend continued beyond the third day, at which time the colostrum should no

longer be a factor, other reasons for the decline need to be addressed. One possibility may be that the need for T_4 and T_3 by the milk secretory cells decreased during galactopoiesis relative to that required for lactogenesis. Second, T_4 and T_3 may be deiodinated in the secretory cell and the metabolic end products are not measured as T_4 or T_3 .

TABLE IV. MILK PRODUCTION (% BODY WT) AND MILK PRODUCTION (% BODY WT^{3/4}) IN FOUR SPECIES

Species	N ^a	Body wt (kg)	Milk production	
			% Body wt	% Body wt ^{3/4}
Cow	6	500	4.00 ± 0.10 ^a	18.95 ± 0.58 ^a
Goat	5	50	5.54 ± 0.20 ^b	14.78 ± 0.56 ^b
Sheep	6	50	2.40 ± 0.09 ^c	6.40 ± 0.23 ^c
Guinea pig	7	0.8	2.50 ± 0.12 ^c	2.40 ± 0.67 ^d

^a Note. Values for milk production are means ± SE.

^{a-c,d} Means within a column followed by the same letter are not significantly different ($P > 0.05$).

^b Number of animals with each representing 21 values.

in the assay. A third possibility is that relatively more T₄ is converted to T₃ by the secretory cell as lactation progresses with the result being less thyroid hormone is required to regulate a given level of metabolism. This suggestion receives additional support from the information on T₄:T₃ ratios, which decrease significantly through the first 21 days of lactation in the goat and the sheep.

Species differences in T₄:T₃ ratios, as opposed to differences through lactation, were observed in the four species studied. Milk of cows and sheep had ratios of T₄ to T₃ which were nearly equal. Milk of goats and guinea pigs had T₄ levels which were 2.5 to 3.0 times the T₃ levels. These differences may reflect abilities of specific tissues to bind thyroid hormones or to convert T₄ to T₃. Wilson and Gorewit (15) reported that T₄ bound with high affinity to receptors in the cytosol of lactating mammary gland secretory cells of cattle. Davis *et al.* (16) found T₄ bound with higher affinity than T₃ to membrane and cytosol receptors. Others found

high affinity T₄ binding sites compared to T₃ binding sites in membranes and cytosol of various tissues (17-19). Degroot and Torre-sani (20) observed high-affinity binding sites in cytosol of liver and kidney cells of rats. T₃ binding sites in the nucleus were also found. Numerous studies have shown a conversion of T₄ to T₃ in the cell (21-25). Since milk from goats and guinea pigs had significantly higher ratios of T₄ to T₃ than milk from cows and sheep, we suggest that T₄ was converted to T₃ in the mammary secretory cells of cows and sheep much more so than in similar cells of goats and guinea pigs.

Since the species in this study had wide ranges in body weights from 500 kg in cattle to 800 g in guinea pigs, an attempt was made to equalize these differences by expressing milk production on a per unit body weight basis. This favored the goat over the cow as the best milk producer. However, since milk production is a function of metabolic rate and since metabolic rate is related to the three-quarters power of body weight, the data were calculated on this basis. The results showed that the cows in this study were superior in milk production to the goats, while the sheep were third and the guinea pigs a distant last. In fairness to the guinea pig as a lactator, it should be pointed out that milk production was averaged over the first 21 days. The three species other than guinea pigs had high milk production throughout this period, while the guinea pig peaked at Day 7 of lactation and was practically dry by Day 21.

This study investigated concentrations and total amounts of T₄ and T₃ transferred to

TABLE V. T₄ AND T₃ IN MILK AVAILABLE TO OFFSPRING OF FOUR SPECIES COMPARED TO NORMAL THYROID HORMONE SECRETION RATES OF EACH SPECIES

Species	Weight of offspring at birth (kg)	Milk consumption/day (10% of body wt)	T ₄ in milk consumed/day (ng)	T ₃ in milk consumed/day (ng)	Total T ₄ equivalents in milk/day (ng)	Normal TSR ^a /day (ng)	T ₄ equivalents produced from milk (%)
Cow	45	4500	4370	4230 ^b	17,060	450,000	4.0
Goat	4	400	496	208	1,120	20,000	5.6
Sheep	4	400	396	316	1,340	20,000	6.7
Guinea pig	0.1	10	14.1	5.3	30	500	6.0

^a TSR = thyroid hormone secretion rate.

^b T₃ was multiplied by 3 to obtain T₄ equivalents.

milk on a daily basis. The data obtained were intended to provide information concerning transfer of T₄ and T₃ via mother's milk to the newborn. This study complemented our previous studies which determined live-birth weights, amounts of milk intake suckled (26), and thyroid hormone secretion rates of the neonate within the first several weeks after birth (10). In the present study we estimated T₃ to be three times as potent as T₄ on an equimolar basis in stimulating metabolism. Thus, the contributions of T₄ and T₃ from milk represent 4 to 7% of the offspring's requirements for thyroid hormone stimulation. This suggests that milk is not a significant source of thyroid hormone for the newborn, although these levels may provide minimal metabolic stimuli to insure survival for some newborns experiencing minimal thyroid function.

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