

## Effect of Acute Increases in Suckling Frequency upon Food Intake and Milk Secretion in the Rabbit<sup>1</sup> (41289)

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**Abstract.** The normal once-a-day frequency of suckling in rabbits was altered on Day 10 (early) and Day 30 (late lactation) by the addition of either one (8 hr after) or two extra sucklings (8 and 16 hr after) the daily suckling. One additional suckling significantly increased milk yield on Day 10 in comparison with the average 4-day milk yield before and after Day 10 whereas two additional sucklings decreased the increment. Either one or two additional sucklings on Day 30 significantly accelerated the already decreasing daily milk yields characteristic of late lactation. Not only was less milk secreted on Day 30 than on Day 10, as expected, but it was also secreted in a less consistent manner throughout the 24-hr period. The lower milk yields in late lactating rabbits were not due to depressed food intake; paradoxically the quantity of food ingested per gram of milk produced was greater at this time. These data suggest that suckling may activate mechanisms in late lactation in the rabbit which offset the stimulatory effects of suckling upon milk secretion.

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Maintenance of milk secretion in the rabbit after parturition depends upon the stimulatory action of prolactin upon the mammary glands (1) and upon the availability of precursors from the diet for milk synthesis (2). During early lactation, a sustained high level of prolactin in the circulation follows suckling (3). This together with an elevated level of responsiveness of the mammary glands to the hormone (4) probably accounts for the copious milk yields characteristic of this stage. In late lactation, however, the levels of prolactin following suckling decline more rapidly than in early lactation (3), and the responsiveness of the mammary glands to either local, neural, or hormonal factors is much reduced (4). Milk yields are considerably lower in comparison to early lactation (5-10).

The initial purpose of the present experiments was to determine if increasing the suckling frequency from the normal once-a-day pattern in the rabbit could prevent the reduced milk yields observed dur-

ing late lactation. We found that increasing the suckling frequency indeed increased milk yields but only during early lactation; the same procedure decreased milk yields in late lactation. We have analyzed some of the possible mechanisms involved in the depressant effect of suckling during late lactation.

**Materials and Methods.** *Animals.* Pregnant nulliparous New Zealand white rabbits (2.5-4.0 kg body wt) were placed in large individual cages in a separate room which had controlled light (14-hr light:10-hr darkness) and temperature (22-23°). They were given food (Conejina, Purina) and water *ad libitum*. Only rabbits with six to eight pups/litter were used in the experiments.

*Measurement of milk yield.* The litters were isolated from their mothers except at the time of the normal once-a-day suckling, which usually took place between 1100 and 1200 hr. Shortly before suckling on each day milk yield was to be recorded, the pups' bladders were manually emptied. They were weighed individually to the nearest 0.1 g, and then were placed on the teats of the mother, who was manually restrained in the supine position. After the pups were attached and actively suckling, an injection of 500 mU oxytocin (Syntoci-

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non, Sandoz) was given through a lateral ear vein of the mother to facilitate milk ejection. The pups required only 3–5 min suckling to empty all the mammary glands of milk, after which they were reweighed to estimate milk yield. This procedure, in contrast with that where the mother has constant access to her pups (11) does not interfere with normal daily gain in weight of the litter (4).

Reference milk yields were taken daily in all rabbits for 4 days before and after Day 10 or Day 30 of lactation. These also served to indicate whether or not each rabbit was lactating normally. Then, on Day 10 (early) or Day 30 (late lactation), groups were suckled again either once (8 hr after) or twice (8 and 16 hr after) the daily suckling. Controls received oxytocin without suckling during these times. Milk yields were obtained during these additional sucklings with the same technique as employed during the control daily sucklings. The daily milk yields on Day 10 and Day 30 then were derived by adding the milk yields obtained at all the sucklings on those days. Food intake was assessed at 8-hr intervals the day the rabbits received the two extra sucklings. Differences between groups were analyzed using Student's *t* test.

**Results.** The daily milk yield increased from 151.3 to 187.3 g from Days 7 to 10 of lactation or an average increase of 12.0 g/day in oxytocin-injected control rabbits (Table I). An additional increase of 4.3 to 191.6 g occurred on Day 10; milk yields then tended to plateau during the subsequent 4 days. In the groups receiving an extra suckling 8 hr after the daily suckling on Day 10, there was a significant increase in milk yield on that day from 167.3 to 208.6 g (+41.3 g) in comparison to the average daily increase of 10.7 g for the previous and 2.6 g for the subsequent day periods. Two additional sucklings at 8-hr intervals on Day 10, however, failed to significantly increase milk yield on that day. The yield increased only 1.6 g (167.7 to 169.3 g), compared to an average daily increase of 16.9 g during the previous 4 days. Milk yields tended to plateau from Days 11 to 14.

Days milk yields of control lactating rabbits fell steadily from the 26th to 34th days

of lactation. One additional suckling on Day 30 resulted in a significantly greater decrease in milk yield on that day (123.1 to 100.9 g, or -22.2 g) than occurred on an average (-4.9 g) during the preceding 4 days. Two extra sucklings on Day 30 also resulted in a significant decrease in milk yield on that day (153.2 to 116.9 g, or -36.3 g) compared with the average decrease during the preceding (-2.4 g) and subsequent (-6.3 g) 4 days.

The milk yield and food intake of rabbits which received two extra sucklings are shown in Table II. The 24 hr milk yield on Day 10 averaged 169.6 g compared with 116.4 g on Day 30. The milk yields on Day 10 were distributed evenly (53–60 g) for the three 8-hr periods, whereas on Day 30, milk yield was 29 g in the first 8-hr period and 42 and 45 g during the second and third 8-hr periods, respectively (Table II).

The mothers each ate an average of 388.6 g of food/24 hr on Day 30 compared with 356.6 g on Day 10. Food intake was greater each of the first two 8-hr periods than it was during the last 8 hr in both groups.

The quantity of food eaten for each gram of milk produced also appears in Table II. The lowest ratio in both early and late lactating rabbits (1.6 and 1.4 g/g, respectively) occurred in the last 8-hr period. The ratios obtained in the late lactating rabbits were 5.7 g/g during the first and 3.8 g/g during the second 8-hr period; these are significantly higher than the ratios of 2.3 and 2.5 g/g for the corresponding periods in early lactating rabbits.

**Discussion.** Milk secretion declined in our rabbits, in confirmation of previous observation by Cowie (9), as lactation progressed chronologically. The cause of the decline does not appear to be related to a corresponding reduction in food intake for the quantity of food ingested actually was greater during late than during early lactation. In addition, less milk was secreted during the first 8 hr following the daily suckling yet this period corresponded to the period of greatest food intake. Thus the less constant milk secretion during late lactation which we and Gachev *et al.* (12) observed does not appear to be influenced by corresponding alterations in food intake. It has

TABLE I. ACUTE EFFECT OF ADDITIONAL SUCKLINGS ON DAY 10 OR DAY 30 UPON MILK YIELD IN RABBITS

		Milk yield (g)									
No. of rabbits		Gain (loss)/day (Days 7-10)					Gain (loss)/day (Day 10)				
		Day 7	Day 10	Gain (loss)/day (Days 7-10)	Day 10	Gain (loss)/day (Day 10)	Day 10	Gain (loss)/day (Day 10)	Day 11	Day 14	Gain (loss)/day (Days 11-13)
<b>Early lactation</b>											
Control	8	151.3	187.3	+12.0 ± 2.7	191.6	+4.3 ± 5.3	190.6	187.6	-1.0 ± 4.6		
One extra suckling	6	135.2	167.3	+10.7 ± 4.6	208.6	+41.3 ± 10.8*	211.2	219.0	+2.6 ± 3.1		
Two extra sucklings	5	117.2	168.0	+16.9 ± 2.3	169.6	+1.6 ± 3.7	169.8	171.3	+0.5 ± 8.8		
<b>Milk yield (g)</b>											
No. of rabbits		Gain (loss)/day (Days 27-29)			Gain (loss)/day (Day 30)			Gain (loss)/day (Days 31-33)			
		Day 27	Day 30	Gain (loss)/day (Days 27-29)	Day 30	Gain (loss)/day (Day 30)	Day 31	Day 34	Gain (loss)/day (Days 31-33)		
<b>Late lactation</b>											
Control	11	133.0	111.7	-7.1 ± 2.5	98.8	-12.9 ± 3.8	96.9	91.2	-1.9 ± 1.7		
One extra suckling	11	137.8	123.1	-4.9 ± 3.4	100.9	-22.2 ± 4.1*	—	—	—		
Two extra sucklings	5	160.4	152.7	-2.4 ± 5.2	116.4	-36.3 ± 6.4*	100.6	81.7	-6.3 ± 3.8		

Note. Single extra suckling applied 8 hr after daily suckling; two extra sucklings applied 8 and 16 hr after daily suckling. Values are means ± SEM.  
 \* Significantly different ( $P < 0.05$ ) from preceding average 4 days milk yield.

TABLE II. MILK YIELD (g) FOOD INTAKE (g), AND GRAMS OF FOOD REQUIRED FOR EACH GRAM OF MILK SECRETED (F/M) OF RABBITS SUCKLED 8, 16, AND 24 hr AFTER DAILY SUCKLING ON DAY 10 OR 30 OF LACTATION

	0-8 hr			8-16 hr			16-24 hr			Total 0-24 hr		
	Food	Milk	F/M	Food	Milk	F/M	Food	Milk	F/M	Food	Milk	F/M
Day 10	119.8 ±7.5			139.9 ±13.5			96.9 ±9.1			356.6 ±25.0		
		52.6 ±5.0			57.0 ±3.7			60.0 ±2.0			169.6 ±9.3	
			2.3 ±0.3			2.5 ±0.3			1.6 ±0.1			2.1 ±0.2
Day 30	164.4 ±12.7			161.2 ±14.7			63.0 ±2.0			388.6 ±19.9		
		28.6 ±9.0			42.4 ±5.8			45.4 ±7.7			116.4 ±18.0	
			5.7 ±2.5*			3.8 ±0.7*			1.4 ±0.1			3.3 ±0.1*

Note. Values are means ± SEM. Five rabbits in each group.

\* Significantly greater ( $P < 0.05$ ) than corresponding ratios on Day 10.

been shown that a short latency exists between food ingestion and its influence upon milk secretion (2) in lactating rabbits.

Prolactin is essential for milk secretion in the rabbit, as shown by replacement studies in hypophysectomized animals (1). It is possible therefore that less prolactin is available during late lactation and/or the mammary gland may become less responsive to the hormone at this time. Plasma prolactin levels remain elevated for a longer period following suckling in early- compared to late-lactating rabbits (3), and there is a decline in the responsiveness of the mammary glands to prolactin in animals deprived of suckling (4) and in animals whose secretion of prolactin has been blocked with bromocriptine (F. Mena, D. Aguayo, C. Clapp, G. Martínez-Escalera, and C. E. Grosvenor, unpublished). In intact lactating rabbits, injections of prolactin stimulate milk secretion during late lactation (5, 9, 10), whereas no effect occurs during early lactation (10). During late lactation in the rat, the secretion rate of prolactin not only is reduced (13), but the minimal effective dose of prolactin capable of fully stimulating milk secretion in the rat is four or five times greater at this time than in early lactation (C. E. Grosvenor and F. Mena, unpublished data).

One would expect, therefore, that increasing the suckling frequency would provide more prolactin and thus increase milk secre-

tion. This indeed appeared to be the case when one extra suckling was applied during early lactation. Also, milk yield and milk lactose concentration, considered as direct indices of prolactin secretion in the rabbit, have been reported to be twice as high in rabbits suckled chronically every 12 hr as in rabbits suckled only once a day (12). On the other hand, the failure to obtain an increase in the rate of milk secretion when two extra sucklings were applied in early lactation suggests that, beyond a certain frequency, suckling and emptying of the mammary glands were adverse to milk secretion. Since only one extra suckling was required to reduce the rate of milk secretion in late lactation, it is possible, moreover, that a lower threshold exists during this stage for any adverse effect to occur. A stressful state may be induced by the extra suckling, and this may be implicated in the mechanism of reduction of milk secretion. Findlay (15) observed that suckling and emptying of the mammary glands of rabbits tended to reduce the willingness of the mothers to nurse their litters 2 and/or 4 hr later. No comparison was made in this regard, however, between early- and late-lactating rabbits. Previously, we presented evidence for both the rat (14) and the rabbit (4) that sympathetic influences may play an inhibitory role in milk secretion. Moreover, reflex activation of the sympathetic system follows mechanical stimulation of the mammary gland area (16)

or electrical stimulation of afferent mammary nerve (17). We have recently shown (unpublished) that substituting young pups for the mother's own litter prevented the reduced milk yields characteristic of late lactation in the rabbit, whereas substitution of older pups for the mother's own litter results in a reduction in milk yields during early to mid lactation. It is possible, therefore, that suckling itself may regulate lactation in the rabbit through antagonistic mechanisms, one involved in the release of prolactin and possibly other galactopoietic hormones, and the other involving activation of the sympathetic-adrenal system. Thus, galactotrophic,<sup>3</sup> i.e., stimulatory, influences of suckling may predominate over galactolytic<sup>3</sup> influences during early lactation, whereas the latter may be predominant during late lactation. Since quantitative changes of suckling do not occur in this species during the course of lactation, it is possible that qualitative changes in suckling associated perhaps with the age and strength of the pups, may be involved in the degree of response of these two systems.

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<sup>3</sup> Since the decline of lactation appears to be an active process, the term "galactolysis" is proposed as synonymous with this process. Also, "galactotrophy" and "galactostasis" probably describe more adequately beginning and established lactation, respectively. "Galactopoiesis" which originally referred to restoration or to the increase in intensity of lactation (18) now often is used synonymously with established lactation.