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Behavioral Interactions Rather Than Milk Availability Determine Decline in Milk Intake of Weanling Rats[1]

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THIELS, E., C. P. CRAMER AND J. R. ALBERTS. *Behavioral interactions rather than milk availability determine decline in milk intake of weanling rats.* PHYSIOL BEHAV 42(6) 507–515, 1988.—We examined the relation between milk availability and milk intake during the period in which rat pups gradually abandon milk as a food source. The amount of milk produced by rat dams does not change from postpartum Day 15 to Day 20, but decreases thereafter and completely disappears around Day 30. In contrast, the amount of milk actually obtained by pups does begin to decline between Days 15 and 20. This decline in milk intake can be attenuated by integrating 20-day-old pups into 15-day-old litters. We concluded that pups do not begin to ingest less milk because of diminishing milk supplies. Rather, the decreased tendency of mothers to nurse older pups and the diminished tendency of older pups to extract available milk, together appear to underlie the decline in pups' milk consumption. Milk supplies decline after changes in behavioral interactions and may play an instrumental role in the eventual abandonment of suckling.

Weaning Milk availability Milk intake Nursing Suckling Behavioral interactions

DURING the first 2 weeks of postnatal life, Norway rat pups subsist exclusively on mother's milk. Beginning around Day 16, their milk intake gradually declines and pups begin to supplement their diet with solid food. By the fifth week postpartum, milk consumption has completely disappeared, and pups subsist exclusively on solid food [5–7, 33, 49].

This dramatic shift from milk to solid food constitutes an integral aspect of mammalian development, and many hypotheses regarding the underlying basis of weaning have been proposed (e.g., [7, 8, 32, 33, 66]). A common hypothesis is that pups give up milk simply because the mother's supply declines and, as a consequence, they must transfer their ingestion to solid food to satisfy their nutritive needs, much as foraging animals shift food patches as the supply in the currently exploited patch diminishes [19, 30, 60]. Although intuitively satisfactory and parsimonious, there is surprisingly little evidence in support of the idea that diminished milk supply drives the pups to solid food.

The lack of evidence stems largely from the assumption that pups' milk intake is isometric with the mothers' milk stores [18]. This assumption may be unwarranted because the amount of milk pups obtain from their mother is determined not only by the amount of milk produced by the dam but also by behavioral variables such as the dam's initiation and/or maintenance of nursing contact, and the pups' active extraction of milk [37]. Therefore, results based on milk consumption (e.g., [6, 7, 11, 43, 44, 47, 48, 54, 55]) provide little information about the pattern of maternal milk supplies across lactation.

Apart from problems in data interpretation, previous studies on milk yield suffer from several methodological shortcomings. For instance, measuring pups' body weights before and after a specified nursing period— the most common technique used to determine milk flow from mother to pups [11, 36, 47, 48, 68]—requires a control for weight loss from heat production, evaporation, and excretion. Measuring the amount of radioactivity in pups suckling from radioisotope-injected mothers [6, 20, 44] requires a control for ingestion of radioactivity containing feces. Measurement of the amount of milk in pups' stomach [33,49] must include information on gastrointestinal absorption and motility. Failure to include appropriate control measures may lead to either over- or underestimates of the actual amounts of milk consumed by the pups.

Some investigators separate mother and young for a lengthy period prior to testing, thereby inducing maternal and food deprivation on the part of the pups and milk accumulation on the part of the mother [36, 43, 47, 48, 55].

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Both of these effects are likely to magnify milk flow from mother to pups during the subsequent testing period, and the results therefore are probably not representative of pups' milk consumption under normal conditions.

Finally, few investigators have measured pups' milk consumption throughout weaning [7, 33, 49]. Thus, there is presently only limited information regarding the amount of milk rat dams have available and the amount of milk pups ingest during late lactation. We therefore conducted a series of experiments to directly assess both maternal milk supplies during the second half of lactation (i.e., Days 15 to 30) and the amount of milk pups obtain during this period.

EXPERIMENT 1

Our first goal was to establish the amount of milk that could be extracted from rat dams at different points during the weaning period. Because we were primarily interested in the amount of milk *stored* in females, rather than the amount that passed to the pups (see Experiment 2 below), we adopted an assessment technique that eliminated both the influence of mothers' nursing behavior and age-related changes in the pups' suckling.

Specifically, we anesthetized 15-, 20-, 25-, and 30-day-postpartum dams and repeatedly injected them with oxytocin, the hormone that induces release of milk into the mammary ducts [64]. We extracted the milk with the aid of milk- and mother-deprived 14- to 16-day-old litters. When deprived as described, pups of this age readily attach to the nipples of anesthetized dams, quickly and effectively extract milk after an ejection, and detach only in the process of nipple-shifting [25,26]. Yet, pups of this age rarely urinate spontaneously, and do not require control for excretory weight loss (Gubernick and Alberts, personal communication). The pups thus served as milking vessels whose intake can be accurately measured. By giving repeated oxytocin injections and using successive sets of pups until weight gain ceased, we could derive our measure of dams' extractable milk supplies.

METHOD

Subjects

A total of 17 multiparous female Sprague-Dawley rats, 35 foster litters (280 pups), and 4 control litters (32 pups) served in this experiment. Mothers were outbred from stock originally obtained from Charles River (Portage, MI) and were born in the Animal Behavior Laboratory colony at Indiana University. Impregnated females were housed in standard polypropylene maternity cages (48×20×26 cm) with Purina rat chow and tap water continuously available. The colony rooms were maintained at 24° to 26°C and were illuminated from 0700 hr to 2300 hr. Cages were checked daily around 1700 hr for birth, and pups found at that time were considered to be born on that day (Day 0). Litters were reduced to 8 pups (4 males and 4 females) on Day 3.

We tested mothers at either 15, 20, 25, or 30 days postpartum; all foster and control litters were 14- to 16-days old on the day of testing. Each dam was tested with 2 foster litters, except for one 15-day-postpartum dam which was tested with 3 foster litters. There were 4 dams in each of the 3 younger lactational age groups, and 5 dams in the 30-day-postpartum group. We excluded the data of one 30-day-postpartum dam because her nipples appeared involuted and difficult for the foster pups to apprehend.

Procedure

Twelve- to 16-hr before testing, we removed foster litters from their mothers and placed them individually in plastic cages (27×18×13 cm) with wire mesh lid and wood shavings covering the floor. No food or water was available in the deprivation cages, which we placed in incubators (Isolette, Air-Shields Inc.) maintained at 31°C.

We separated test dams from their litters between 0800 and 0900 hr and allowed 3 hr for milk accumulation before testing. (We thereby approximated both milk accumulation and test times used in Experiment 2, below.) Thirty min before testing, we anesthetized test dams with Chloropent (3 mg/kg, IP), made a 3 cm longitudinal incision along the base of the tail, exposing the ventral vein. After puncturing the vein, we carefully inserted a PE-10 cannula (Adams Clay) approximately 1 cm into the vein, secured the cannulation with sutures 0.5 cm rostral and caudal to the puncture, and sutured the incision closed. This preparation is a valid and efficient alternative to fermoral vein cannulations [14]. The cannula was attached to a 1-ml syringe containing oxytocin (Syntocinon, Sandoz) in isotonic saline (1.5 U/100 ml). After surgery we placed dams supine in individual plastic tubs (27×18×13 cm).

Pups were removed from the incubator and voided by gently stroking the anogenital region with a soft paper tissue; we then recorded their combined body weight (accurate to 0.1 g) and placed them with the cannulated dam. Pups were allowed to attach freely, though we occasionally guided them toward a nipple to accelerate this part of the procedure. During testing of 30-day-postpartum dams, it was necessary not only to help pups locate the dams' nipples, but also to extrude and moisten dams' nipples in order to facilitate attachment. One min after 6 to 8 pups were attached, we began a series of threshold dose infusions of oxytocin (0.15 ml), delivered at 2-min intervals. Milk ejection was indicated by pups' synchronous display of a whole-body stretch accompanied by vigorous suckling and followed by a burst of rapid nipple-shifting [42]. Two min after the tenth infusion, we briefly removed the pups to determine litter weight gain and returned them for a second series of infusions. We designated a litter weight gain of less than 0.3 g after a series of infusions as criterion for depletion of a dam's milk stores. Fifteen-, 20-, and 25-day-postpartum dams received 5 additional threshold dose infusions before reweighing litter weight gain. Because pups suckling from 30-day-postpartum dams gained no weight during the first infusion series, these dams received one final large dose of oxytocin (0.05 ml of 1.5 U) to verify that shifts in the threshold of the milk ejection reflex could not account for the observed absence or low level of milk flow from dam to pups.

Dams that were not milk-depleted by the end of the second infusion series received a second, identically prepared foster litter. If criterion was attained at the end of the third infusion series (i.e., a total of 25 infusions), we omitted administration of the fourth series. For one 15-day-postpartum dam, it was necessary to add a fifth series with a third, identically prepared foster litter. In all but this one dam, body weight gains of the second litter were considerably lower than those of the first litter, and in some cases we recorded no weight gain or a weight loss during the final infusion series, confirming the validity of our criterion, i.e., that dams' current milk supply was nearly or completely depleted at the end of testing.

Control litters were treated identically to foster litters, except that after recording litter body weight, we returned them to their deprivation cages and maintained them at room temperature for a total of 1.5 hr. We determined litter weight change after 0.5 hr and at the end of the recording session to assess insensible weight loss in the absence of any milk receipt during a time period roughly equivalent to testing of 30-day-postpartum, and 15-, 20-, and 25-day-postpartum dams, respectively.

Data Analysis

To obtain for each dam an estimate of their total milk supply, we first combined litter weight changes of all litters tested with the same dam. Because every control litter lost a measurable amount of weight after both 0.5 hr (M=0.48 g) and 1.5 hr (M=1.15 g) at room temperature, we subtracted 0.50 g from each combined litter weight change computed for 30-day-postpartum dams, and 1.15 g from each combined litter weight change computed for 15-, 20-, and 25-day-postpartum dams. The resultant values provided corrected estimates of females' milk supplies. We compared milk supplies between the 4 groups with a Kruskal-Wallis test by ranks [34] and subsequently conducted individual comparisons based on Kruskal-Wallis rank sums [34].

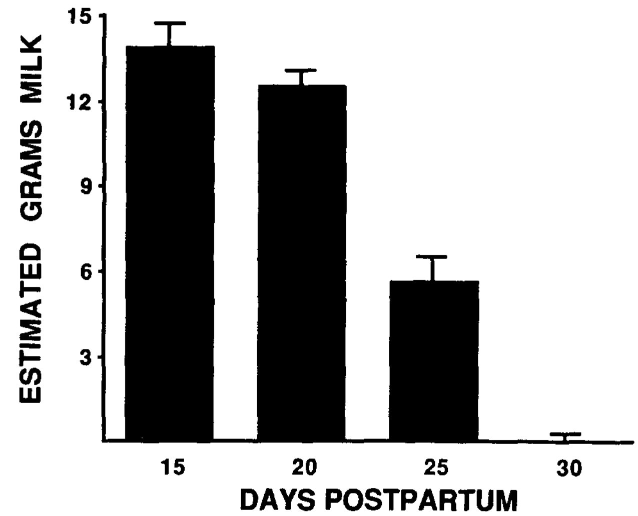

FIG. 1. Mean (±SEM) estimates of the amount of milk available in either 15-, 20-, 25-, or 30-day-postpartum dams (n=4 per lactational age) after a 3- to 4-hr accumulation period.

RESULTS AND DISCUSSION

The amount of milk stored in rat mothers changed significantly across lactational age, H[4(4,4,4)]=13.50, $p<0.01$. Fifteen-day-postpartum dams contained a mean of 13.98 g of milk compared to 12.58 g contained in 20-day-postpartum dams, 5.68 g in 25-day-postpartum dams, and no measurable stores of milk in 30-day-postpartum dams. Litters suckling from dams of this oldest lactational age group lost, on the average, exactly as much weight as litters not permitted to suckle at all during an equivalent time period (see Fig. 1).

Comparison of rank values revealed that milk stores did not significantly change from lactation Day 15 to Day 20, [absolute rank sum difference (critical difference=34), $p>0.05$]. However, reliable decreases in the amount of stored milk occurred between Days 20 and 25, and between Days 25 and 30; there was no overlap between any rank values of either 15- or 20-day-postpartum dams, and 25-day-postpartum dams, and the rank values of either 15-, 20-, or 25-day-postpartum dams and 30-day-postpartum dams.

The observed lack of change in milk supplies between Days 15 and 20 agrees well with findings of relatively stable mammary structure and cellular functioning during this period [58, 59, 62]. However, the pattern of results deviates sharply from the commonly observed reduction in pups' milk consumption during the corresponding postpartum period ([7, 33, 49]; but see [20]) and indicates that maternal milk supplies may not be the limiting factor of pups' milk intake during that period.

The decline in milk supplies observed in Day-25 dams agrees with both structural and physiological indices of mammary performance and pups' milk intake patterns (e.g., [7,49]). Late in lactation, *after* pups have begun to gradually shift from milk to solid food, the amount of milk produced by their mother may indeed limit milk intake.

We never recorded weight gain in pups tested with 30-day-postpartum dams, suggesting that little or no milk had accumulated in dams of such advanced lactational stage. Pups suckling from 30-day-postpartum dams never displayed stretch responses following oxytocin injections. The stretch response is a postural reflex reliably elicited by the release of milk into the mammary ducts [38,42]. In addition, these pups engaged in a high frequency of nipple-shifting and some abandoned the nipple entirely. High levels of nipple-shifting typically occur when there is little or no milk to be extracted [16]. Measurements of pups' milk consumption by other methods also suggest that by Day 30 postpartum only insignificant amounts of milk are ingested [5, 49, 62].

The difficulties pups exhibited in locating and attaching to the nipples of 30-day-postpartum dams—never a problem with dams of any of the other lactational age groups—suggest that these females, in addition to having no milk, no longer possess the stimulus characteristics that usually guide nipple attachment behavior (e.g., [9,13]). This is surprising in the light of other findings demonstrating that in the nest 30-day-old pups continue to spend about 2.5 hr per day attached to their mothers' nipples [17,61].

EXPERIMENT 2

The results of the previous experiment demonstrated that the amount of milk that *can* be extracted from rat mothers does not change between Day 15 and Day 20 postpartum. Nevertheless, numerous studies show that both the amount of time mother and pups engage in nursing contact [1, 17, 23, 39, 40, 46] and the amount of milk pups ingest ([6, 7, 33, 44, 49]; but see [20]) decrease from Day 15 to Day 20 postpartum. The results of Experiment 1 suggest that around the time pups begin to shift from milk to solid food, they may not always obtain all the milk the mother is able to provide.

To determine whether maternal milk supplies around the time of weaning exceed pups' milk consumption and, if so, whether such imperfect overlap between stored and obtained milk is dependent on pups' age, we measured the amount of milk 15- or 20-day-old pups obtained from either 15-, 20-, or 25-day-postpartum dams during a 4-hr nursing test. Measurement of milk intake of 25-day-old pups was, unfortunately, impractical for reasons detailed below. Testing dams of the most advanced lactational age group used in Experiment 1 (i.e., 30-day-postpartum dams) was superfluous in the light of the findings of Experiment 1.

As have many previous investigators, we used pup weight gain as an index of milk intake. However, we added the following features to enhance the accuracy of our measures: (1) We acutely sealed pups' excretory openings, thereby preventing weight loss due to urination/defecation. (2) We measured the amount of milk passing from mother to pups during a time period (4-hr) sufficiently long to allow dams and litters to settle and engage in nursing (as verified in informal observations during experimentation), but at the same time sufficiently short for pups to consume only milk produced by stimulation prior to the test period. In rats, latency of milk secretion in response to suckling stimulation is approximately 4 to 6 hr [21, 22, 28]. A 4-hr test period thus minimized possible confounding differences in milk secretion due to differentially intensive suckling by the various test litters [3]. We also obtained estimates of the amount of milk required to maintain pups' body weight during testing by measuring body weight loss during a 4-hr period without a caretaker.

METHOD

Subjects

A total of 55 primi- and multiparous female Sprague-Dawley rats and 604 pups, most of which were born to the test females, served in this experiment. Animals' housing and maintenance conditions were the same as described in Experiment 1. We tested mothers when their litters were 15, 20, or 25 days of age with either a 15- or 20-day-old litter. Due to difficulties in preventing pups from urinating (and therefore losing unknown amounts of weight) during testing, we had to delete 7 mother/litter pairs leaving a final number of 8 pairs (8 mothers and 64 pups) in each of the 6 groups outlined below. Eight additional litters, 4 at 15 days of age and 4 at 20 days of age served in control groups.

Procedure

Between 0730 and 0830 hr, 1 hr before testing, we separated test dams from their litter and prepared them for testing. After tranferring dams to standard maternity cages (48×20×26 cm) with fresh bedding (test arena), we voided pups' excretory passages by gently stroking their anogenital region with a Q-tip and sealed their excretory openings by applying small amounts of quick binding adhesive (Krazy Glue). Immediately before placing treated litters with test dams, we recorded pups' individual body weights accurate to 0.1 g and removed all food but not water from test arenas. Animals were arranged into 6 groups as follows: 15-day-postpartum dams with either 15- or 20-day-old litters (15D/15L and 15D/20L), 20-day-postpartum dams with either 15- or 20-day-old litters (20D/15L and 20D/20L), and 25-day-postpartum dams with either 15- or 20-day-old litters (25D/15L and 25D/20L). We attempted to include groups with 25-day-old litters; however, many of these older pups chewed the seals from their excretory openings, thereby not only injuring themselves but also reducing the accuracy of our measurement. All pairs were foster arrangements; that is, we never tested a dam with her own litter.

During the 4-hr testing period, mother-litter pairs were left undisturbed with the exception of a brief (10- to 15-min) interruption after 2 hr to record pups' body weight and reinforce the seals on their excretory openings. At the end of the 4-hr period, we reweighed pups and determined their individual body weight changes. Control litters were treated identically, except that they spent the test period in plastic tubs (27×18×13 cm) in an incubator (Isolette, Air-Shields Inc.) maintained at 28°C. Tub floors were covered with fresh bedding, and a water bottle was available. Control litters were used to determine body weight change under thermal conditions similar to the nest but with no milk provision.

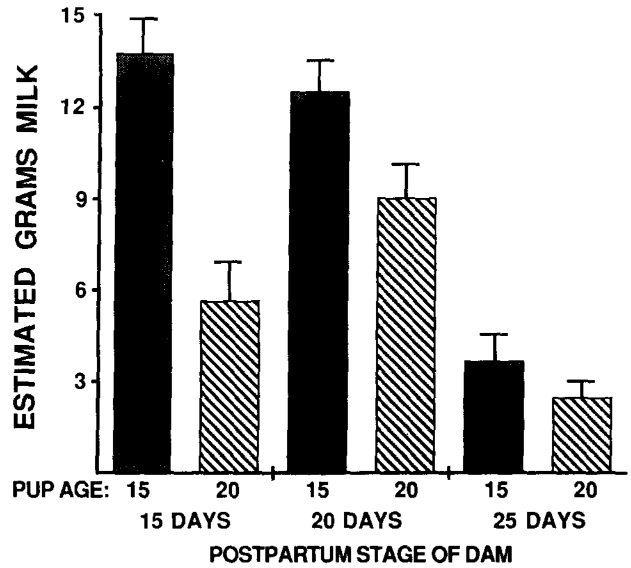

FIG. 2. Mean (±SEM) estimated milk transfer from either 15-, 20-, or 25-day-postpartum dams to either 15- or 20-day-old litters of 8 pups during a 4-hr test period (n=8 dams per condition).

Data Analysis

Both 15- and 20-day-old control pups lost significant amounts of body weight during the 4-hr test period, $t(31)=41.74$, and $t(31)=4.80$, both $ps<0.01$, one-tailed, for 15- and 20-day-olds, respectively. We subtracted the average litter weight loss of 15- or 20-day-old control litters (−3.18 g or −2.95 g, respectively) from the litter weight changes we observed in experimental litters of the same age. Litter weight changes were calculated by summing across littermates body weight changes during the combined, first and second 2-hr periods. The corrected litter weight gains provided for each dam an estimate of the total amount of milk the pups obtained during the 4-hr test period. We compared estimates of milk transfer between the 6 groups with a 3×2 ANOVA (Lactational Age × Pup Age; [67]) and used Tukey's HSD method [29] for subsequent comparisons of specific interest.

RESULTS

Dams' lactational age significantly affected the amount of milk transferred from mother to litter during the 4-hr test period, $F(2,42)=39.64$, $p<0.01$. A significant Lactational Age × Pup Age interaction, $F(2,42)=7.08, p<0.01$, indicates that the magnitude of the effect depended on the age of the suckling litter. Overall, 20-day-old litters obtained significantly less milk than 15-day-old litters, $F(1,42)=31.00$, $p<0.01$.

Figure 2 illustrates the estimates of milk transfer for each Lactational Age and Pup Age. Individual post hoc comparisons revealed that milk transfer did not differ between 15-

and 20-day-postpartum dams, but that 25-day-postpartum dams provided significantly less milk compared to the average amount provided by 15- and 20-day-postpartum dams during the 4-hr test period. This pattern of results was found both in dams nursing 15-day-old litters and in dams nursing 20-day-old litters; 15-day-old groups: 15D/15L vs. 20D/15L, Tukey's HSD (critical difference=3.97), $p>0.05$; combined 15D/15L and 20D/15L vs. 25D/15L, Tukey's HSD (critical difference=5.11), $p<0.01$; and 20-day-old groups: 15D/20L vs. 20D/20L, Tukey's HSD, $p>0.05$; combined 15D/20L and 20D/20L vs. 25D/20L, Tukey's HSD, $p<0.01$.

Milk flow from mother to litter depended not only on dams' lactational status but also on pup age. When lactational and pup age were matched, the amount of milk transferred during the 4-hr suckling test did significantly diminish from Day 15 (M=13.78 g) to Day 20 (M=9.05 g) postpartum, Tukey's HSD, $p<0.05$. In general, 20-day-old pups tended to receive less milk than 15-day-olds except when suckling from 25-day-postpartum dams which provided relatively little milk to litters of either age group. On lactation Day 15, older pups obtained, on the average, only 5.65 g compared to the average of 13.78 g received by 15-day-olds, Tukey's HSD, $p<0.01$. On lactation Day 20, the difference was less pronounced, M(15)=12.53 g vs. M(20)=9.05 g, and barely failed to reach statistical significance using Tukey's HSD method (critical difference=3.97), $p>0.05$. However, we considered sufficiently interesting the unexpected outcome that 20-day-old pups tended to receive less milk than 15-day-olds during 4-hr suckling from a 20-day-postpartum dam to pursue it further, both statistically and experimentally (see Experiment 3). Accordingly, we compared the results of only these two groups (20D/15L and 20D/20L) with a t-test for independent groups and found the difference in milk intake between 15- and 20-day-olds to also be significant in testing with 20-day-postpartum dams, $t(14)=2.50$, $p<0.05$. On lactation Day 25, the amount of milk obtained by 15- and 20-day-old litters did not differ significantly from one another, M(15)=3.67 g and M(20)=2.95 g, Tukey's HSD, $p>0.05$.

DISCUSSION

When dams' lactational and pups' chronological age were matched, milk flow from mother to pups significantly decreased from Day 15 to Day 20 postpartum. Despite their overall greater food requirements for maintenance and growth [10,24], 20-day-old pups suckling from 20-day-postpartum dams obtained considerably less milk (35%) during the 4-hr test period than 15-day-old pups suckling from 15-day-postpartum dams.

Although we did not measure milk consumption of 25-day-old litters suckling from 25-day-postpartum dams, the results of the two younger age groups paired with 25-day-postpartum dams suggest that the amount of milk pups obtain from their mother continues to significantly decrease (at least 67%) from Day 20 to Day 25 postpartum. Our weight gain data thus confirm the pattern of milk intake observed by other investigators using different assessment techniques ([6, 7, 33, 49]; but see [20]).

When only dams' lactational age was varied and pups' chronological age held constant, a different pattern of results emerged: milk flow from mother to pups did not change from lactation Day 15 to 20. Fifteen-day-old litters suckling from 20-day-postpartum dams obtained just as much milk as 15-day-old litters suckling from 15-day-postpartum dams. This lack of a maternal effect clearly demonstrates that the decline of pups' milk intake from Day 15 to 20 does not arise from growing limitations in mothers' potential lactational performance. Rather, the lower milk intake of 20-day-old litters compared to 15-day-old pups suggests that attributes specific to the older pups have suppressive effects on successful nursing encounters.

Relating the present values to those of Experiment 1, we first note that 15-day-old pups suckling from 15-day-postpartum dams extracted about all milk that was secreted or initiated for secretion prior to test onset. In contrast, 20-day-old litters appeared to have received only about 75% of the milk potentially obtainable from their 20-day-postpartum test mothers. Pups tested with 25-day-postpartum dams also extracted all available milk; however, whether under these circumstances 25-day-old pups would extract all milk or, like 20-day-olds suckling from 20-day-postpartum dams, would capture only a fraction of the actual amount we cannot estimate on the basis of the present results.

Thus, prior to weaning and possibly late during weaning, pups' milk consumption and the amount of milk available match; around the onset of weaning, pups' consumption falls short of and hence is *not* limited by the amount of milk their mothers can supply.

EXPERIMENT 3

In the previous experiment, we found that 20-day-old pups suckling from 20-day-postpartum dams obtain less milk than 15-day-olds suckling from 20-day-postpartum dams. Because the test duration was sufficiently short to minimize the effects of differential milk production during testing, this subpotential milk delivery to, or extraction by 20-day-old pups compared to 15-day-old pups on lactation Day 20 appears to have arisen from other, behavioral and/or physical factors specific to the older pups. For instance, dams paired with 20-day-old litters may have spent less time soliciting pups to suckle and/or more time engaged in behaviors (including postures) incompatible with nursing, than dams tested with 15-day-olds [51,53]. Furthermore, they may have terminated nursing episodes earlier than dams feeding 15-day-old litters, as 20-day-old pups are more thermogenic and hence more stressful to nurse for prolonged time periods [3, 40, 57]. Nevertheless, we have found that in a free-nesting situation, the duration of nursing episodes does not decline between Days 15 and 20 [17].

Alternatively, older pups may have actively lowered milk transfer by less frequently initiating nipple attachment or deserting the nipples sooner than younger pups. In the absence of maternal- and milk-deprivation, as was the case in Experiment 2, 20-day-old pups typically spend less time attached to a dam's nipples than 15-day-olds [16,26]. Clearly, 20-day-old pups' milk intake was not depressed due to feeding alternate nutritives during testing, for we removed all food from the test arenas prior to pairing dams and litters.

To determine the relative contribution of mothers and pups to the depressed milk intake of 20-day-old pups, we paired 20-day-postpartum dams with litters comprised of six 15-day-old and two 20-day-old pups. We assumed that the predominance of 15-day-old pups would lead mothers to engage in maternal behaviors appropriate to an entire Day 15 litter. Comparison of the amount of milk consumed by the 20-day-old pups of the present experiment with that of 20-day-old pups suckling from 20-day-postpartum dams of Experiment 2 would reveal whether milk intake of the latter group of pups was lower because of maternal factors or regardless of maternal differences (i.e., because of factors intrinsic to the pups).

METHOD

Subjects

A total of 14 primi- and multiparous female Sprague-Dawley rats and 112 pups served in this experiment. Animals were housed and maintained under the same conditions as described in Experiment 1. We tested females when their young were 20 days of age, whereas test pups were either 15 (84 pups) or 20 (28 pups) days of age. Test pups originated from 28 different litters, 14 per age group. Six 15-day-old siblings (3 females and 3 males) together with 2 of 20-day-old siblings (1 female and 1 male) constituted a test litter. Because of problems relating to the effectiveness of the sealing procedure (see below), we had to delete 4 dams and their test litters. The final sample consisted of 10 mothers and 80 pups, 20 at 20 days of age and 60 at 15 days of age.

Procedure

The testing procedure was the same as described in Experiment 2. Briefly, we separated test mothers from their litter between 0730 and 0830 hr, placed mothers into test arenas, and prepared pups for testing. After voiding pups' excretory passages, we sealed the openings with quick binding adhesive glue, recorded their individual body weights, and placed them with test mothers for a 4-hr test period. No food was available during this period, which we briefly (10- to 15-min) interrupted at midpoint to measure pups' body weights and reinforce the seals of their excretory openings. At the end of testing, we reweighed pups and determined their individual 4-hr body weight gains.

Data Analysis

Three comparisons of milk intake were of particular interest to us: intake of (1) 15- vs. 20-day-old pups suckling together from the same 20-day-postpartum dam; (2) 15-day-olds suckling with vs. without 20-day-old foster siblings from 20-day-postpartum dams; and (3) 20-day-olds suckling with vs. without 15-day-old foster siblings from 20-day-postpartum dams. We therefore calculated for each test litter 2 estimates of *litter* weight gain, one based on the average 4-hr body weight change of the 6 15-day-old pups and one based on the average body weight change of the pair of 20-day-old pups. As in Experiment 2, we corrected weight gain measures for pups' weight maintenance requirements during the test period. For this purpose, we subtracted the average body weight loss of 15-day-old control litters (−3.18 g; see Experiment 2) from every weight gain estimate for 15-day-old litters, and the average weight loss of 20-day-old control litters (−2.95 g) from every weight gain estimate for 20-day-olds. We thus obtained for each dam and test litter 2 measures of milk intake, one estimating intake of 8 15-day-old pups, and one estimating intake of 8 20-day-old pups.

We compared these estimates (i.e., 15- vs. 20-day-old litters) with a matched groups t-test [29], using dams as the matching variable. For comparison of milk consumption within pup age groups (i.e., with vs. without different-aged pups), we performed two independent groups t-tests [29], one for 15-day-old pups and one for 20-day-old pups.

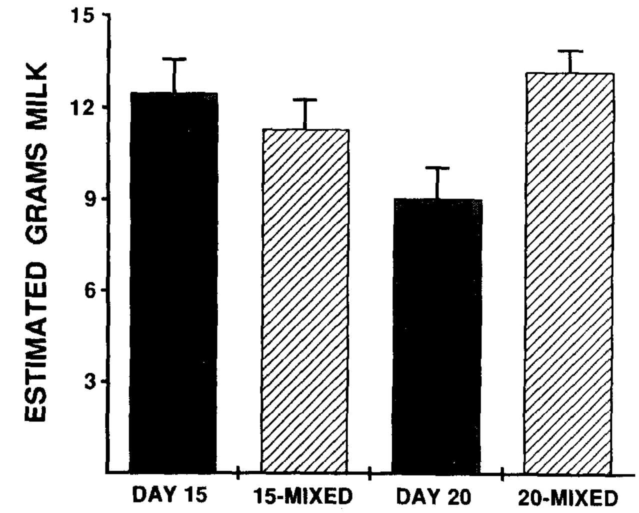

FIG. 3. Mean (±SEM) estimated milk flow from 20-day-postpartum dams to either 15- or 20-day-old pups during a 4-hr test period under either uniform or mixed litter conditions (Uniform = all 8 pups are either 15- or 20-days old; Mixed = 6 pups are 15- and 2 pups 20-days old). Estimates reflect transfer to a standard-sized 8-pup group (n=10 per condition).

RESULTS AND DISCUSSION

As in the preceding experiment, we found that milk flow from 20-day-postpartum dams to pups significantly depended on the age of the suckling young, $t(9)=2.53, p<0.03$, two-tailed. However, in contrast to our findings of Experiment 2, 20-day-old pups obtained *more* milk per pup than their 15-day-old foster littermates when the foster litter was predominantly comprised of 15-day-old pups. On the average, 20-day-olds extracted 13.19 g of milk per 8 pups during the 4-hr test period, whereas 15-day-olds extracted only 11.27 g of milk per 8 pups.

The reversed relation in the amount of milk obtained from 20-day-postpartum dams between the two pup ages originates from significantly higher milk intake by 20-day-old pups tested together with younger sucklings compared to those tested together with their own, same-aged littermates (M=9.05; see Experiment 2), $t(16)=3.55$, $p<0.03$, two-tailed. In contrast, milk consumption by 15-day-old pups was relatively unaffected by the age of companion pups, $t(16)<1$. Figure 3 illustrates the effects of the presence or absence of different-aged pups on milk transfer from 20-day-postpartum dams to 15- and 20-day-old pups.

At first glance, our findings seem to provide compelling evidence for maternal dictation of milk transfer. Younger pups are known to elicit higher levels of maternal care than older pups, whereas older pups are more likely to provoke rejection and evasive behavior by lactating females [50, 52, 53]. Therefore, the 20-day-old pups that were placed among 15-day-old pups probably experienced more maternal care and opportunity to suckle than pups from an entire Day 20 litter. Like younger pups, 20-day-old pups presumably extract all available milk if provided with the opportunity to do so. The increased milk intake by 20-day-old pups tested together with 15-day-old pups observed in the present experiment exemplifies such a notion (compare to Experiment 1).

In accordance with our results and this interpretation, Cramer and Blass [14,15] found that when the dams' behavioral contributions to nursing interactions are blocked by anesthesia, 20-day-old pups consume as much milk from the nipple as 15-day-old pups. It thus appears that reductions in dams' tendency to nurse their young are the source for the decline of milk consumption from postpartum Day 15 to 20.

Informal observations of both the mixed and uniform litter situations led us to entertain an alternative explanation of our results: 20-day-old pups placed among 15-day-old pups appeared to be more docile and oriented toward the dam than 20-day-old pups without younger littermates. That is, 20-day-old pups in the mixed litter situation appeared to behave like the younger pups. This suggested the possibility that the elevated milk consumption by 20-day-old pups we observed in the present experiment was due to social facilitation of suckling induced by the relatively more zealous 15-day-old pups.

Two sets of findings support such an alternative explanation. First, it has been well established that 15-day-old pups are more prone to spontaneously (i.e., without deprivation) attach to the nipple than 20-day-old pups, even in the absence of any behavioral contribution by dams. Under test conditions in which mothers' behavior was blocked by anesthesia, the proportion of pups attaching to the dams' nipples was higher and the latency to the first attachment shorter in 15- compared to 20-day-old pups [25–27, 66].

Second, it has recently been demonstrated that social stimulation from younger pups can override the relatively diminished tendency of older pups to attach to the nipple. Individual postweaning-age pups, housed with a constant series of preweaning-age mothers and their 16- to 21-day-old litters, will continue to suckle until they are up to 70 days of age, well beyond the time of normal weaning [41,45]. It is noteworthy that older pups never attached to a nipple in the nest when none or less than half of the young pups were attached [45].

Given the feasibility of this alternative explanation, we assessed attachment to the nipples of anesthetized 20-day-postpartum dams by 20-day-old pups with and without the presence of 15-day-old littermates. We reasoned that if intake levels of 20-day-old pups tested in the company of 15-day-olds were indeed elevated due to social facilitation (as opposed to enhanced milk accessibility), then these facilitative effects on suckling should persist even under conditions of equalized suckling opportunities. Using anesthetized dams enabled us to create equal suckling opportunities between the two litter situations (i.e., uniform 20-day-old litters and mixed litters consisting of two 20-day-old and six 15-day-old pups); however, anesthesia can interfere with milk ejection [42], and we therefore measured latency to the first nipple attachment and total attachment time during a 1-hr test rather than milk intake.

Because the data were neither normally distributed nor equally variable between groups, we analyzed them with the Wilcoxon test [34]. Based on 10 independent observations per condition, we found a relatively reliable effect of litter type on attachment latency, but not total attachment time. Median attachment latency of 20-day-old pups tested with younger littermates was 5.47 min compared to 8.60 min required by 20-day-old pups tested with their biological littermates, $W(10,10)=1.59$, $p<0.06$. The median attachment times of the respective groups of pups were 53.25 min and 48.75 min, $W(10,10)=1.29$, $p<0.10$. Of course, total attachment time was constrained by the particular test duration we used, and the statistical evaluations of this measure therefore should be interpreted cautiously.

In general, 20-day-old pups, when among agemates, spent the first 5- to 15-min playing and exploring the test arena without specifically attending to the dams' exposed nipple regions. We frequently observed 20-day-old pups that did not attach at all during the 1-hr period. In contrast, 20-day-old pups tested in the company of 15-day-old pups quickly concentrated their explorations onto females' ventrum and frequently seized nipples that 15-day-old pups had just begun to suckle.

We interpret these results as indicating that at least the attachment component of suckling behavior by 20-day-old pups may be sensitive to social stimulation; nipple attachment can be slightly enhanced by testing with younger pups. However, because the evidence for social facilitation of suckling of 20-day-old pups by younger pups was not very strong, we believe that primarily maternal factors such as females' accessibility for milk extraction contributed to the higher milk consumption of 20-day-old pups in the present experiment compared to Experiment 2. We therefore conclude that the subpotential milk intake of 20-day-old pups suckling from 20-day-postpartum dams arises from depressed willingness of dams to nurse older pups and, to a lesser degree, from dissipating interest of older pups in attaching to the nipple.

GENERAL DISCUSSION

The results of the present series of experiments have important implications for our understanding of the dynamics that underly the decline and eventual disappearance of milk consumption by young rats. Experiment 1 established that the amount of milk that *can be* extracted from rat mothers does not change significantly from postpartum Day 15 and 20, begins to wane sometime between Days 20 and 25, and is negligible by postpartum Day 30.

Experiment 2 showed that the amount of milk pups consume nevertheless begins to decline between postpartum Days 15 and 20, i.e., several days before there is measurable reduction in maternal milk supply. Experiment 2 also showed that 20-day-postpartum dams are as able to release milk as are 15-day-postpartum dams; we found that 15-day-old litters consumed equal amounts of milk suckling from either a 15- or 20-day-postpartum dam.

Experiment 3 demonstrated that the decline in pups' milk consumption from Day 15 to 20 can be attenuated by integrating 20-day-old pups into the nursing interactions that take place between 15-day-old litters and 20-day-postpartum dams. Additional manipulations revealed that the attenuation effect is partially due to social dynamics: 15-day-old pups have a subtle facilitative effect on nipple attachment by 20-day-old pups that is not mediated by maternal factors.

This combination of results led us to the following conclusions. First, the *onset* of the decline in milk intake cannot be attributed to changes in either the amount of milk available in rat mothers or their ability to transfer the available milk to suckling young. That is, contrary to common belief (e.g., [19, 30, 60]), pups do not begin to ingest less milk because the supply diminishes.

Rather, the findings of Experiment 3 suggest that the decline of pups' milk consumption is initiated by changes in both dams' nursing and pups' suckling behavior. Pups seem to begin ingesting less milk after postpartum Day 15 primarily because mothers are less likely to make the available milk accessible to the young as they grow older. In addition, pups' milk intake decreases after postpartum Day 15 because older pups are less likely to create or take advantage of suckling opportunities.

The first of these two conclusions is supported by numerous demonstrations that dams spend progressively more time away from the litter and thereby increasingly deny pups access to their milk resources over the course of lactation [23, 39, 40, 46, 53]. This development in dams' behavior is mediated by age-related changes in the pups, for replacing pups by younger ones attenuates the decline of females' attendance to the young [12, 50, 51, 53, 65]. Thus, it is diminishing milk *accessibility* that underlies the decline in pups' milk consumption after postpartum Day 15. The diminution of milk accessibility appears to be provoked by the pups themselves (i.e., their physical, physiological, and/or behavioral make-up as perceived by the dams) and commences well before any changes in maternal milk availability.

Decreasing milk accessibility does not appear to be the only factor that contributes to the change in pups' feeding habits. During the first 25 days of their lives, rat pups undergo tremendous structural, physiological, and sensorimotoric changes that permit, if not increasingly favor, interaction with the environment outside the nest (e.g., [2, 4, 31]). As the array of possible activities broadens, the relative attractiveness of suckling decreases, and pups no longer pursue every opportunity to attach to the nipple and extract milk. Suckling regains relative attractiveness when all potential play partners are engaged in suckling and/or few alternative behaviors are available ([35, 41, 45, 56], Experiment 3).

Although maternal milk supplies play no role in the onset of the decline in milk intake, they may be instrumental in the eventual *disappearance* of milk consumption. Because we could only assess the amount of milk available in 25- and 30-day-postpartum dams, but not the amount that 25- and 30-day-old litters actually obtain, we do not know whether milk availability is the limiting factor of pups' intake late during lactation. Obviously, as milk supplies begin to progressively decline after Day 20, pups are forced to give up milk as a food source and instead increasingly rely on alternate ingesta.

In summary, behavioral elements associated with the transfer of milk from mother to pups appear to play a critical role in the initial decline of pups' milk consumption, whereas the amount of milk available in rat mothers may critically contribute to the complete disappearance of suckling and replacement by solid food intake.

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