NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

Factors Influencing the Self-Selection of Calcium in Lactating Rats

Lisa Millelire

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montréal, Québec, Canada

April 1988

ISBN 0-315-44838-5

ABSTRACT

Factors Influencing the Self-Selection of Calcium in Lactating Rats

Lisa Millelire

Factors influencing the self-selection of calcium in lactating rats were investigated. In Experiment 1, two groups of lactating rats, one having a litter size adjusted to four pups (n = 10) on the day after parturition and the other a litter size adjusted to 16 pups (n = 9), were given ad lib access to a 2.4% solution of calcium lactate, demineralised water, and a calcium deficient diet. Calcium, water and food intake were compared for these two groups both before impregnation and during 16 days of lactation. Females nursing 16 pups increased their calcium and food intake over the course of lactation more than did mothers nursing four pups. In Experiment 2, a similar procedure was employed to determine whether milk delivery was a necessary prerequisite for an increase in calcium intake during lactation. Female rats were divided into four groups consisting of 10 nonimpregnated, 10 impregnated, 10 impregnated galactophore-cut, and nine sham operated impregnated animals. Litters of all reproducing females were adjusted to eight pups per litter on the day after parturition. To maintain pup health and thus equivalent suckling stimulation among groups litters were switched between galactophore-cut, impregnated and colony foster mothers every twelve hours. Over the course of lactation galactophore-cut dams showed an increase in calcium intake compared to nonlactating females. Further, galactophore-cut females took in similar quantities of calcium as both intact and sham

operated impregnated animals in the first week of lactation. These studies showed that female rats do selectively increase their calcium intake during lactation. Moreover, this increase was found to vary as a function of litter size and persist in the absence of milk delivery. The results of these studies are discussed in terms of potential mechanisms.

Acknowledgements

I would like to thank my thesis supervisor, Dr. Barbara Woodside, for her guidance, support, and incredible patience.

I appreciate the many helpful comments and suggestions from my committee members, Drs. Elizabeth Henrik and Dwight Mazmanian.

A special thank you to Dr. Jim Jans for reading the final version of this thesis.

Lastly, I would like to thank Tom for drawing the figures and keeping me happy, my parents for their encouragement, and my friends, Deirdre, Nora, Paul and Madeleine for the good times.

TABLE OF CONTENTS

LIST OF FIGURES

PAGE

Rats are an example of an altricial species, that is, a species that is totally dependent on its mother for sustenance in early life. Moreover, the female rat reproduces a number of times during her life (Galef, 1981). Consequently during any one reproductive episode the female rat must partition both her time and energy resources in the most efficient way in order to guarantee the survival of her young, without compromising her own future reproductive success. A successful outcome to a reproductive episode is achieved by a complex interaction of both physiological and behavioral mechanisms.

The provision of warmth and adequate nutrition by the rat dam to her young are two of the most important activities she will engage in to ensure the survival of her offspring, and both are potentially costly with respect to resource allocation. Keeping the pups warm is primarily a drain on maternal time. Rat pups have very limited thermoregulatory abilities and the major sources of warmth in their environmentare maternal body heat and the warm milk delivered by the dam. If ambient temperature is low, then the dam spends almost all day (1200 minutes) in contact with the pups (Jans & Leon, 1983). This cost can be offset by maternal behavior. For example, the dam may construct a nest for her pups that effectively raises the ambient temperature of the immediate environment. Increasing the ambient temperature at which the dam rears her young has a variety of effects on pup development (Jans, deVillers, & Woodside,1985) and for the dam herself (Jans & Woodside, 1987). Providing the pups with a warm environment reduces the total amount of time that the dam spends in contact with her young by as much as 50% when compared to females that have reared their young in cooler environments (Jans & Leon, 1983). In spite of this reduction

in mother-young contact, however, pup growth is maintained because pup energy expenditure associated with thermoregulation is minimized. As well, increased ambient temperature decreases the latency to the first milk ejection and thus facilitates milk delivery in the dam (Jans & Woodside, 1987). Further, dams raising their pups in a warm nest show reduced food intake and a shorter period of lactational diestrus than those rearing their pups in a cooler ambience. Thus the behavior of nest building and its interaction with physiological mechanisms may significantly reduce the cost of reproduction for the dam.

Providing adequate nutrition for the young is the other major energetic cost of lactation. Brody, Riggs, Kaufman, and Herring (1938) have estimated that the metabolic rate of females at the period of peak lactation is three times that of the nonpregnant rate. This high energetic cost is again offset by both physiological and behavioral mechanisms. Throughout lactation anatomical changes in the digestive tract occur, where the alimentary canal progressively increases in weight and size and thus absorptive capacity (Cripps & Williams, 1975). Accompanying these physiological changes are behavioral modifications, for example, the female rat meets the nutritional demands of her young by changing her feeding behavior. In particular she may alter the amount and type of food that she selects. During lactation the female rat may increase her food intake by three times as much as that eaten prior to mating (Fleming, 1976) and this increase has been found to be proportional to the number of pups she suckles (Ota & Yokoyama, 1967). Further, Leon and Woodside (1983) have found that when lactating rats are given a diet diluted with a non-nutritive substance, they will increase their diet intake in order to consume as much of the nutrient

fraction as controls.

The factors mediating the hyperphagia of lactation are unclear, however, the events accompanying the nursing situation suggest a number of possible mechanisms. Lactation in the female rat is initiated and sustained by the suckling stimulation received from her young. This suckling stimulation not only provides a means for milk withdrawal but also stimulates the release of hormones from the anterior pituitary needed for continuation of lactation. In particular, prolactin and adrenocorticotrophic hormone serve to stimulate milk synthesis and the development of the mammary gland (Simpson, Simpson, Sinha, & Schmidt, 1973). Further, the release of oxytocin from the posterior pituitary is stimulated which in turn is essential for milk let down, while gonadotrophic activity is inhibited (Turner, 1966). Cotes and Cross (1954) have suggested that the suckling stimulus alone and/or its induced release of hormones are responsible for the observed hyperphagia in lactating rats. Lactating dams that receive suckling stimulation from their young but are prevented from delivering milk as a result of galactophore ligation continue to show a significant increase in food intake over non-suckled galactophore-cut animals whose litters have been removed at birth (Cotes & Cross, 1954).

Regardless of the etiology of lactational hyperphagia these findings suggest that in terms of providing adequate nutrition to their offspring female rats can meet the increased caloric demands of lactation. Different stages of the reproductive episode, however, may call for expenditure of specific nutrients; the question of how well the female rat may be able to select such nutrients has been less well investigated. One nutrient that is known to be of particular

importance during a reproductive episode is calcium (Garel, 1987). In general, calcium is an important and necessary mineral to the body and is involved in many physiological functions. In the human body, calcium is the most abundant cation consisting of 2% of total body weight. As much as 99% of all calcium is contained in bone. The remaining calcium is involved in the stimulation of muscle contraction, regulation of synaptic transmission, blood coagulation, myocardial function and activation of enzymes all of which in turn are required for normal behavior (Czarnecki & Kritchevsky, 1980).

During lactation a specific demand is placed on the female rat for calcium which is delivered to the young in large quantities through the dam's milk (Czarnecki & Kritchevsky, 1980). For example, in the lactating rat the daily loss of calcium in milk is approximately 100 mg, which is 30 times more than the daily urine excretion. Over 21 days of lactation this is equivalent to the transfer of 60% of the calcium content of the maternal skeleton to the litter; yet, the lactating dam may lose only 15% of her calcium bone stores (Garel, 1987). If there is insufficient calcium in the diet of a lactating rat then this shortfall is compensated for by the resorption of large amounts of calcium from the maternal skeleton, such that 50% of the initial bone calcium content is lost (de Winter & Steendijk, 1974).

The female rat is a particularly good candidate in which to study mechanisms of calcium homeostasis during lactation because of the much higher rate of calcium excretion in the milk relative to body size when compared to humans, where the daily loss of calcium in the milk is about 1000 mg. When expressed per kilogram of body weight the calcium loss in the lactating rat is approximately 60 times greater than that

of the lactating woman (Toverud, Boass, Haussler, & Pike, 1983). This great stress placed on the homeostatic mechanism in the rat in turn accentuates hormonal and behavioral adjustments. The next question then, is what metabolic and behavioral changes in lactating rats allow them to meet this specific demand?

The homeostatic mechanism used to regulate calcium works within extremely narrow limits (Lloyd, McDonald, & Crampton, 1978). Upon calcium consumption from the diet, the level of serum calcium in the body is controlled by a hormonally mediated mechanism involving parathyroid hormone (PTH), vitamin D and calcitonin. During hypocalcemia the secretion of PTH from the parathyroid gland is increased. This increase in turn stimulates the synthesis of 1,25-dihydroxyvitamin D3 (the metabolically active form of vitamin D) in the kidney by activating the enzyme 25-hydroxyvitamin D3 1-hydroxylase. Parathyroid hormone in conjunction with 1,25-dihydroxyvitamin D3 enhances calcium absorption across the intestinal tract, increases bone resorption and kidney calcium absorption, thus increasing calcium serum levels. Under conditions of hypercalcemia the parathyroid stimulates the thyroid gland to secrete increased amounts of calcitonin. Calcitonin has the opposite effects from PTH in that it functions to decrease blood levels of calcium. Calcitonin exerts its effects by promoting the absorption of calcium into bone and by preventing the mobilization of calcium from the bone (Freed, Perlow, & Wyatt, 1979).

During lactation adaptive changes to this mechanism are made; for example, the percentage of net calcium absorption across the intestine may rise up to three fold (Fournier & Sousbielle, 1952; Kostial, Gruden, & Durakovic, 1963), an effect that depends on the high serum

levels of prolactin during lactation which, in turn, lead to high levels of 1,25 dihydroxyvitamin D3 (Robinson, Spanos, James, Pike, Haussler, MaKeen, Hillyard, & Macintyre, 1982). As well, 15% of maternal skeletal calcium may be depleted during lactation (Warnock & Duckworth, 1944) and urinary calcium excretion is decreased (Fournier & Susbielle, 1952). In spite of these mechanisms, serum calcium is somewhat lower in lactating rats than in nonlactating females and it has been suggested that this reflects an increase in skeletal conservation of calcium in the lactating female (Toverud, Cooper, & Munson, 1978).

A number of investigators have shown that when calcium metabolism is disturbed, for example, by parathyroidectomy, rats are able to compensate by selectively increasing their intake of calcium solution (Richter, 1937). It seems reasonable, therefore, to suppose that the lactating rat would show a similar change in behavior to augment the physiological mechanisms that appear to conserve calcium during lactation.

Indeed, the early cafeteria-selection studies of Richter and Barelare (1938) showed that during pregnancy and lactation rats selected a diet rich in protein, fat, and calcium and were able to maintain a pattern of nutrient intake conducive to normal growth and reproduction. These authors suggested that the particular selection of these nutrients indicated their importance during reproduction. Other researchers, for example, Tribe, (1955) unfortunately have not been able to replicate the results of Richter and Barelare and the differences in results have been attributed to problems of palatibility with specific nutrients.

Recently Woodside and Millelire (1987) used a two-choice technique adapted from Richter and Ekert (1937) to investigate calcium self-selection during reproductive episodes in female rats. This study was designed to determine whether female rats given access to demineralised water, a 2.4% solution of calcium lactate and a calcium deficient diet would demonstrate selective intake of calcium by increasing their intake of the calcium solution during pregnancy and lactation over those levels observed in nonimpregnated females on the same diet and impregnated animals given access to a calcium sufficient diet and the same fluid choices. The results of this study suggest that female rats do selectively increase their calcium intake during lactation. The reproducing female rats on the calcium deficient diet took in more of the calcium solution than the nonreproducing females on a similar diet. Further, these females increased their intake of calcium solution relatively more than their increase of water. Moreover, impregnated females on the calcium deficient diet showed a greater intake of calcium solution than those reproducing females having access to a calcium sufficient diet. These data strongly suggest that rat dams selectively increase calcium intake during lactation.

Given that this selective increase does occur the next question is the mechanism by which this change in intake is brought about. Specifically, two questions remain: first, how does the animal select a specific nutrient; and second , what factors influence the amount of intake of a particular nutrient displayed within a particular manipulation.

The first of these two questions has been addressed extensively in the area of specific hungers. Generally, in the specific hunger

literature substances have been divided into two categories, those for which the animal has an innate hunger, for example sodium, and those for which the specific hunger is learned, for example, thiamine (Rozin & Kalat, 1971).

It has been thought that the learned preference for a specific nutrient is based on the associations between the taste of a food that contains a needed dietary substance and the beneficial effects of its ingestion (Rozin, 1976). On the other hand, an abundance of literature (Nachman, 1962; Handal, 1965; Richter, 1936, 1939) has suggested that the appetite for some substances, in particular, sodium, is not learned but rather is innately or genetically predetermined. In the case of calcium, however, many conflicting findings exist as to its learned or innate properties (Rodgers, 1967; Frumkin, 1975) and no conclusive evidence has as yet has been reported.

The studies described below are concerned with the second of the two questions outlined above, that is, which factors within a particular manipulation may lead the animal to display a specific hunger.

Richter and Eckert, (1937) suggested that a selective increase in intake of a particular nutrient may simply be the direct response to a deficit of a specific nutrient. An animal that is deficient in a particular substance simply eats more of that substance to compensate for its deficiency. Such a deficiency could be produced in a number of ways: by removing a specific nutrient from the animals diet, increasing the loss of that nutrient by either increasing output, as in lactation, or by reducing the means of conserving a particular nutrient, as after adrenalectomy. Thus, in the case of lactation,

females simply increase their intake in order to compensate for deficiency induced by increased loss of a specific nutrient, milk. It has recently been suggested, however, that a selective increase in intake of a particular nutrient can be obtained by directly stimulating the neural substrate mediating the appetite for that substance; for example, Epstein (1982) has shown that intraventricular administration of angiotensin II in sodium-replete rats results in an increased appetite for sodium. Such a mechanism would allow for a preventative increase in intake of a particular nutrient; that is, these data raise the possibility that a hormonal change is sufficient to obtain a change in appetite.

In the present paper, I address the question: what are the critical stimuli present during lactation that promote or instigate the display of the specific hunger for calcium ? Specifically, does the female lactating rat increase her dietary preference for calcium as a result of her hormonal status, suckling stimulation, and/or milk delivery per se ? To investigate this problem I determined whether relative calcium intake would increase with litter size; that is, can lactating females selectively increase their calcium intake as a function of litter size? In the second experiment I determined whether milk delivery was a necessary prerequisite for the female to show a selective increase in calcium intake.

Experiment 1

Generally it has been reported that female rats nursing large litters eat more than those nursing small litters (Leon & Woodside, 1983). Moreover, Ota and Yokoyama (1967) have shown that dams nursing either two, four, eight or twelve pups increased their food intake in proportion to the number of pups they were suckling. These authors further suggested that the number of suckling pups may affect the rate of secretion of pituitary hormones associated with the development of the mammary gland and thus the amount of milk produced may be directly regulated by the number of suckling pups (Ota & Yokoyama, 1967).

Given that female rats can selectively increase their calcium intake during lactation (Woodside & Millelire, 1987) it was of interest to me to see if calcium intake, as with food intake, would vary as a function of litter size. In the present experiment, therefore, animals were randomly divided into two groups and the number of pups per litter was adjusted on the day after parturition to four or 16 respectively. It was expected that dams nursing 16 pups would self-select greater amounts of calcium lactate than those dams nursing only four pups.

Method

Subjects

Nineteen virgin female Wistar rats obtained from Charles River Breeding Farms (St. Constant, Quebec) served as subjects. All animals were approximately three months of age and weighed between 240 and 290 grams.

Procedure

All animals were housed in individual plastic cages, 38cm x 33cm x 17cm in dimension with Beta chip bedding. They were kept in a 12 hour light/dark cycle room having a temperature of 20°C ± 2°C. All animals had ad lib access to a 2.4% calcium lactate solution, demineralised water and a calcium deficient diet (ICN, Nutritional Biochemicals, Cleveland, Ohio) as shown in Appendix A.

Both water and the calcium lactate solution were presented in 100 mL graduated cylinders equipped with rubber stoppers and metal spouts. The calcium deficient diet was served in glass jars affixed to metal supports which in turn were secured to the side of the cage. A Sartorius scale (Model no. 1206 BMP3) and an Ohaus triple beam balance were used to weigh food and animals respectively. In order to avoid a response bias to the position of the water and calcium lactate, tubes were alternated daily, while the position of the calcium deficient diet remained the same. Each container was refilled when necessary; approximately every two days.

Impregnation was achieved by introducing males into the home cages of the females. Vaginal smears were taken each morning until the presence of spermatazoa confirmed impregnation. On the day after parturition animals were randomly assigned to one of two groups, one

including 10 dams with a litter size adjusted to four pups and the other consisting of nine dams each having a litter size adjusted to 16 pups.

Intake measurements of the three nutrient fractions, as well as female and where appropriate, pup weight were recorded bidaily between 0900 and 1200 hours. Data for both groups were collected for eight days prior to mating and for 16 days of lactation. While data were not collected during pregnancy animals were maintained on the same dietary regimen throughout this period.

Data were collected bidaily and a mean bidaily intake for blocks was calculated for each animal. Blocks consisted of eight days baseline, days one through eight of lactation and days nine through 16 of lactation. In order to account for variation between groups observed in the baseline period, data were analysed as absolute change from baseline for the two eight day blocks of lactation. A two-way split-plot analysis of variance with Litter Size as the between groups factor and Time as the within groups factor was carried out on the blocked data for calcium, water and food intake. All source tables for analyses of variance are shown in Appendix B.

Results

Calcium Intake

Figure 1 shows the mean bidaily change in intake of calcium solution expressed as change from baseline and averaged for blocks for both groups over the course of lactation. Both groups of animals increased their calcium intake over the 16 days of lactation (significant main effect for time; $F(1,17) = 35.49$, $p<.001$). Overall, animals nursing 16 pups took in more of the calcium solution during

lactation than did those animals nursing four pups (significant main effect for groups; $F(1,17) = 6.03$, $p<.05$), however, most of the increase of the calcium solution by dams nursing 16 pups ocurred in the second week of lactation (significant interaction of Groups x Time; $F(1,17) = 10.16$, $p<.01$).

When these data are expressed as relative change in calcium solution, that is, change in the percentage of total fluid intake ingested as calcium solution, the analysis of variance reveals a statistically significant effect of time ($F(1,17) = 27.22$, $p<.001$). The main effect for groups did not reach statistical significance but the Groups x Time interaction did ($F(1,17) = 6.25$, $p<.05$), that is, dams nursing 16 pups showed a significantly higher relative change in intake of calcium solution in the second week of lactation compared to dams nursing four pups.

Gram Calcium Intake

The calcium intake data above, expressed as grams of calcium ingested, show that dams nursing four pups on the average took in 0.09 $\pm$ 0.07 grams of calcium, bidaily, above baseline levels in the first week of lactation and 0.29 $\pm$ 0.09 grams of calcium in week two of lactation. Animals nursing 16 pups increased their bidaily intake by 0.19 $\pm$ 0.07 grams of calcium during week one of lactation and by 0.84 $\pm$ 0.15 grams of calcium in the second week of lactation.

Water Intake

Mean bidaily intake of water expressed as change from baseline and averaged per block of lactation is shown in Figure 2. Water intake increased significantly over the period of lactation in both groups of animals (significant main effect for time; $F(1,17) = 6.17$, $p<.05$). No

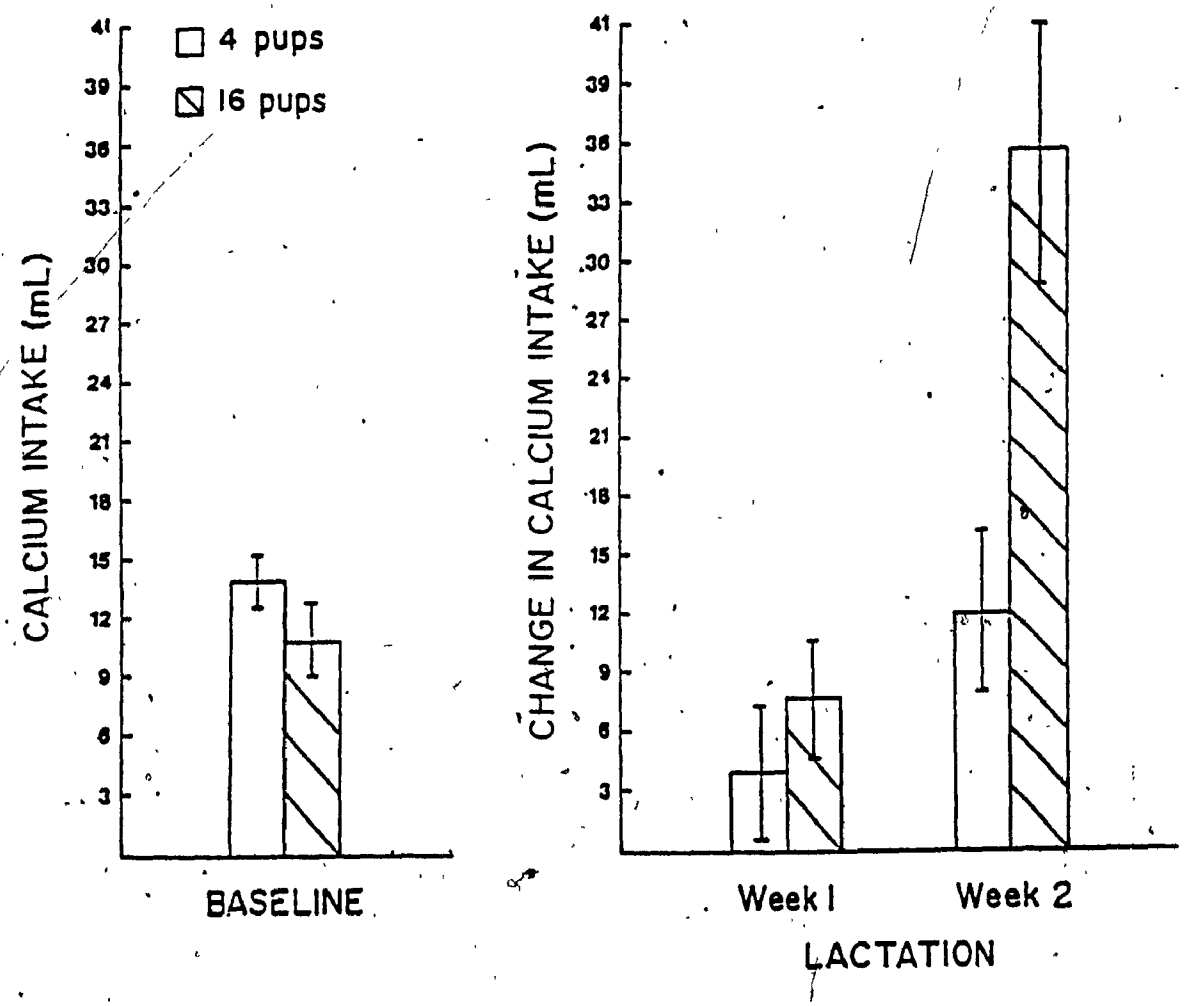

Figure 1. Bidaily intake of calcium solution (mL) for dams nursing four pups and dams nursing 16 pups over the course of lactation, expressed as change from baseline and averaged over eight day blocks (means ± S.E.M.'s) are shown. The left hand panel shows the baseline levels for the two groups (means ± S.E.M.'s) are shown.

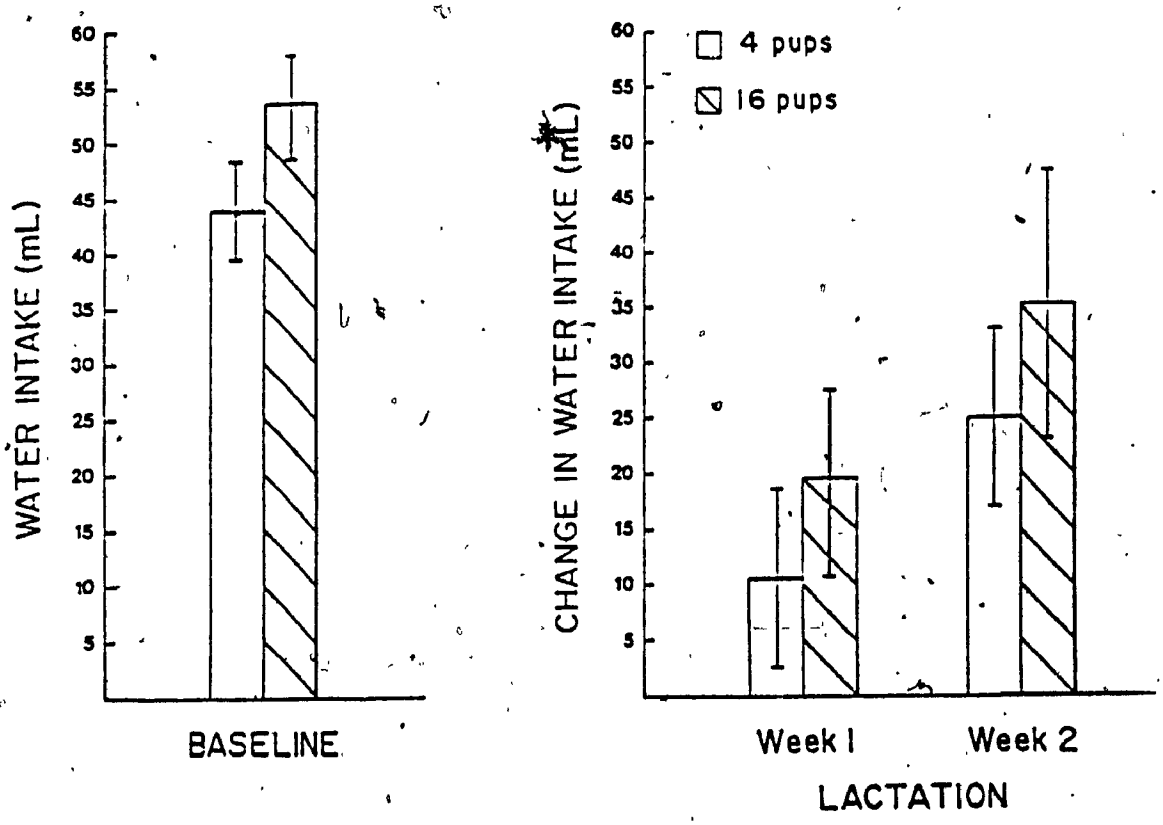

Figure 2. Bidaily water intake (mL) for dams nursing four pups and dams nursing 16 pups over the course of lactation, expressed as change from baseline and averaged over eight day blocks (means ± S.E.M.'s) are shown. The left hand panel shows the baseline levels for the two groups (means ± S.E.M.'s) are shown.

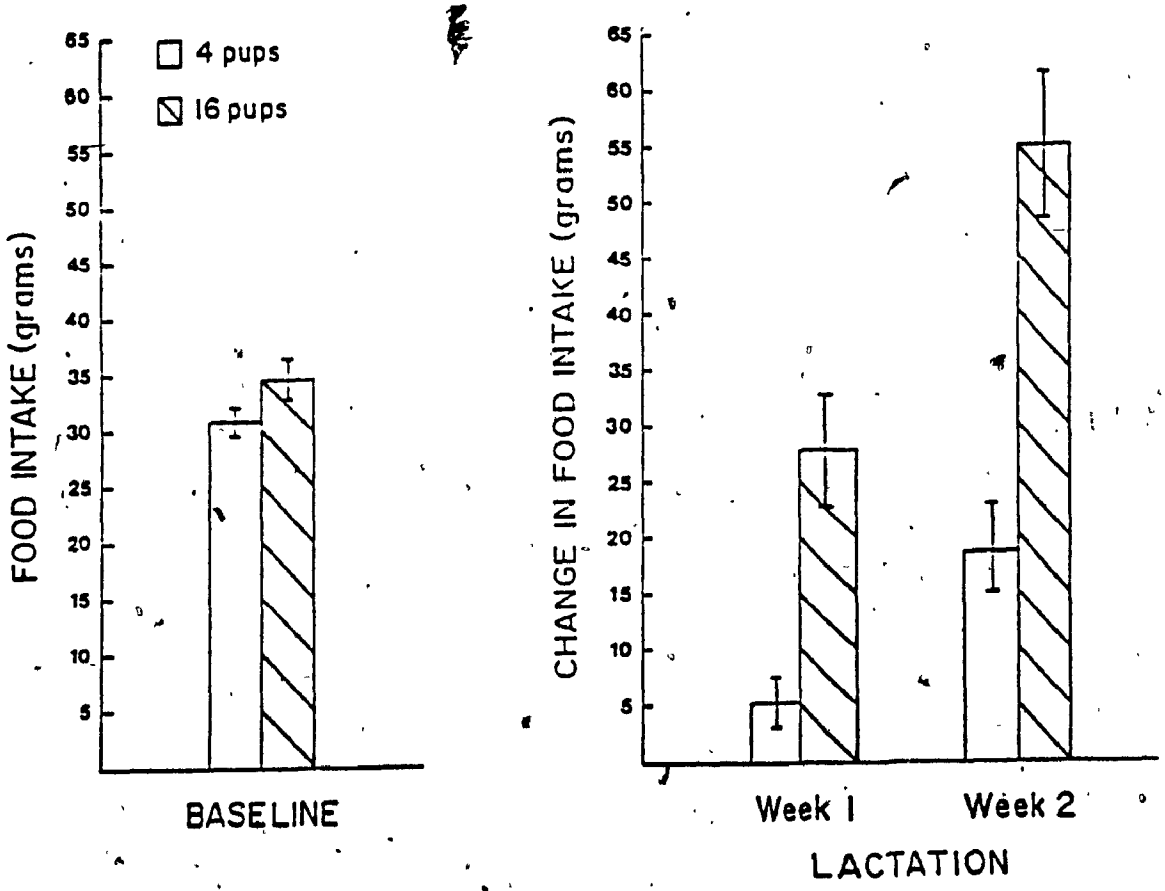

Figure 3. Bidaily food intake (g) for dams nursing four pups and dams nursing 16 pups over the course of lactation expressed as change from baseline and averaged over eight day blocks (means ± S.E.M.'s) are shown. The left hand panel shows the baseline levels for the two groups (means ± S.E.M.'s) are shown.

other statistically significant effects were obtained.

Food Intake

Figure 3 shows the mean bidaily food intake, expressed as change from baseline and averaged per block of lactation. Dams nursing 16 pups and those nursing four pups ate increased amounts of food over the course of lactation (significant main effect for time; $F(1,17) = 111.64$, $p<.001$). Animals suckling 16 pups, however, ate more in both the first and second week of lactation than did those suckling four pups, (significant main effect for groups; $F(1,17) = 21.96$, $p<.001$ and significant interaction of Groups x Time; $F(1,17) = 13.2$, $p<.01$).

Reproductive Outcome Measures

There were no differences between dams nursing four pups and dams nursing 16 pups in number of pups born (litter size of 16; mean = 11.8 $\pm$ 0.9, litter size of four; mean = 9.5 $\pm$ 1.03, $t(17) = -1.65$, $p>.05$), weight of newborn pups (litter size of 16; mean = 6.28 $\pm$ 0.2, litter size of four; mean = 6.4 $\pm$ 0.28, $t(17) = 0.31$, $p>.05$) or percent mother weight change over the course of lactation, (litter size of 16; mean = -8.1 $\pm$ 3.34, litter size of four; mean = -5.7 $\pm$ 2.23, $t(17) = 0.59$, $p>.05$). Differences, however, were found between the two groups in weight of pups on Day 16 postpartum (litter size of 16; mean pup weight = 20.43 $\pm$ 1.57, litter size of four; mean pup weight = 31.75 $\pm$ 2.53, $t(17) = 3.79$, $p<.05$).

Discussion

The results obtained from my first experiment confirm the hypothesis that lactating dams nursing different size litters and given the chance to self-select for calcium will increase their intake as a function of litter size. Overall, dams nursing 16 pups increased their

calcium intake more than did mothers nursing four pups. This increase was observed mostly in the second week of lactation where dams with litters of 16 pups took in almost three times the amount of calcium solution (when expressed as change from baseline) than did dams nursing only four pups, while intake of calcium solution in the first week of lactation by dams nursing large litters was close to double that of dams nursing four pups.

Consistent with previous data (Ota & Yokoyama, 1967), mothers nursing 16 pups ate considerably more food than mothers nursing small litters. Nearly five times as much food was consumed (when expressed as change from baseline) by the mothers nursing 16 pups in the first week of lactation and almost three times as much in the second week. As has also been previously reported, however, the extra food ingested by the dams nursing large litters was not sufficient to result in equal pup growth between pups in litters of 16 and those in litters of four.

Thus, my findings indicate that calcium intake, as with food intake, does vary as a function of litter size. Dams nursing 16 pups deliver more milk than those nursing four pups; thus, these data are consistent with Richter's suggestion that lactating females increase their intake of specific nutrients so as to meet the requirements of milk production. But, as Ota and Yokoyama (1967) have suggested, the rate of hormone secretion may be directly regulated by the number of suckling pups, and levels of prolactin do seem to increase with increasing litter size (Ford & Melampy, 1973). These data, then, are also consistent with the notion that a hormonal mechanism may be mediating the selective increase in calcium intake seen in lactating rats.

In the next experiment, therefore, I examined calcium self-selection in postparturient female rats which were not delivering milk, but in which the hormonal state typical of lactation was maintained.

Experiment 2

Past evidence has suggested that the suckling stimulation and or its induced release of hormones rather than milk delivery per se are directly responsible for part of the enhanced food intake observed in the lactating rat (Cotes & Cross, 1954). These authors have shown that lactating female rats whose galactophores have been cut to prevent milk delivery continue to suckle their young and show a significant increase in food intake and body weight as compared to galactophore-cut animals whose litters have been removed at birth. The increase in food intake by suckled galactophore-cut females, however, was not found to be as great as the increase seen in intact lactating rats.

In the present experiment, in order to disentangle the initial question of whether the hormonal status of the lactating dam, the suckling stimulation, and/or milk delivery per se leads to the selective increase in calcium intake, I determined whether milk let down was an essential prerequisite for the female to show a selective increase in calcium intake.

I employed a procedure similar to that used by Cotes and Cross (1954). Nursing dam's galactophores were cut to prevent the withdrawal of milk, yet suckling stimulation received from her pups was maintained in order to sustain a normal lactating hormonal status. One difference in this study, however, was the time at which galactophore ligation was performed; specifically, in this experiment surgery was carried out one week prior to impregnation as compared to 36 hours postpartum in the study of Cotes and Cross. We reasoned that if milk delivery alone was a necessary factor contributing to the selective increase in calcium intake, then dams whose galactophores had been cut should take in the

same amounts of calcium as their nonlactating controls. If, however, the dam's suckling stimulus alone or resulting change in hormonal status is the crucial factor necessary to instigate the selective increase of calcium, then galactophore-cut females should consume similar quantities of calcium as their intact lactating controls.

Method

Subjects

Thirty-nine virgin female Wistar rats obtained from Charles River Breeding Farms (St. Constant, Quebec) were used. All rats were approximately three months of age and weighed between 230 and 260 grams.

Surgical Procedures

Galactophore ligation was carried out one week prior to the beginning of the baseline period. Under metofane anesthesia two midline incisions were made exposing the galactophores of all mammary glands. All galactophores were cut and the midline incisions closed. The success of the operations were verified by weighing litters before and after suckling and by postmortem examination of the mammary glands.

Identical procedures were followed for sham operated animals, but galactophores were left intact.

Procedure

Animals were divided into four groups consisting of 10 non-impregnated, 10 impregnated, 10 impregnated galactophore-cut and nine sham operated impregnated animals.

The nonimpregnated and sham operated animals served as controls for the remaining two groups. Since no significant statistical differences were found on any measures between the impregnated and sham operated animals, these groups were combined to form one lactating control group (n = 19). Animals were assigned randomly to each of the groups.

For all animals, housing conditions, nutrient choice, equipment, and mating procedures were identical to those procedures used in

Experiment 1.

During the baseline period intake measurements of the three nutrients and female weight were recorded bidaily between 0900 and 1200 hours. During lactation, the nutrient measures as well as female and where appropriate pup weight were recorded daily at 0900 hours. In addition, to ensure that equal suckling stimulation was received by all impregnated females and to prevent starvation of the litters of galactophore-cut dams , litters were rotated between galactophore-cut, impregnated and colony foster dams respectively every 12 hours (0900 and 2100 hours).

A mean bidaily intake was calculated for each animal. Blocks comprised eight days of baseline, days one through eight of lactation and days nine through 16 of lactation. Again data were expressed as absolute change from baseline to account for any discrepencies in intake between groups during the baseline period. A two-way split plot analysis of variance with Groups as the between groups factor and Time as the within groups factor was carried out on the blocked data for calcium, water and food intake. All source tables of analyses of variance are shown in Appendix C.

Results

Calcium Intake

Figure 4 shows the mean bidaily change in intake of the calcium solution expressed as change from baseline and averaged for blocks for all groups over the course of the experiment. Overall, the impregnated galactophore-cut and lactating control animals increased their calcium intake across the whole of lactation more than did the nonimpregnated animals (significant main effect for groups; $F(2,36) = 9.24$, $p<.001$).

The main effect for time did not reach statistical significance but the Groups x Time interaction did ($F(2,36) = 3.02$, $p<.06$). Scheffe post hoc comparisons showed that both the galactophore-cut and lactating control groups increased their intake of calcium solution to a significantly greater extent than the nonimpregnated group in both the first and second weeks of lactation. Further, in the first week of lactation galactophore-cut dams took in similar quantities of the calcium solution to the lactating control dams. By the second week, however, lactating control animals were drinking significantly more of the calcium than the galactophore-cut group.

Analysis of these data when expressed as relative change in calcium solution reveals a statistically significant main effect for groups ($F(2,36) = 5.72$, $p < .01$). Scheffe post hoc comparisons showed that the percentage change in calcium solution intake during lactation is higher for the suckled animals (both galactophore-cut and lactating control groups) than for the nonimpregnated group. None of the other effects reached statistical significance.

Gram Calcium Intake

The calcium intake data above, expressed as grams of calcium ingested, shows that galactophore-cut animals on the average took in 0.25 $\pm$ 0.08 grams of calcium bidaily above baseline levels in the first week of lactation and 0.23 $\pm$ 0.03 grams of calcium in week two of lactation. Lactating control animals increased their bidaily intake by 0.24 $\pm$ 0.06 grams of calcium during week one of lactation and by 0.48 $\pm$ 0.08 grams of calcium in the second week of lactation. The non-impregnated group did not show any change in gram calcium intake in either week one or week two of lactation.

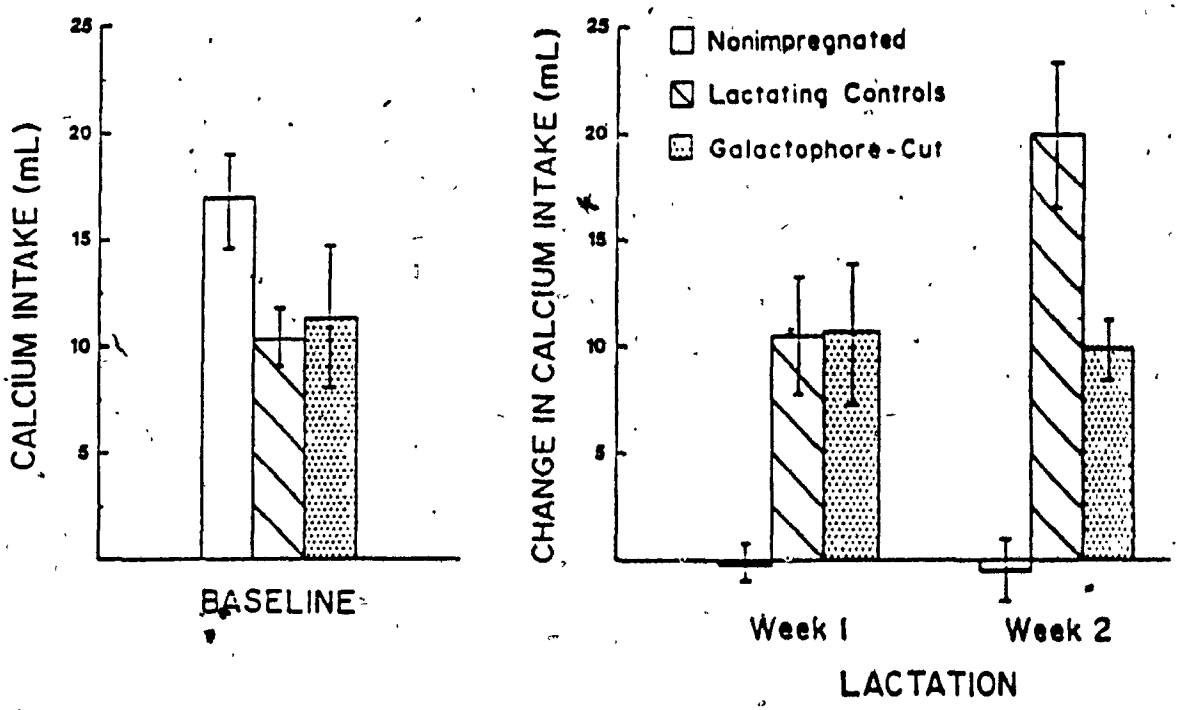

Figure 4. Bidaily intake of calcium solution (mL) for nonimpregnated, lactating control, and galactophore-cut animals over the course of lactation expressed as change from baseline and averaged over eight day blocks (means + S.E.M.'s) are shown. The left hand panel shows baseline levels for all three groups (means ± S.E.M.'s) are shown.

Time Course

In Figure 5 we show the absolute intake of calcium solution for the galactophore-cut and lactating control groups for lactation only plotted bidaily. When the lactational data for these two groups are compared using a two-way analysis of variance with one between factor (Groups) and one within factor (Time) the results show a significant effect for Time (F (7,189) = 3.26, p,<.01) and a Time x Groups interaction (F(7,189) = 2.11, p<.05). The main effect for Groups did not reach statistical significance.

Water Intake

The mean bidaily water intake expressed as change from baseline and averaged per week of lactation is shown in Figure 6. Overall, the lactating control females increased their water intake across the two weeks of lactation more than did the nonimpregnated or galactophore-cut dams, (significant main effect for Groups; F(2,36) = 8.75, p< .001; Time; F(1,36) = 19.13, p<.001, and significant interaction of Groups x Time F(2,36) = 7.84, p<.001).

As shown by Scheffe post hoc comparisons, the lactating control group took in significantly more water than did the galactophore-cut and nonimpregnated groups, in both the first and second weeks of lactation. Over the course of lactation galactophore-cut animals actually took in less water than they had during the baseline period. In fact, in the first week of lactation they were consuming significantly less water than the nonimpregnated animals; however, by week two of lactation they increased their water intake up to levels similar to those of the nonimpregnated females.

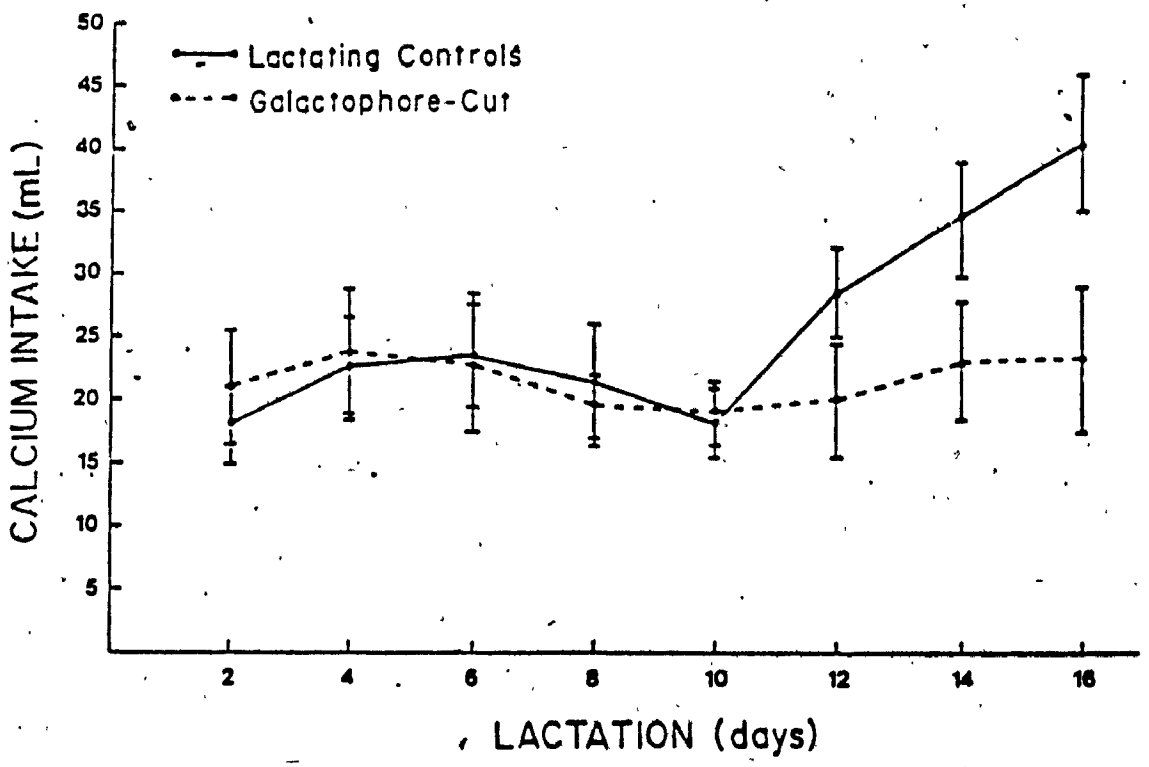

Figure 5. Mean bidaily calcium intake (mL) of lactating control and galactophore-cut animals over 16 days of lactation (means ± S.E.M's) are shown.

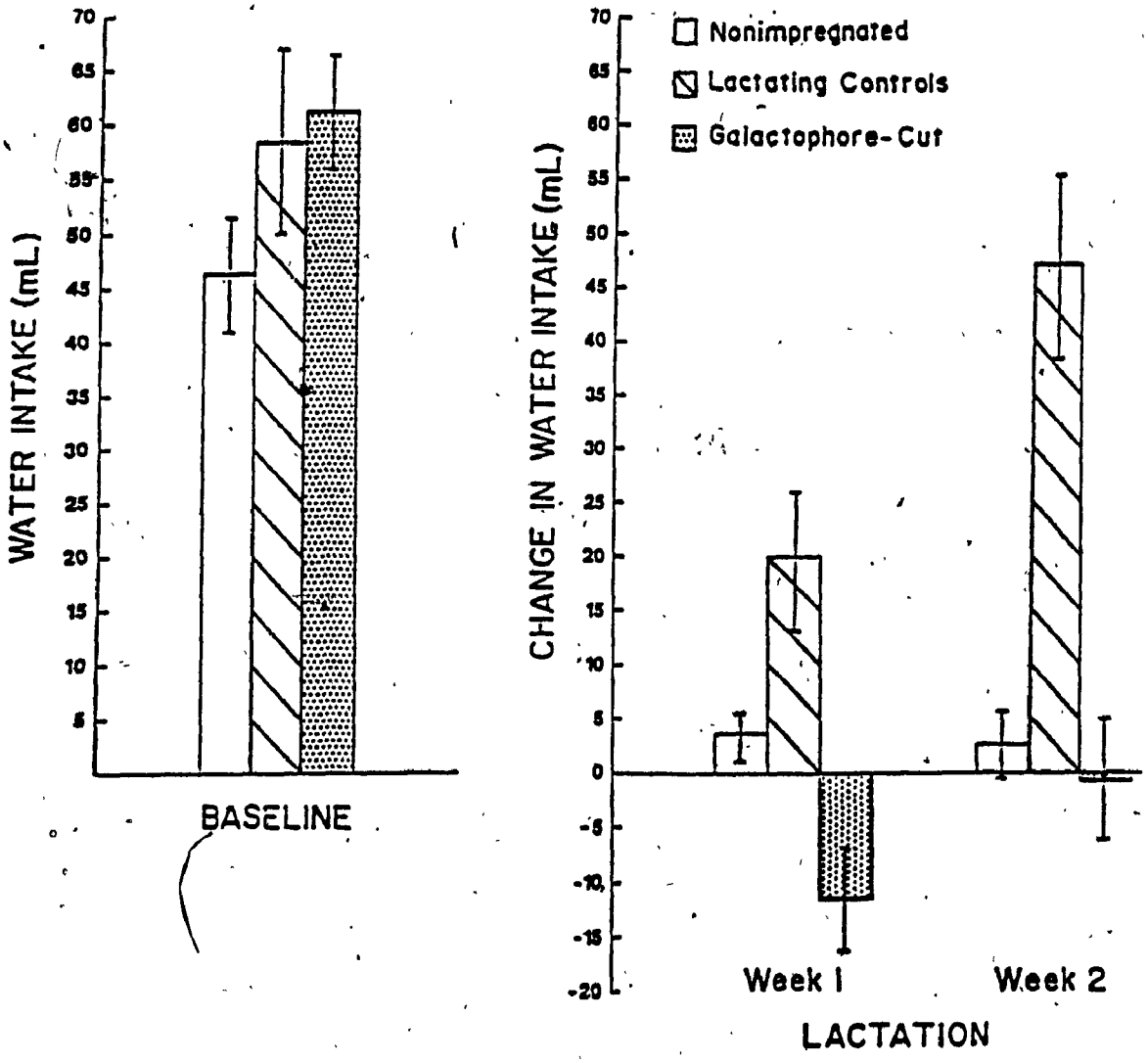

Figure 6. Bidaily water intake (mL) for nonimpregnated, lactating control, and galactophore-cut animals over the course of lactation expressed as change from baseline and averaged over eight day blocks (means + S.E.M.'s) are shown. The left hand panel shows baseline levels for all three groups (means + S.E.M.'s) are shown.

Food Intake

Food intake expressed as change from baseline averaged bidaily and per block of lactation is shown in Figure 7. Animals in the lactating control group showed a greater change in food intake over the two weeks of lactation than did both the nonimpregnated and galactophore-cut animals. The analysis of variance revealed significant main effects for groups and time as well as a significant interaction of these two factors: $F(2,36) = 56.79$, $p<.001$, $F(1,36) = 72.48$, $p<.001$, and $F(2,36) = 30.11$, $p<.001$, respectively.

The lactating control group increased their intake of food to a significantly greater extent than the other two groups in both weeks of lactation as indicated by Scheffe post hoc comparisons. In addition, only during the second week of lactation did the galactophore-cut animals ingest significantly more food than the nonimpregnated animals.

Reproductive Outcome Measures

Both impregnated groups showed no differences in number of pups born (galactophore-cut group; mean = 11.7 $\pm$ 0.9[illegible] lactating control group; mean = 11.1 $\pm$ 0.57, $t(27) = 0.49$, $p>.05$) or weight (galactophore-cut group; mean = 5.95 $\pm$ 0.24, lactating control group, mean = 6.3 $\pm$ 0.16, $t(27) = 1.2$, $p>.05$). One-way analysis of variance and Scheffe post hoc comparisons showed that galactophore-cut dams had a higher percent weight change over the course of lactation than both the lactating control and nonimpregnated animals (significant effect of groups; $F(2,36)=3.73$, $p<.05$). Nonimpregnated and lactating control animals, however, were similar in percentage weight change over the time period of lactation. Of particular importance to this study was the assurance that galactophore-cut mothers were in fact not secreting

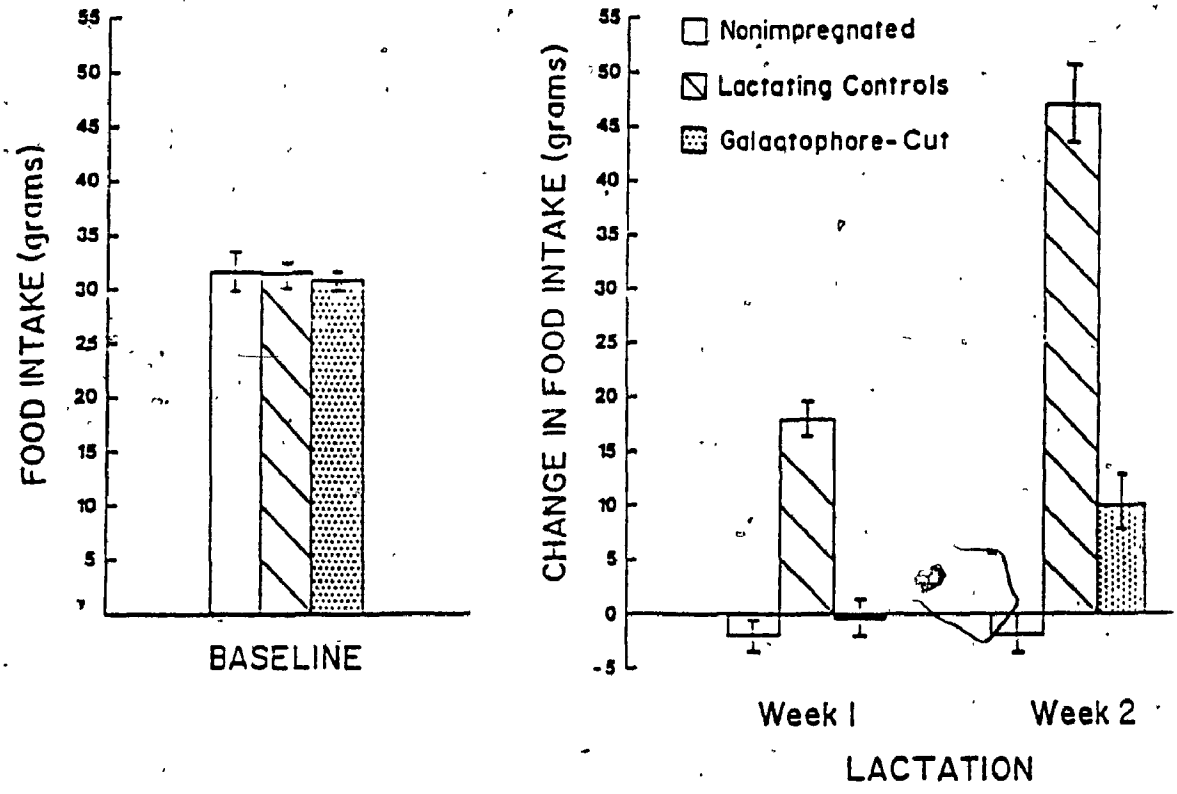

Figure 7. Bidaily food intake (g) for nonimpregnated, lactating control, and galactophore-cut animals over the course of lactation expressed as change from baseline and averaged over eight day blocks (means + S.E.M.'s) are shown. The left hand panel shows baseline levels for all three groups (means ± S.E.M.'s) are shown.

any milk despite intense suckling from their litters. Pups nursed by galactophore-cut dams lost weight after every 12 hour rotation period and litters averaged over the entire 16 days of lactation a mean daily loss of 11.21 ± 1.03 grams as compared to pups nursed by lactating control females that gained on the average 17.92 ± 0.9 grams per day per litter.

Discussion

Data obtained from my second experiment demonstrate that suckled dams whose galactophores have been cut to prevent milk delivery continue to show an increase in calcium intake compared to nonlactating females. Both the lactating control and galactophore-cut groups took in similar quantities of calcium in the first week of lactation. By the second week galactophore-cut females maintained their calcium intake while lactating control mothers showed a further increase in calcium probably due to the timing of peak milk production.

These data indicate that suckling stimulation alone is indeed sufficient to cause a selective increase in calcium intake. The increase in calcium intake, however, can be even further augmented if dams are delivering milk, as is the case seen here for the lactating control animals during the second week of lactation. Thus, it appears that both factors; milk delivery and suckling stimulation, contribute to the overall increase in calcium intake during lactation, but, it is clear that milk delivery alone is not a necessary factor.

In the first week of lactation galactophore-cut mothers were actually taking in less water than they had during the baseline period. Interestingly, the actual total fluid intake of calcium solution and water combined was the same as during baseline.

My results for food intake are similar to those of Cotes and Cross (1954) for food intake where galactophore-cut suckled females show an increase in food intake in the absence of milk delivery. The pattern of these results can be compared to calcium intake where galactophore-cut females took in more of the calcium solution over the two weeks of lactation than did the nonimpregnated controls..

These data support the hypothesis that suckling stimulation itself and/or the hormonal status it induces, is sufficient to augment a relative increase in calcium intake in postparturient female rats. Before this conclusion can be drawn, however, some alternative explanations have to be considered.

First, it is possible that galactophore-cut animals increase their self-selection of calcium during lactation as a result of incurring a calcium debt during pregnancy and are thus compensating for this debt during lactation. This possibility, however, seems unlikely as the dam usually experiences an increase in bone mass and consequently calcium stores during pregnancy due to the action of estrogen (Miller, Schupem, Redd, Miller & Omura, 1986). Furthermore, if the observed increase in calcium intake ocurred as a result of an acquired calcium debt during pregnancy one would expect the pattern of calcium intake over the course of lactation to appear quite different from the pattern that I obtained. In particular, one might expect to see a large increase in calcium intake at the very beginning of lactation followed by a slow steady decrease in intake for the remainder of lactation, rather than the steady level of calcium intake observed throughout both weeks one and two of lactation in the galactophore-cut females in this study.

To test the hypothesis that the female rat increases her calcium intake during lactation as a response to a calcium debt acquired during pregnanacy a few groups could be added to the existing experimental design. Specifically, we could look at calcium intake in galactophore-cut and sham operated animals whose pups have been removed at partutrition. The addition of a thalectomized control group of animals would provide additional information concerning calcium intake in the absence of milk delivery and suckling stimulation, that is, to simply look at the presence of pups as a function of calcium intake. If these three groups showed no increase in calcium intake during the time period of lactation then we could suggest that the increased calcium intake observed in galactophore-cut and lactating control mothers whose pups are present is not a response to compensate for a calcium debt acquired during pregnancy but rather a result of factors present during lactation.

Second, cutting the galactophores of female rats did indeed prevent the delivery of milk to the suckling pups since litter weights decreased after each 12 hour period spent with galactophore-cut dams. Preventing milk delivery, however, does not exclude the possibility that some residual milk production is occurring which may be sufficient to maintain the observed increase in calcium intake by galactophore-cut dams during lactation.

According to Selye (1934) milk production does persist in suckled dams after galactophore ligation. The mammary gland alveoli of the galactophore-cut females in Selye's study were found to be still engorged with milk 11 days after galactophore ligation was performed.

In this study autopsies were carried out on Day 16 of lactation

for both the sham operated and galactophore-cut animals and, upon gross inspection, the mammary glands of galactophore-cut animals appeared flat and pale yellow in color, a very different appearance from those of sham operated animals which were pink and swollen.

The existing discrepencies between our observations and those of Selye (1934) may be due to the different conditions under which galactophore ligation was performed. In particular, the animals in Selye's experiment underwent galactophore ligation on Day 3 of lactation, after milk production had commenced, while in our study surgery was performed one week prior to impregnation.

Even if a small amount of milk production was occurring in our galactophore-cut females the demand for calcium in sustaining this production is questionable. If milk was being produced and was not being delivered it is presumably being resorbed back into the system, and thus the galactophore-cut animal is experiencing no net loss of calcium and would still be ingesting more than is required. The question then arises as to what the animal is doing with the extra intake of calcium. It would be interesting, therefore, to analyze the urinary and bone calcium content of these galactophore-cut females.

In order to eliminate the possibility that residual milk production leads to an increase in calcium intake observed in galactophore-cut females, one could compare the calcium intake of these females to a group of mammectomized females. Mammectomy would ensure the absence of milk production without altering the hormonal status of the dam (Moltz, Geller, & Levine, 1967). If mammectomized females consumed similar quantities of calcium as their galactophore-cut counterparts then the increase in calcium intake could be attributed to

factors other than a need of calcium for the production of milk.

As these alternatives appear unlikely, then how might the suckling stimulation and/or hormonal status produce these effects ?

The first step in unravelling this question might be to determine whether this behavior is indeed hormonally mediated. Since eliminating the neural input from suckling inevitably results in a disruption of the females hormonal status, the more straight forward approach would seem to be the selective observation of the effects of elimination and replacement of the hormones of lactation or self-selection of calcium.

Given that many of the hormones that are elevated during lactation have also been implicated in the control of calcium metabolism, for example, prolactin, PTH, and calcitonin, it seems most probable that the relative increase in calcium intake in lactating females is hormonally mediated. If this is indeed so, how might the hormonal status of the dam be producing these effects?

One possibility is that the selective increase in calcium intake by galactophore-cut and lactating control animals may be the result of one of the calcium regulating hormones having a direct effect on the neural substrate mediating calcium appetite; much like the effect found by Epstein (1982) with Angiotensin II and sodium appetite. As of yet, however, little or no evidence has been reported concerning this possibility and in particular, the existence of a calcium taste receptor.

A final alternative is that the hormonal status of lactating animals is changed in such a way that there is a resulting shift in calcium homeostasis so that the dam is always in a calcium need state. Indeed, Toverud, Harper and Munson (1976) have shown that intact

lactating rats are hypocalcemic. Moreover, they have provided evidence showing that iv administration of calcitonin decreases serum calcium levels in lactating rats but not in nonlactating rats. Perhaps, then, galactophore-cut females, as intact lactating females, have high circulating levels of calcitonin, are in a hypocalcemic state, and thus increase their calcium intake in response to low serum levels of calcium. This possibility could be further examined by comparing the serum calcium and calcitonin levels of galactophore-cut, lactating control and nonimpregnated animals. It would also be of interest to compare serum calcium and calcitonin levels in dams nursing 16 pups and those nursing four pups to see if these levels differed as a function of amount of milk produced and amount of suckling stimulation received.

The other side of the question, of course, is the role that milk delivery plays in contributing to the overall increase in calcium intake by female rats during lactation. It is presumed, however, that the mechanism by which this factor serves to increase calcium intake is one and the same as that just previously described. Perhaps, as the amount of milk delivered by the dam increases over the course of lactation the level of serum calcium decreases to an even greater extent than that seen in galactophore-cut dams. Thus, lactating dams may show a further increase in calcium intake in response to very low serum levels of calcium.

Summary and General Discussion

It is clear from the results of the experiments described above that female rats nursing large litters increase their intake of calcium lactate to a greater extent than do dams nursing small litters. Moreover, results from our second experiment indicate that the amount of milk delivered does not seem to be an essential factor controlling the increased calcium intake observed in dams nursing 16 pups, since suckled dams whose galactophores have been cut to prevent milk delivery continue to show an increase in calcium solution intake comparable to their intact lactating controls. In addition, galactophore-cut dams nursing eight pups took in similar amounts of calcium to intact females nursing four pups. The pattern of calcium intake over lactation for these two groups, however, was quite different; galactophore-cut dams maintained a consistent level of calcium intake throughout lactation, while dams nursing four pups showed a progressive increase in calcium intake over time, reaching levels similar to galactophore-cut dams by the second week of lactation.

Potential explanations and mechanisms mediating the increase in calcium intake in the abscence of milk delivery in the female rat have been suggested and include: a) the female incurring a calcium debt during pregnancy resulting in a compensation for this debt during lactation, b) the occurrence of residual milk production sufficient enough to maintain an increase in calcium intake, c) a direct hormonal effect on a possible neural substrate mediating calcium appetite, d) an overall change in hormonal status resulting in a shift in calcium homeostasis so that the dam is constantly in a calcium need state. A review of current knowledge suggests that the latter is the most likely

hypothesis.

Clearly other studies are needed, some of which have been discussed, to further establish the precise mechanism underlying the self-selection of calcium solution in lactating rats.

The question still remains, however, as to how and when female rats learn to distinguish between the calcium lactate solution and demineralised water. Results of previous studies have shown that rats made calcium deficient by either parathyroidectomy (Richter & Eckert, 1937) or by maintenance on a low calcium diet (Scott, Verney, & Morrisey, 1950) develop a preference for a 2.4% calcium lactate solution and a calcium sufficient diet repectively. These authors suggest that calcium deficient rats learn to develop a preference for calcium as a means to help correct the deficiency.

It is presumed, however, that animals in the present study are not in a calcium deficient state. Previous findings (Woodside & Millelire, 1987) have shown that female nonimpregnated rats maintained on a calcium deficient diet and given ad lib access to a 2.4% calcium lactate solution do not appear to compensate for the absence of calcium in their diet by taking in more calcium than nonimpregnated animals on a calcium sufficient diet. Animals in both diet conditions took in similar amounts of the calcium lactate solution, although, animals on the calcium sufficient diet also obtained from their food an amount of calcium (when measured in grams) equivalent to that amount sampled from the calcium lactate solution. It is assumed then that animals maintained on the calcium deficient diet obtained as much calcium as is normally required by nonlactating females, while animals given the calcium sufficient diet were taking in more than normal requirements.

Given these findings, it seems clear that animals in the present study do not learn about calcium as a result of being calcium deficient.

The fact that intake of calcium solution in baseline is much lower than that of water would suggest that the animals are in fact discriminating between the two - presumably on a taste cued - and that they find the taste of the calcium solution mildly aversive. The question then is when do the animals learn to associate taste with post-ingestional consequences, as it is presumably this association that mediates the increase in calcium intake during lactation. One possibility is that they only learn about post-ingestional cues when they become deficient. To test this hypothesis, a series of flavor experiments could be conducted where flavors of equal preference are added to both the demineralised water and calcium lactate solutions prior to lactation. Once lactation had commenced, the flavors added to the two liquids could be interchanged. If animals show an increase in intake of the flavored solution initially associated with calcium then it may be assumed that an association between the post-ingestional effects of calcium and the taste of the solution were made prior to lactation.

In the context of maternal behavior, it seems that we again have a situation similar to that of nest building where the dam is meeting the demands of her young by utilizing both physiological and behavioral mechanisms. Specifically, the reproducing dam experiences both physiological (an increase in absorptive capacity of the small intestine) and behavioral (a three fold increase in overall food intake) changes which help meet the increased nutritive demands of her young. Thus, from the results of the present study the dam seems to

behaviorally augment physiological mechanisms by not only increasing her overall food intake during lactation but also by increasing her relative intake of specific nutrients, such as calcium. Moreover, when the dam is faced with the increased demands of a large litter she attempts to compensate by further increasing her calcium intake in addition to her overall food intake.

Overall, it appears that the self-selection of calcium solution by lactating rats is one of a variety of strategies that the dam employs which serves to offset the cost of reproduction.

References

Brody, S., Riggs, J., Kaufman, K., & Herring, V. (1938). Energy metabolism levels during gestation, lactation and post-lactation rest. Research Bulletin 281 of the University of Missouri Agricultural Experiment Station, 1-43.

Cotes, M., & Cross, B. (1954). The influence of suckling on food intake and growth of adult female rats. Endocrinology, 10, 363-367.

Cripps, A.W., & Williams, V.J. (1975). The effect of pregnancy and lactation on food intake, gastrointestinal anatomy and the absorptive capacity of the small intestine in the albino rat. British Journal of Nutrition, 33, 17-32.

Czarnecki, S.K., & Kritchevsky, D. (1980). Trace elements. In R.B. Alfin-Slater, D. Kitchevsky (Eds.), Nutrition and the adult micronutrients (pp. 319-329). New York: Plenum press.

de Winter, F.R., & Steendijk, R. (1975). The effect of a low-calcium diet in lactating rats; observations on the rapid development and repair of osteoporosis. Calciferous Tissue Research, 17, 303-316.

Epstein, A.N. (1982). Mineralocorticoids and cerebral angiotensin may act together to produce sodium appetite. Peptides, 9, 493-494.

Fleming, A.S. (1976). Control of food intake in the lactating rat: Role of suckling and hormones. Physiology and Behavior, 17, 841-848.

Ford, J.J., & Melampy, R.M. (1973). Gonadotropin levels in lactating rats: Effect of ovariectomy. Endocrinology, 93, 540-547.

Fournier, P., & Susbielle, H. (1952). Les echanges de calcium chez le rat au cours de la gestation de la lactation et du serrage. I. Influence d'un regime riche en calcium. Journal of Physiology, (Paris), 44, 123-134.

Freed, W.J., Perlow, M.J., & Wyatt, R.J. (1979). Calcitonin: inhibitory effect on eating in rats. Science, 206, 850-852.

Frumkin, K. (1975). Failure of sodium- and calcium-deficient rats to aquire conditioned taste aversions to the object of their specific hunger. Journal of Comparative and Physiological Psychology, 89, 329-339.

Galef, B.G. (1981). The ecology of weaning: Parasitism and the achievement of independence by altricial mammals. In D.J. Gubernick & P.H. Klopfer (Eds.), Parental care in mammals (pp. 211-241). New York: Plenum Press.

Garel, J.M. (1987). Hormonal control of calcium metabolism during the reproductive cycle in mammals. Physiological Reviews, 67, 1-66.

Handal, P.J. (1965). Immediate acceptance of sodium salts by sodium deficient rats. Psychonomic Science, 3, 315-316.

Jans, J.E., de Villers, S., & Woodside, B.C. (1985). The effects of rearing environment on pup development. Developmental Psychobiology, 18, (4), 341-347.

Jans, J.E. & Leon, M. (1983). Determinants of mother-young contact in Norway rats. Physiology and Behavior, 21, 919-935.

Jans, J.E., & Woodside, B.C. (1987). Effects of litter age, litter size, and ambient temperature on the milk ejection reflex in lactating rats. Developmental Psychobiology, 20, (3), 333-344.

Kostial, K., Gruder, N., & Durakovic, A. (1969). Intestinal absorption of calcium-47 and strontium-85 in lactating rats. Calciferous Tissue Research, 4, 13-19.

Leon, M., & Woodside, B. (1983). Energetic limits on reproduction: maternal food intake. Physiology and Behavior, 30, 945-957.

Lloyd, L.E., McDonald, B.E., & Crampton, E.W. (1978). Fundamentals of nutrition (2nd ed.). San Francisco: W.H. Freeman and Company.

Miller, S.C., Schupe, J.G., Redd, E.H., Miller, M.A., & Omura, T.H. (1986). Changes in bone mineral and bone formation rates during pregnancy and lactation in rats. Bone, 7, 283-287.

Moltz, H., Geller, D. & Levine, R. (1967). Maternal behavior in the totally mammectomized rat. Journal of Comparative and Physiological Psychology, 64, 225-229.

Nachman, M. (1962). Taste preferences for sodium salts by adrenalectomised rats. Journal of Comparative and Physiological Psychology, 55, 1124-1129.

Ota, K., & Yokoyama, A. (1967). Body weight and food consumption of lactating rats nursing various sizes of litters. Journal of Endocrinology, 38, 263-268.

Richter, C.P. (1936). Increased salt appetite in adrenalectomised rats. American Journal of Physiology, 115, 155-161.

Richter, C.P. (1939). Salt taste thresholds of normal and adrenalectomised rats. Endocrinology, 24, 367-371.

Richter, C.P., & Barelare, B. (1938). Nutritional requirements of pregnant and lactating rats studied by the self-selection method. Endocrinology, 23, 15-24.

Richter, C.P., & Eckert, J.F. (1937). Increased calcium appetite of parathyroidectomised rats. Endocrinology, 24, 50-54.

Robinson, C.J., Spanos, E., James, M.F., Pike, J.W., Haussler, M.R., Mackeen, A.M., Hillyard, C.J., & MacIntyre, I. (1982). Role of prolactin in vitamin D metabolism and calcium absorption during lactation in the rat. Journal of Endocrinology, 94, 443-453.

Rodgers, W.L. (1967). Specificity of specific hungers. Journal of Comparative and Physiological Psychology, 64, (1), 49-58.

Rozin, P. (1976). The selection of foods by rats, humans, and other animals. In J. Rosenblatt, R. Hinde, & E. Shaw (Eds.), Advances in the study of behavior (pp. 21-75). New York: Academic Press.

Rozin, P., & Kalat, J.W. (1971). Specific hungers and poison avoidance as adaptive specializations of learning. Psychological Review, 78, 459-486.

Scott, E.M., Verney, E.L., & Morrisey, P.D. (1950). Self-selection of diet. XI. Appetites for calcium, magnesium and potassium. Journal of Nutrition, 78, 187-202.

Selye, H. (1934). On the nervous control of lactation. American Journal of Physiology, 107, 535-538.

Simpson, A.A., Simpson, MH.W., Sinha, Y.N., & Schmidt, G.H. (1973). Changes in concentration of prolactin and adrenal corticosteroidsin rat plasma during pregnancy and lactation. Journal of Endocrinology, 58, 675-676.

Toverud, S.U., Boass, A., Haussler, M.R., & Pike, J.W. (1983). Circulating levels and function of 1,25 $(OH_2)D_3$ in lactation. Journal of Steroid Biochemistry, 19, 505-510.

Toverud, S.U., Harper, C. & Munson, P.L. (1976). Calcium metabolism during lactation: Enhanced effects of thyrocalcitonin. Endocrinology, 99 (2), 371-378.

Toverud, S.U., Cooper, C.W., & Munson, P.L. (1978). Calcium metabolism during lactation: Elevated blood levels of calcitonin. Endocrinology, 103, 472-479.

Tribe, D.E. (1955). Choice of diets by rats: The choice of purified

constituents during growth, pregnancy and lactation. British Journal of Nutrition, 9, 103-109.

Turner, C.D. (1966). General Endocrinology, Fourth edition. Philadelphia: W.B. Saunders Company.

Warnock, G.M., & Duckworth, J. (1944). Changes in the skeleton during gestation and lactation in the rat. Biochemistry Journal, 38, 220-224.

Woodside, B.C., & Millelire, L. (1987). Self-selection of calcium during pregnancy and lactation in rats. Physiology and Behavior, 39, 291-295.

Appendix A: Dietary Constituents of Calcium Deficient Diet.

Calcium Deficient Diet

Composition	g/kg
Vitamin Free Caesin	240
Sucrose	680
Corn Oil	50
Calcium Free Salt Mixture Plus Special ICN Vitamin Diet Fortification Mixture	30

Vitamins:	mg
Added to each 100 grams of caesin:	
Thiamine Hydrochloride	1.46
Riboflavin	1.46
Pyridoxine Hydrochloride	1.46
Nicotinic Acid	10.4
Calcium Pantothenate	8.3
Choline Chloride	417
Inositol	4.7
Para-Aminobenzoic Acid	126

Salt Mixture:	g
Potassium Phosphate (dibasic)	645
Sodium Phosphate (monobasic)	126
Magnesium Sulfate	100
Sodium Chloride	282
Ferric Citrate	55
Potassium Iodide	1.6
Manganese Sulfate	9
Zinc Chloride	1
Cupric Sulfate	0.6

Vitamins:	mg
Added to each 100 grams of corn oil:	
Beta Carotene	22
Irradiated Ergosterol	6000 I.U.
2-Methyl-1,4-Naphthoquinone	12
Alpha-Tocopherol	100

Appendix B: Source Tables of Analyses of Variance (Experiment 1).

Table 1

Change in Calcium Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	1751.61	1	1751.61	6.03 *
Error	4934.14	17	290.24	
Within Subjects/Blocks				
Time	3089.49	1	3089.49	35.49 ***
Groups x Time	884.70	1	884.70	10.16 **
Error	1479.52	17	87.03	
Total	12139.46	37		

* $p < .05$
** $p < .01$
*** $p < .001$

Table 2

Change in Relative Calcium Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	506.47	1	506.47	0.92
Error	9392.92	17	552.52	
Within Subjects/Blocks				
Time	893.24	1	893.24	27.22 ***
Groups x Time	205.03	1	205.03	6.25 *
Error	557.88	17	32.82	
Total	11555.54	37		

* $p < .05$
*** $p < .001$

Table 3

Change in Water Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	2172.03	1	2172.03	1.29
Error	28521.67	17	1677.74	
Within Subjects/Blocks				
Time	856.00	1	856.00	6.17 *
Groups x Time	6.93	1	6.93	.05
Error	2358.21	17	138.72	
Total	33914.84	37		

* $p < .05$

Table 4

Change in Food Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	8167.58	1	8167.58	21.96 ***
Error	6323.28	17	371.96	
Within Subjects/Blocks				
Time	3925.71	1	3925.71	111.64 ***
Groups x Time	464.25	1	464.25	13.20 **
Error	597.79	17	35.16	
Total	19478.61	37		

** $p < .01$
*** $p < .001$

Appendix C: Source Tables of Analyses of Variance (Experiment 2).

Table 5

Change in Calcium Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	2988.20	2	1494.10	9.24 ***
Error	5818.91	36	161.64	
Within Subjects/Blocks				
Time	151.14	1	151.14	2.19
Groups x Time	418.02	2	209.01	3.02 +
Error	2487.43	36	69.09	
Total	1183.7	77		

\+ $p = .06$
*** $p < .001$

Table 6

Change in Relative Calcium Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	2686.18	2	1343.09	5.72 **
Error	8444.95	36	234.58	
Within Subjects/Blocks				
Time	27.15	1	27.15	0.26
Groups x Time	97.75	2	48.87	0.47
Error	3706.47	36	102.96	
Total	14962.50	77		

** $p < .01$

Table 7

Bidaily Calcium Intake During Lactation

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	1024.46	1	1024.46	0.91
Error	30328.80	27	1123.29	
Within Subjects/Blocks				
Time	3754.33	7	536.33	3.26 **
Groups x Time	2431.98	7	347.43	2.11 *
Error	31100.95	189	164.55	
Total	68640.52	231		

* $p < .05$
** $p < .01$

Table 8

Change in Water Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	20264.24	2	10132.12	8.75 ***
Error	41690.68	36	1158.07	
Within Subjects/Blocks				
Time	2826.14	1	2826.14	19.13 ***
Groups x Time	2317.15	2	1158.57	7.84 ***
Error	5317.46	36	147.71	
Total	72415.67	77		

*** $p < .001$

Table 9

Change in Food Intake

Source	SS	df	MS	F
Between Subjects/Blocks				
Groups	15799.11	2	7899.55	56.79 ***
Error	5007.24	36	139.09	
Within Subjects/Blocks				
Time	3124.04	1	3124.04	72.48 ***
Groups x Time	2595.41	2	1297.70	30.11 ***
Error	1551.70	36	43.10	
Total	28077.50	77		

*** $p < .001$

Table 10

Percent Mother Weight Change Over Lactation

Source	SS	df	MS	F
Groups	342.32	2	171.16	3.73 *
Error	1652.05	36	45.89	
Total	1994.37	38		

* $p < .05$