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Effect of Litter Size Upon Milk Yield and Litter Weight Gains in Rats. (32363)

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In a series of studies from this laboratory, the effect of a number of hormones on milk secretion and removal has been reported. In these studies the litter size was reduced to 6. The milk yield was frequently increased significantly over the control groups; however, the mean litter weight in most experiments did not increase with increasing milk yield. In a study of this problem it was suggested that when milk secretion is intense the litters do not obtain all of the milk available due to the limited amount of oxytocin discharged and its short biological half-life. When 1 μ of oxytocin was injected at 6-hour intervals/day to enable the nursing young to obtain more of the available milk, the mean litter weight was increased 29 g, a highly significant increase of 13% (1).

In this and all previous studies, the number in the litter has been 6 pups. In an extension of the study to determine the relation between milk yield and litter growth, it seemed desirable to determine the effect of litter size. It was thought that such a study would contribute also to the problem of more complete milk removal when milk secretion was increased by one or more exogenous hormones.

Materials and methods. Lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, and fed Purina Lab Chow and water *ad libitum*. The animals were kept under standardized conditions of temperature and light (78 $\pm$ 1°F, 14 hours light and 10 hours darkness). On day 1 of lactation, the pups were adjusted in each litter from 2 to 12. The pups and mothers were weighed daily. On days 14, 16, 18 and 20 of lactation, milk yields were estimated from the increase of litter weights during a 30-minute nursing period following 10 hours of isolation from the mother. One USP unit of oxytocin‡ was injected subcutaneously into the dams immediately before nursing and a second unit after 15 minutes of nursing to aid in complete milk removal. On day 20 of lactation the dams were killed after nursing. Six posterior mammary glands were removed and DNA was determined by the Webb and Levy method (2). Ribonucleic acid (RNA) was determined by measuring the total nucleic acids (TNA) and obtaining RNA by the difference between TNA and

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TABLE I. Effect of Litter Size on Milk Yield and Litter Weight Gains in Normal Rats from Day 14 to Day 20 of Lactation.

No. of rats	Litter size	Mean milk yield after 10 hr separation (g) Day 14	Day 16	Day 18	Day 20	Mean litter wt (g) Day 14	Day 16	Day 18	Day 20
22	2	5.59 ± .36	5.59 ± .48	5.23 ± .49	4.64 ± .38	68.32 ± 3.31	71.36 ± 3.52	76.09 ± 4.05	86.27 ± 4.84
20	4	9.20 ± .72	11.30 ± .75	12.35 ± .82	9.00 ± .49	150.50 ± 5.32	161.80 ± 5.53	177.55 ± 6.61	194.00 ± 7.76
20	6	10.50 ± .52	13.35 ± 1.05	14.45 ± .96	9.90 ± .84	194.60 ± 7.13	204.20 ± 8.32	219.65 ± 9.48	242.55 ± 10.30
19	8	13.58 ± .89	16.11 ± 1.08	16.11 ± .90	14.84 ± .99	220.84 ± 5.07	232.63 ± 6.99	248.95 ± 2.80	276.95 ± 9.66
20	10	15.90 ± .84	19.15 ± 1.33	19.90 ± 1.10	20.30 ± .97	264.60 ± 6.01	280.90 ± 6.65	302.50 ± 8.25	338.65 ± 10.89
23	12	16.36 ± 1.15	19.68 ± 1.18	22.23 ± 1.58	21.82 ± 1.84	287.18 ± 6.49	297.86 ± 7.53	330.77 ± 10.17	367.86 ± 12.59
		Mean milk availability per pup and its weight g							
	2	2.80	2.80	2.62	2.32	34.16	35.68	38.05	43.14
	4	2.30	2.83	3.09	2.25	37.63	40.45	44.39	48.50
	6	1.75	2.23	2.41	1.65	32.43	34.03	36.61	40.43
	8	1.70	2.01	2.01	1.86	27.61	29.08	31.12	34.62
	10	1.59	1.92	1.99	2.03	26.46	28.09	30.25	33.86
	12	1.36	1.64	1.85	1.82	23.93	24.82	27.56	30.66

DNA. The procedure has been reported previously(3). The amount of DNA/100 g bw was used as an index of the extent of mammary gland growth and the amount of RNA/100 g bw or RNA/DNA ratio was used as index of mammary gland protein synthetic activity during lactation.

Results. On day 14 of lactation, the mean milk yield gradually increased with increasing litter size from 5.6 g with 2, to 16.4 g with 12 in the litter (Table I). The mean milk yield increased in most groups until the 18th day, then decreased on day 20. The milk yield available/pup obtained on day 14 was greatest for the 2-pup litters with 2.8 g. As the litter size increased, the amount declined gradually to 1.4 g for the 12-pup litter. These amounts also increased until day 18, then declined on day 20.

The mean litter weight on day 14 was 68.3 g for litters of 2. With increasing numbers in the litters there was a gradual increase in weight to 287.1 g for the litters of 12. In each group litter weight increased to the 20th day. On day 14 the mean weight/pup was 34.2 g for litters of 2 and increased to 37.6 g for litters of 4, then declined gradually with increasing litter number to 24 g for litters of 12. The mean weight/pup also increased from the 14th to the 20th day of lactation but the litters of 4 showed the greatest weight/pup.

At the end of the experiment, the mothers were sacrificed and the DNA and RNA/100 g bw determined (Table II). The total DNA/6 mammary gland for the litters of 2 was 8.9 mg/100 g bw. It increased gradually with increasing litter size to 13.6 mg for the litters of 12. The total RNA also increased with increasing litter size up to 10.

Discussion. The normal rat possesses 12 nipples and 12 individual mammary glands. She therefore has the capacity to nurse 12 pups. It is not known to what extent these 12 mammary glands differ in size and milk secreting capacity. In studies of mammary gland growth of rats the 6 abdominal-inguinal glands are used. The 6 pectoral were shown to be quite similar in total DNA (pectoral 9.01 mg/100 g bw and abdominal-inguinal 8.46 mg/100 g bw) in pregnant animals(4).

TABLE II. Effect of Litter Size upon Mammary Gland DNA and RNA on Day 20 of Lactation.

No. of pups/litter	No. of lactating rats	Final body wt (g)	Mean DFFT (mg)*	Total DNA (μg/mg DFFT) Mean ± S.E.	Total DNA/100 g body wt (mg) Mean ± S.E.	Total RNA (mg /100 g body wt) Mean ± S.E.	RNA/DNA Mean ± S.E.
2	22	308	907.00	27.04 ± 1.01	8.92 ± .31	7.88 ± .83	.85 ± .09
4	20	306	1130.29	31.21 ± 1.14	10.28 ± .34	15.21 ± .95	1.47 ± .09
6	20	293	1179.60	31.78 ± 1.63	10.87 ± 1.33	17.21 ± .96	1.56 ± .13
8	19	278	1176.21	31.43 ± 1.02	11.44 ± .46	17.99 ± .91	1.58 ± .08
10	20	286	1378.55	35.59 ± 1.60	12.42 ± .33	24.26 ± 1.29	1.91 ± .10
12	23	285	1455.41	38.70 ± 1.65	13.56 ± .43	20.54 ± 1.19	1.55 ± .07

DFFT = Dry fat-free tissue. S.E. = Standard error of mean. * Six posterior mammary glands.

In swine with 12 normal glands it has been reported by several investigators that pigs attempt to nurse the anterior glands rather than the posterior glands and it has been suggested that the anterior glands are more productive. It has been reported that once a pig selects a nipple there is a tendency to suckle the same nipple(5). It is interesting in both rats and swine that the nursing characteristics are quite similar. They nurse for a few minutes at about hourly intervals.

In the present study of rats, it was observed in the group nursing only 2 pups that apparently the posterior glands were preferred since the anterior glands showed extensive signs of involution on day 20 of lactation. When litters of 4 or more were nursed, both anterior and posterior glands were lactating.

It is interesting to note that litters of 2 and 4 show the greatest capacity to remove milk at the test milkings on days 14 to 20. It suggests that they have the capacity to select and nurse the most productive glands. It is possible also, that more than one gland would be nursed during the nursing period. This is not believed to be probable due to the short biological half-life of oxytocin(6). An alternative would be the possibility that the pups would nurse one gland at one nursing period and a second gland at the next. In this way the milk secreted in a 2-hour period would be removed instead of at a one-hour period. When the number in the litter was increased to 6 or more, there was a gradual reduction in the mean removal of milk. This decline in milk yield/pup could be explained on the basis of a gradual reduction in the size of the available glands and milk yield which the increase in litter size forced the pups to nurse.

In previous studies on the influence of one or more hormones on milk secretion, the number of pups in the litter has been restricted to 6. As the number of hormones injected together has been increased, the milk yield has increased very markedly. However, it seemed possible that not all of the increased milk stimulated could be removed by 6 pups and therefore, larger numbers of pups should be included. The present study shows that 12 pups increased milk yield by 56% over 6 pups on day 14, 47% on day 16, 54% on day 18 and 120% on day 20. These data indicate that by doubling the number of pups, it is possible to increase milk yield by about 50% up to the 20th day. The greater increase on day 20 is due to the decline in milk yield on day 20 of the rats with 6 in the litter; whereas, in the groups with 10 and 12 in the litter milk yield remained high.

From these observations indicating that milk yield does not increase in direct proportion to litter size, studies in which hormones are administered to increase milk yield should utilize litters of 10 to 12 to demonstrate the value of the hormones.

In previous studies from this laboratory it has been shown that administration of exogenous hormones to lactating rats with litters of 6 caused marked increases in milk yield on days 14 to 20. However, the gains in litter weight of the experimental groups are quite similar to the control groups. If more milk is being secreted why do the litters show no response by improved weight gains? Due to these observations, considerable doubt has been expressed that the increase in milk yield claimed for exogenous hormone treatment is true. To explain why litter weight gains should not increase in response to increased availability of milk, the normal nursing habits of the rat must be understood. The pups normally nurse their mothers for about 1 to 5 minutes at hourly intervals. If the amount of milk present in the glands is limited all the milk present may be removed at each hourly nursing period and the litter weight gain would be related to total milk yield. As the amount of milk present at nursing time increases due to exogenous hormone stimuli, the milk present would not be removed during the normal short nursing period, and the growth rate of the litter would not be increased above the control group because the excess of milk would not be removed at each nursing period. This implies that the exogenous hormones administered, which increase milk secretion, do not stimulate an increased secretion of oxytocin, which, if present, would lengthen the nursing period and thus permit more milk to be obtained at each hourly nursing period.

In addition to the limited nursing period regulated by the release of oxytocin, it is possible that the voluntary consumption of milk by the nurslings varies just as in growing and mature rats, and an abundance of available milk will not increase their intake or influence their growth rate(7).

In a previous study it was indicated that there may be genetic and endocrinological limits in the rate of growth of nurslings, for it was shown that litter weight increased in dams secreting increased amounts of milk up to a point beyond which, though milk yield was greater, no parallel increase in litter weight was observed(8). For these reasons we agree with the conclusions of Reddy and Donker(9) that litter weight gain is a poor indicator of milk production.

At the end of previous lactation experiments (day 20) the dams have been sacrificed and the DNA of the 6 posterior glands determined to observe the effect of the exogenous hormone treatment upon the maintenance of the lobule-alveolar cells. In these studies little difference in the mean DNA of the control and experimental groups given various hormones has been observed. In the present study, litters of 2 decreased the DNA. This was observed also in the appearance of the anterior glands. As the litter number increased, the mean DNA also increased, indicating that constant nursing of the glands tended to maintain the cell number. The RNA, indicating the intensity of protein synthesis, also increased with litter number up to 10. No explanation can be offered for the decrease with 12 in the litter.

Summary. A study is reported concerning the influence of litter size in rats on the estimated milk yields and litter weight gains on days 14 to 20, and on the DNA of the mammary glands on day 20. On day 14 of lactation, the mean milk yield increased from 5.6 g with 2 to 16.4 g with 12 in the litter. The milk yield in most groups increased until the 18th day, then decreased on day 20. The milk available/pup on day 14 was 2.8 g for the 2-pup litters and declined gradually to 1.4 g for the 12-pup litters. The litter weight on day 14 was 68.3 g for litters of 2 and increased gradually to 287.1 g for litters of 12. The gain per pup was greatest for litters of 2 and then declined with increasing litter number. At the 20th day, the DNA for litters of 2 was 8.9 mg/100 g bw and increased gradually with increasing litter size to 13.6 mg for litters of 12. The RNA also increased with increasing litter size up to 10.

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Relationship of Renal "Net Acid" Excretion to Titratable Ash-Acidity (Ash-TA) in Diet and Feces.* (32364)

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Recent studies concerning quantitative relationships between endogenous acid production and renal "net acid" excretion have been referred to elsewhere(1), and earlier work in this area has been reviewed by Elkinton(2). It is generally accepted that renal "net acid" excretion can be quantitatively evaluated in terms of urinary titratable acidity (TA), ammonium and bicarbonate as represented in Equation 1, Table I. However, evaluations of endogenous acid production have been unsatisfactory from both conceptual and methodological standpoints. Goodman *et al*(3) equated endogenous acid production solely to the sum of values for urinary sulfate and organic anions, although the earlier procedure of Relman *et al*(4) included also the calculated value for acid phosphorus from the diet as a component of the endogenous acid production value. More recently, Lennon *et al* (5) modified the method of Goodman *et al*(3) by including the value of absorbed (dietary minus fecal) chloride and phosphorus minus absorbed sodium, potassium, calcium and magnesium as an additional component of the endogenous acid production value. All of these procedures yield values which appear to represent endogenous acid production under the described conditions(3-5) since the values agree satisfactorily with renal "net acid" excretion values for normal, unstressed human subjects. However, it has subsequently been pointed out(1) that the values for urinary sulfate and organic anions must inevitably constitute components of the corresponding renal "net acid" values regardless of endogenous acid production (see Equations 1-3, Table I). On this basis, it was shown(1) that an endogenous acid production value calculated as described by Lennon *et al*(5) must unavoidably tend to equal the corresponding renal "net acid" value, regardless of actual endogenous acid production, providing balance is maintained between mineral intake and excretion. The same must also apply to endogenous acid production values calculated as described by Relman and his associates(3,4).

Although there is no evidence that either mineral intake or excretion can provide an adequate basis for estimating true endogenous acid production, the relationships shown in Equation 3, Table I, suggested that the renal "net acid" excretion value might be determined largely by mineral composition of the urine, and data presented earlier(1) verified that the extent to which cation-forming mineral elements are absorbed from the gut can considerably influence the extent of renal "net acid" excretion. The present report is concerned with both cation-forming and anion-forming mineral elements in terms of a parameter which we have named titratable

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