Mammary Growth and Milk Yield as Related to Litter Size* (33734)

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Selye (1) first demonstrated the importance of the nursing stimulus in the maintenance of mammary parenchyma during lactation. He showed that involution which normally occurs upon removal of young from the mother could be delayed despite the fact that milk removal from the gland was prevented by ligation of galactophores. Moon (2) determined the deoxyribonucleic acid (DNA) content of galactophore-ligated and nonligated rat mammary glands and found that the DNA content of ligated glands was significantly less than that of the contralateral nonligated glands, irrespective of the number of pups per litter. If, however, the suckling stimulus was sufficiently intense as was the case for rats nursing 12 young, mammary DNA and histological structure of galactophore-ligated glands was maintained at a level comparable to that of rats nursing 6 young but without galactophore ligation. Since lactational performance based on litter weight gain has yielded conflicting results (3, 4), it was of interest to determine the relationship existing between mammary gland cellular content as estimated by mammary DNA, milk yield, and litter weight gain in rats nursing litters of different size.

Methods. Litters of primiparous lactating rats of the Holtzman strain were adjusted to contain either 6, 9, or 12 young on the day of parturition. On day 13 postpartum, each lactating rat was isolated from her litter for 16 hr. At the end of the isolation period (day 14 postpartum), each dam was anesthetized by injecting intraperitoneally 3.5 mg/100 g of sodium pentobarbital. The teats of the pectoral glands were covered with tape to prevent the young from suckling these galnds. Each dam was then weighed to the nearest 0.1 g. The pups were returned to their anesthetized mothers and allowed to suckle the abdominal-inguinal glands. At this time, each dam received an intraperitoneal injection of one international unit of oxytocin followed by one additional unit of oxytocin 10 minutes later. After a nursing period of approximately 20-30 minutes or until the offspring appeared satiated or disinterested, each dam was reweighed. The difference between the prenursing and postnursing weights of the dam was used as an index of milk yield. After obtaining the milk yield, each dam was sacrificed and the abdominal-inguinal glands were removed for determination of DNA. Details of the procedure for extraction and determination of DNA has been reported previously (5). Glands in which milk was visible at sacrifice were not included in the milk yield data.

Results. The data on DNA, milk yield, and litter weight gain are shown in Table I. As indicated, DNA content of glands of rats nursing 9 or 12 young was 22 and 39% greater, respectively, than that of glands of rats nursing only 6 offspring. Furthermore, DNA content of glands of dams nursing 12 young was significantly greater than that of dams nursing 9 young. Milk yield (difference between prenursed and postnursed weight of dam expressed as g/100 g) of rats nursing 6 young was 3.80 ± 0.19 g/100 g. Milk yield of rats nursing 9 and 12 young was increased 20 and 47%, respectively, when compared with the milk yield obtained in rats nursing 6 young. Although the gain in litter weight between day 1–13 postpartum increased with an increase in litter size, a significant difference in litter weight gain was only evident between litters of rats nursing 6 and 12 young.

Discussion. Several investigations (6) have

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TABLE I. Relationship of Litter Size to Mammary Gland DNA and Milk Yield of Lactating Rats at a Single Nursing on Day 14 Postpartum.

No. of young in litter	No. of lactating rats	Body wt. of dams (g)	DFFT[a] (mg)	DNA/mg DFFT (μg)	Total DNA, abdominal-inguinal glands (mg/100 g)	Milk yield, abdominal-inguinal glands (g/100 g)	Total litter wt. gain, day 1–13 postpartum (g)
6	10	324.8	1296.2	20.04 ± 0.83	8.02 ± 0.50[1][b]	3.80 ± 0.19[4]	129.0 ± 7.0[7]
9	12	295.7	1336.1	21.76 ± 0.67	9.73 ± 0.51[2]	4.55 ± 0.20[5]	144.7 ±10.1[8]
12	10	272.4	1237.8	24.50 ± 0.46	11.11 ± 0.38[3]	5.60 ± 0.40[6]	168.7 ± 7.1[9]

[a] DFFT = Dry, fat-free tissue; values are means ± SE.
[b] Student's t test, level of significance: 1–2, $p < .050$; 1–3, $p < .001$; 2–3, $p < .050$; 4–5, $p < .025$; 4–6, $p < .005$; 5–6 and 7–8, not significant; 7–9, $p < .005$; and 8–9, not significant.

shown that mammary cellular proliferation continues well into lactation and that a sufficiently intense suckling stimulus maintains mammary parenchymal tissue even though milk is not removed from the gland (2). The data of the present study support our previous findings (2) using galactophore-ligated glands in that an increase in the suckling stimulus (increase in litter size) results in an increase in the cellular content of the gland (increase in DNA content). Furthermore, the increase in mammary cellular content is reflected in an increase in milk yield. Although milk yield at a single nursing may not be an adequate criterion of the lactational performance of the animal, it does at least give some indication of the rate of milk synthesis and the capacity of the gland to contain milk. Inasmuch as litter weight gain increases with an increase in litter size from 6 to 12 pups, the milk yield data become even more significant. It is apparent, however, that the increase in litter weight gain is not as precise in estimating lactational performance as milk yield and may account for the conflicting results of other investigations (3, 4).

It is probable that the more intense or more frequent suckling stimulus which has been shown to occur in rats nursing the larger litters (2) results in a greater, more frequent or prolonged release of adenohypophyseal somatotropin and prolactin which are known to be both mammogenic and galactopoietic in rats. This interpretation is strengthened by recent studies (7, 8) showing that both pituitary somatotropin and prolactin are released in increasing amounts as a result of an increase in the number of suckling young.

Summary. Litters of primiparous lactating rats were adjusted to contain either 6, 9, or 12 young on the day of parturition. On day 13 postpartum, each rat was isolated from her litter for 16 hrs. On day 14 postpartum, each dam was anesthetized with sodium pentobarbital and weighted. The pups were then returned to their mothers, allowed to suckle the abdominal-inguinal glands, and each dam was reweighed. The difference between the prenursing and postnursing weights of the dam was used as an index of milk yield. Deoxyribonucleic acid (DNA) of the abdominal-inguinal glands was also determined. The DNA content of the glands of rats nursing 9 or 12 young was 22 and 39% greater, respectively, than that of rats nursing 6 offspring. Milk yield by rats nursing the larger litters was also significantly greater. A significant increase in litter weight gain was evident between litters of rats nursing 6 and 12 pups. These data suggested that an increase in the suckling stimulus (larger litters) resulted in an increase in mammary gland cellular content (DNA) which was reflected in an increase in milk yield.

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Association of Placental Alkaline Phosphatase Activity with Preparations of the Human Placental Inhibitor to Hemagglutination by H-1 Virus* (33735)

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Recent studies (1, 2) described the purification of a glycoprotein observed by Toolan (3) in all human placental fluids, that inhibits hemagglutination by H-1 and HB viruses, but not hemagglutination by the closely related RV or H-3 viruses. The inhibitor contains 73–78% protein, 9–11% hexose, 7–8% hexosamine, and 4–6% sialic acid (1). Purified preparations, when subjected to acrylamide or paper electrophoresis, show one band near the origin in the gel and one band which coincides with the slow alpha or fast beta globulin region on paper (1, 2). After purification by the use of sulfosalicylic acid, the inhibitor exhibits heterogeneity when examined by the analytical centrifuge. Such heterogeneity could be explained on the basis of polymerization of a single species (2). Preparations obtained with more gentle procedures and centrifuged in a sucrose gradient with alpha$_2$M macroglobulin, a 19S serum protein used as a marker, show inhibitor activity in the same area as the alpha$_2$M (2). The glycoprotein is very stable to heat and a wide pH range.

In a recent investigation, Ghosh and Fishman (4, 5) purified and studied the properties of molecular weight variants of human placental alkaline phosphatase. The molecular weights for the two major variants, designated A and B, were 69,000 and over 200,000 respectively, on the basis of sucrose density gradient ultracentrifugation. Placental alkaline phosphatase was reported to be a sialoprotein (6, 7) containing a large carbohydrate moiety (5). It was observed (8) that highly purified preparations of placental alkaline phosphatase contain glucosamine, galactosamine, mannose, galactose, fucose, and glucose in addition to terminal neuraminic acid residues. These placental phosphatases appear in starch gel patterns near the origin (5) and also in the alpha$_2$ and beta globulin zones. The thermal stability of the placental enzyme (9) is a property that has been exploited to inactivate contaminating phosphatase and other enzymes (5). Heat stability of the enzyme is also used to detect, measure, and characterize placental isoenzyme of alkaline phosphatase in pregnancy sera (9–11).

The similarities in physical and chemical properties between the human placental alkaline phosphatases and the hemagglutination inhibitor raises the question of whether they might be the same glycoprotein. The present report describes the association of placental alkaline phosphatase activity with purified preparations of human placental in-

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