from 4 animal species against EEE, 57 from 9 species against VEE and 29 from 7 species and 89 from 10 species against WEE and SLE respectively. In addition to these reactors, 162 other serum samples were found to be positive with two or more of these arbovirus antigens. Only WEE and SLE are known to be present in this region based upon known outbreaks and previous studies of others.

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1. Vest, E. D., Lundgren, D. L., Parker, D. D., Johnson, E. D., Morse, E. L., Bushman, J. B., Sidwell, R. W., Thorpe, B. D., Am. J. Trop. Med. & Hyg., in press.

2. Sidwell, R. W., Lundgren, D. L., Thorpe, B. D., Am. J. Trop. Med. & Hyg., 1964, v13, 591.

3. Sidwell, R. W., Lundgren, D. L., Bushman, J. B., Thorpe, B. D., ibid., 1964, v13, 754.

4. Graham, J. K., Bradley, I. E., Collett, G. C., Mosquito News, 1960, v20, 200.

5. Olitsky, P. K., Clarke, D. H., in Viral and Rickettsial Infections of Man (3rd ed.), T. M. Rivers and F. L. Horsfall, ed., Lippincott, Philadelphia, 1959, p305.

6. Olitsky, P. K., Casals, J., ibid., p292.

7. Kissling, R. E., Chamberlain, R. W., Nelson, D. B., Stamm, D. D., Am. J. Hyg., 1955, v62, 233.

8. Work, T. H., Science, 1964, v145, 270.

9. Chamberlain, R. W., Sudia, W. D., Coleman, D. N., Work, T. H., ibid., 1964, v145, 272.

10. Scherer, W. I., Dickerman, R. W., Chia, C. W., Ventura, A., Moorhouse, A., Geiger, R., Najera, A. D., ibid., 1964, v145, 274.

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Mammary Growth in Rats Treated with Somatotropin During Pregnancy and/or Lactation.* (29791)

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Several studies have indicated that somatotropin (STH) exerts a galactopoietic effect in the cow, sheep and goat(1). In the rat, STH has been reported to have either no effect(1) or to increase(2) lactational performance depending on the method employed in assessing milk secretion.

Moon(3) has shown that STH enhanced mammary growth induced in ovariectomized rats with ovarian steroids and that concomitant injections of estrogen and STH into the spayed rat resulted in formation of alveoli containing a considerable quantity of secretory material. Furthermore, a positive correlation between mammary gland cell content and amount of milk obtained at a single nursing has been reported(4).

In the present study, we were interested in determining whether exogenous STH administered during pregnancy and/or lactation would enhance mammary growth and if so, whether the increased mammary growth would be reflected in the lactational performance of the animal.

Material and method. Adult primiparous and primiparous lactating rats weighing 220-240 g when bred were housed individually in a room maintained at a temperature of 76-78°F and artificially illuminated during normal daylight hours. Animals received Wayne Lab Blox and fresh water *ad libitum*. The day sperm were observed in the vaginal smear was designated as *day 1* of pregnancy whereas the day of parturition was considered as *day 1* of lactation. Rats were divided into groups and received daily subcutaneous injections of 2.0 mg STH‡ from either day 3-19 gestation or day 1-13 lactation. Another group received graded increments (0.5-3.0 mg) of the hormone every 6 days during both pregnancy and lactation. Other groups of rats served as pregnant and lactation controls.

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TABLE I. Effect of Somatotropin (STH) Administered During Pregnancy (P) and/or Lactation (L) Upon Mammary Gland DNA and Quantity of Milk Obtained at a Single Nursing on Day 14 of Lactation.

Treatment amt/day	No. of rats	Body wt day 3 pregnancy (g)	DFFT* (mg)	DNA/mg DFFT (μg)	Total DNA (mg/100g)	Total milk (g/100g)
Pregnant controls	12	226.5	634.3	24.51	6.42 ± .78[1]	—
2.0 mg STH, day 3-19 P	10	221.2	818.1	22.54	8.69 ± .48[2]	—
Lactation controls	16	235.3	1427.1	17.32	10.48 ± .42[3]	7.64 ± .24[6]
2.0 mg STH, day 3-19 P	5	240.0	1834.1	16.78	12.81 ± .61[4]	8.04 ± .77
2.0 mg STH, day 1-13 L	11	240.1	1620.5	17.88	12.00 ± .43	8.02 ± .32
Graded increments STH every 6 days†	7	246.7	1834.7	16.64	12.34 ± .68[5]	9.01 ± .67[7]

Values are means ± S.E. of mean.
* Dry, fat-free tissue.
† 0.5 mg, day 3-8 P; 1.0 mg, day 9-14 P; 1.5 mg, day 15 P-parturition: 2.0 mg, day 1-6 L; 3.0 mg, day 7-13 L.

Student's t test, level of significance.
1-2 .5%
2-3 2.5%
3-4 2.5%
3-5 5 %
6-7 2.5%

Litters of rats allowed to undergo lactation were reduced to 6 young on day 2 postpartum. On day 14 postpartum, each dam was isolated from her litter for 10 hours. At the end of the isolation period, each dam was anesthetized with Nembutal and milk yield was determined by the method described in detail by Grosvenor(5). Body weight of dams and litters was recorded daily.

Immediately after determination of milk yield or one day after last injection of STH, rats were sacrificed by an overdose of ether. Animals were skinned rapidly and the abdominal-inguinal mammary glands were removed and immediately frozen until assayed for deoxyribonucleic acid (DNA). Details of the procedure for extraction and determination of DNA have been reported(6).

The product of the quantity of DNA/mg of dry fat-free tissue (DFFT) and total quantity of DFFT was used as an estimate of the total amount of DNA present in the abdominal-inguinal glands. The total DNA/100 g body weight was used as an index of the degree of cellular proliferation of the abdominal-inguinal glands for reasons outlined previously(7). Since body weight of rats treated with STH increased more rapidly than those which did not receive the hormone, DNA/100 g was based on body weight at *day 3* of pregnancy.

Results. Daily administration of 2.0 mg STH from day 3-19 gestation resulted in a significant increase in mammary gland DNA when compared with that of glands of untreated rats at day 20 of pregnancy (Table I). Rats which received no treatment but were allowed to deliver and undergo 14 days of lactation exhibited a mammary DNA content which was 63% greater than that of pregnant controls. Furthermore, DNA content of glands of non-treated lactating animals was significantly higher than that of glands of rats receiving STH and killed at day 20 of pregnancy. A significant elevation in mammary DNA above the lactation control level was also evident in animals treated with STH and carried to day 14 lactation irrespective of whether the animals received the hormone during either pregnancy or lactation, or during both pregnancy and lactation.

Milk yield of rats receiving a constant daily dose of STH during either pregnancy or lactation did not differ from that of control lactators. However, a significant increase in milk yield was observed in animals treated with graded increments of the hormone during both pregnancy and lactation but no difference in litter weight gain was evident between the treatment groups.

Discussion. Lyons, *et al*(8) have shown that histologically complete lobule-alveolar development of the mammary gland of the hypophysectomized-adrenalectomized-ovariectomized (triply operated) immature rat requires the synergistic activity of both the ovarian steroids and 2 adenohypophyseal hormones, STH and prolactin. Talwalker and Meites(9) have induced alveolar growth in the triply operated adult rat with large quantities of only STH and prolactin. Moreover,

Moon(3) has shown that exogenous administration of STH to the ovariectomized rat with an intact pituitary enhances mammary growth induced with the ovarian steroids. Thus, it is apparent that somatotropin is of prime importance in the development of the rat mammary gland.

In the present study, a significant increase in mammary gland DNA is evident in rats receiving STH during pregnancy when compared with that of the pregnant controls. Such an increase in mammary DNA suggests that during pregnancy the endogenous production and/or release of STH is not optimal for mammary gland growth. Although the endogenous production and/or release of STH may be maximal during this period, the mammary parenchyma is still capable of responding to additional hormone supplied exogenously. Furthermore, the enhanced mammary growth which results from administration of STH during pregnancy does not obscure the cellular proliferation which normally occurs during lactation(6,10), for mammary DNA of lactating rats treated with the hormone during pregnancy or lactation is significantly greater than that of control lactators and STH treated pregnant rats. Whether the spurt in mammary gland cellular proliferation during lactation is due to an increased production and/or release of adenohypophyseal hormones of mammogenic potency or due merely to a change in ratio of ovarian steroids to adenohypophyseal hormones is not known. Nevertheless, administration of STH results in significantly greater mammary growth than that which is evident at mid-lactation irrespective of whether the hormone is administered during pregnancy, during lactation or during both pregnancy and lactation

Although DNA content of glands of lactating rats treated with a constant dose of STH (2.0 mg) and those receiving graded increments of STH (0.5-3.0 mg) is comparable, milk yield is elevated only in rats of the latter group. It appears, therefore, that the increased number of cells present in glands of lactators treated with a constant dose of STH during either pregnancy or lactation are not functional. It is possible that STH requirements for galactopoiesis are greater than that which is necessary to stimulate mammary cellular proliferation. If this is so, then an increase in number of mammary gland cells may occur without a concomitant increase in milk yield. The observation that an elevation in milk yield is obtained in rats injected with graded increments of STH during pregnancy and lactation is consistent with this interpretation.

Summary. Groups of rats received daily injections of 2 mg somatotropin (STH) from either day 3-19 of pregnancy or day 1-13 of lactation. Another group of rats received graded increments (0.5-3.0 mg) of STH every 6 days during both pregnancy and lactation. Other groups of rats served as pregnancy and lactation controls. Mammary gland deoxyribonucleic acid (DNA) content was determined in all rats and milk yield was determined in all rats carried to day 14 postpartum. Significant increases in mammary DNA occurred in all rats treated with STH irrespective of dose or schedule of injections when compared to gestation controls. A significant elevation in mammary DNA above the lactation control level was also evident in rats receiving the hormone and carried to day 14 of lactation. Milk yield of rats treated with graded increments of STH was significantly greater than that of control lactators but milk yield of rats receiving a constant daily dose of the hormone did not differ from that of controls.

1. Meites, J., Milk: The Mammary Gland and Its Secretion, S. K. Kon, and A. T. Cowie, Ed., New York, Academic Press, 1961, vI, 321.

2. Grosvenor, C. E., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1959, v100, 158.

3. Moon, R. C., Am. J. Physiol., 1961, v201, 259.

4. Moon, R. C., Fed. Proc., 1960, v19, 149.

5. Grosvenor, C. E., Endocrinol., 1962, v70, 75.

6. Moon, R. C., Am. J. Physiol., 1962, v203, 939.

7. Moon, R. C., Griffith, D. R., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1959, v101, 788.

8. Lyons, W. R., Li, C. H., Johnson, R. E., Recent Progr. Hormone Res., 1958, v14, 219.

9. Talwalker, P. K., Meites, J., Proc. Soc. Exp. Biol. and Med., 1961, v107, 880.

10. Tucker, H. A., Reece, R. P., ibid., 1963, v112, 409.

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