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Effect of Oxytocin Upon Litter Weight Gain in Rats.* (31405)

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In a series of studies from this laboratory, the influence of one or more hormones upon the yield of milk of rats on days 14 to 20 has been reported(1,2,3,4,5). The method used was to separate the mother from the young for a period of 10 hours on each test day. The young and mothers were then weighed and placed with the mothers for a nursing period of 30 minutes. In the initial experiment, it was observed that the endogenous oxytocin discharged was inadequate to permit complete milk removal since the injection of oxytocin during the nursing period increased the mean milk yield 50%(3). In subsequent studies, oxytocin was injected when the young were returned to the mother and a second injection was given after 15 minutes. The milk yield was then determined either by the increase in weight of the young or by the decrease in weight of the mother or both.

In these studies, it was shown that individual hormones and combinations of hormones injected from day 7 to 20 of lactation stimulated marked increases in milk yield on the test days. However, the litter weights of the experimental groups in most experiments were not significantly greater than the control groups. If, as is claimed, the milk secretion of the experimental groups is markedly increased, why then do not the litters take advantage of the increased available milk and grow faster than the controls?

To seek an answer to this problem, it is necessary to understand the nursing habits of the rat. Normally, the young nurse their mothers for approximately 1 to 5 minutes at about hourly intervals. At the nursing period, the neural stimulus causes the release of oxytocin and permits the removal of milk. If the amount of milk present in the gland is limited, it may be completely removed before the oxytocin is inactivated‡. If the amount of milk present increases, then increasing amounts of milk present in the glands may not be removed by this short nursing period and the growth rate of the litter would not be increased accordingly even though increased amounts of milk were being secreted

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‡ Study of the biological half-life ($t_{1/2}$) of oxytocin in the rat indicates that it is very short. One earlier estimate indicated a $t_{1/2}$ of 1 minute and 40 seconds(6). In a recent study of Aroskar *et al*(7), it was suggested that the rapid disappearance of oxytocin from the blood of the rat was not due to its inactivation by the blood, but rather to the uptake of the hormone by the kidneys and liver. That the $t_{1/2}$ of oxytocin in the rat is shorter than indicated above is suggested by the report of Folley and Knaggs (8), that the $t_{1/2}$ is only 1 minute and 22 seconds in the goat.

under the stimulus of the hormones.

If the inability of the nurslings to remove the available milk at each hourly nursing period due to the short half-life of oxytocin were one factor influencing the litter weights at weaning time, then if the endogenous oxytocin were to be supplemented with exogenous hormone at intervals during the day, the mean litter weight at weaning time should be increased. The experiments described here were conducted to gain information as to this problem.

Materials and methods. Seventy-eight lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, fed Purina Lab Chow and water *ad libitum.* The animals were kept under standardized conditions of temperature and light (78 ± 1°F; 14 hours light—10 hours dark). On day 1 of lactation, litters were adjusted to 8 young and on day 4 reduced to 6 young. From day 7 to 19 of lactation, the dams were injected subcutaneously every 6 hours as follows: (1) 24 controls were injected with 0.05 ml of physiological saline; (2) 24 rats were injected with 1 USP unit of oxytocin.§ The dams and litters were weighed daily from day 7 of lactation.

A group of 30 lactating rats was raised similarly with 6 young per litter. On day 19 of lactation, the milk yield was estimated as follows by increasing hourly intervals: The dams and litters were separated for an hour. The litters were weighed on scales accurate to 1 g and put with their mothers to nurse.

After 15 minutes, the litters were reweighed. The difference between the pre- and post-nursing weight was considered the yield of milk suckled by litters in 15 minutes. Then the mothers were injected with 1 USP unit of oxytocin and the young were allowed to nurse for 15 minutes and the litters were reweighed. After this period the mothers and litters were separated for 2 hours. The milk yield was again determined with the aid of 1 USP unit of oxytocin.

Results. The mean litter weight of control rats on day 7 of lactation was 95±2.09 g and on day 20 of lactation was 217±5.90 g. The mean litter weight of experimental rats on day 7 of lactation was 92±2.47 g and on day 20 of lactation was 246±6.18 g. The mean litter weight gain in the experimental group was 29 g greater than the control litters on day 20 of lactation, a highly significant increase of 13% ($P<0.001$).

Of the 30 lactating rats whose litters were separated for one hour, milk let-down was observed only in 8 without oxytocin during a 15-minute nursing period. The mean milk yield for the 8 rats was 2.25±0.25 g. The rest of the dams did not release milk. When the rats were then injected with 1 USP unit of oxytocin, the mean milk yield after 15 minutes was 7.33±0.38 g. After an hour separation, the mean milk yield with the aid of oxytocin was 1.67 ± 0.23 g. The increase in milk yield following a 2-hour separation the mean milk yield with the aid of oxytocin was increased to 2.87 ± 0.22 g. The increase in milk yield following a 2 hour separation period was highly significant in comparison with 1 hour ($P>0.001$).

Discussion. The use of growth curves of the litters of rats as an index of the intensity of milk secretion has been suggested by Daggs (9) and Cowie and Folley(10). In nutrition or endocrine studies where reduced lactation is expected, a reduction in the mean litter weight would be expected and this index might serve a useful purpose. When an increase in the intensity of milk secretion by hormone therapy is attempted, an increase in litter weight at weaning time has frequently not been observed in spite of the fact that, by the method developed in this laboratory, increased milk yield is indicated.

To explain why litter weight gain above the controls should not be expected, even in the presence of an increased availability of milk in the mammary glands of the dams, the following suggestions are offered. First, it is suggested that the voluntary consumption of milk by the nurslings varies just as in growing and mature rats and an abundance of milk will not increase their intake(11). Second, there are genetic and endocrine limits to the rate of growth in the presence of an excessive milk supply(3). Third, the short biological half-life ($t_{1/2}$) of oxytocin will limit the

§Oxytocin was kindly supplied by Armour-Baldwin Laboratories, Omaha, Neb.

amount of milk which can be removed at each nursing period.

That the amount of milk obtained and litter growth rate of even normal rats is limited by the amount of oxytocin discharged and its short $t_{1/2}$ is shown by the present experiment. When the experimental group of dams was injected with 1 USP unit of oxytocin at six-hour intervals during the day and the nurslings at these periods were able to remove a larger proportion of the available milk, each litter gained a mean of 29 g or 13% more than did the control group.

In a further experiment, a group of rats was separated from the litters for 1 hour. Of a group of 30 rats, only 8 were stimulated sufficiently for the young to obtain milk. These nurslings obtained a mean of 2.25 g of milk. That the milk obtained did not represent all the milk present was shown by separating the young for 1 hour, then 1 unit of oxytocin was administered. At this time a mean of 7.33 g of milk was removed. To obtain information on the rate of milk secretion, the same rats were then separated for 1 hour and 1 unit of oxytocin was given. The young removed a mean of 1.67 g of milk. After a 2-hour interval, the mean milk yield was 2.87 g.

It is concluded from these observations that the amount of oxytocin released and its short $t_{1/2}$ may limit the amount of milk which is removed at each nursing period in the rat. If the secretory capacity of the dam is limited, most of the available milk may be removed at nursing time and this milk will permit normal growth of the young. In normal rats whose intensity of milk secretion is greater and in rats stimulated by one or more hormones, all of the available milk is not removed at each nursing period, and, therefore, their litter weight growth does not represent the capacity of the dams to secrete milk. If exogenous oxytocin were injected at each nursing period to supplement the endogenous supply, litter growth rate would be increased up to the capacity of the litters to consume milk and their genetic limitation in growth rate.

In the light of these observations, it is not believed possible for litter growth to be a satisfactory index of the intensity of milk secretion. On the other hand, by the method used in this laboratory, at the test milkings with the aid of oxytocin, complete milk removal is effected and the yield of milk removed indicates the intensity of milk secretion during the period of 10 hours.

Summary. The influence of one or more hormones on the intensity of milk secretion in rats has been determined by a method developed in this laboratory. Significant increases in milk yield have been observed. However, the litter weights of the control and experimental groups have been quite similar. In explanation of these observations, it has been suggested that when milk secretion is intense that 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 unit 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%. It was shown also that not all of the milk is removed at the regular hourly nursing period, by the removal of 7.33 g of milk when 1 unit of oxytocin was injected at nursing time compared to 1.7 g of milk without exogenous oxytocin.

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