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Estimation of Amount of Oxytocin Released as Result of Nursing Stimuli in Lactating Rat.* (23142)

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There seems to be little doubt that the posterior lobe of the pituitary gland plays an important role in milk let-down(1). It is also generally accepted(2-4) that oxytocin from this gland is responsible for contraction of myoepithelial elements surrounding alveoli and small ducts of the mammary gland thus making available milk for withdrawal. Amount of oxytocin required by various species for milk let-down has been investigated to some extent for the rabbit(5-8), sheep(9), goat(9,10), sow(11) and human(12). However, the amount required by the rat has not been adequately determined. Folley(10), reporting upon unpublished work by Cowie, found that young of anaesthetized lactating rats were able to obtain milk, as determined by test weighing, after a single i.p. injection of .025 I.U. oxytocin into the mother. Grosvenor and Turner(13) found that .2 I.U./kg subc. restored milk let-down in lactating rats in which normal let-down had been inhibited by ergotamine treatment.

In the present study the amount of oxytocin required for normal milk let-down in lactating rats has been determined by injecting various levels of oxytocin i.v. into anaesthetized mothers and comparing weight of milk obtained by the young with normal values previously obtained(14).

Materials and methods. Forty-one lactating rats, each with its first litter and weighing 190-320 g, were housed in individual cages and fed Purina Lab Chow and water *ad libitum.* Shortly after birth each litter was reduced to 6 young and when 14 days old was isolated from the mother for 10 hours. Each mother was weighed and injected with Nembutal (3 mg/100 g) i.p. 10-20 minutes prior to end of isolation period. When completely anaesthetized, she was laid on her side and her litter replaced. Twenty-seven animals received oxytocin‡ in dose of .02, .05 or .1 USP/kg injected i.v., in a volume not exceeding .06 ml, into the superior epigastric vein a few minutes after the young were replaced and while they were actively sucking.

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TABLE I. Milk Removal by Litters of Anaesthetized Nursing Rats Injected with Oxytocin on 14th Day Postpartum.

Treatment	No. of rats	Avg wt of litters (g)	Avg wt of milk (g)	Avg % Wt of milk / Wt of litter
Control*	40	144.0	5.5	3.8 ± .19
Nembutal	14	164.5	.0	.0
Nembutal + .02 USP/kg oxytocin	11	159.0	4.7	3.0 ± .82
Nembutal + .05 USP/kg oxytocin	11	164.2	6.3	3.8 ± .20
Nembutal + .1 USP/kg oxytocin	5	168.8	9.8	5.9 ± .45

* Data from Grosvenor and Turner(14).

Eight similarly received .06 ml distilled water; the remaining were uninjected. After each litter had been nursed for 30 minutes they were removed, weighed, killed by decapitation and stomach contents removed and weighed.

Results. Nembutal anaesthesia blocked the let-down reflex in lactating rats and prevented removal of milk by young during 30 minutes nursing (Table I). Oxytocin injected i.v. a few minutes after nursing had commenced, resulted in milk let-down after a latent period of 5-10 seconds and seemed to persist 4-5 minutes. These times were obtained by observing the young; when milk let-down had started the young immediately began sucking actively. After milk flow had apparently ceased they became quiet and sucked only intermittently during the remainder of the experiment. Amount of milk obtained expressed as percent litter body weight secured during 30 minutes by young of anaesthetized lactating rats injected with oxytocin is shown in Table I. Values obtained with doses of oxytocin of .02, .05 and .1 USP/kg were 3.0 ± .82, 3.8 ± .20 and 5.9 ± .45, respectively. Control injections of distilled water were without effect.

Discussion. Our previous results(14) obtained with 40 litters, each with 6 young, on 14th day postpartum, indicated that weight of milk in stomachs of young expressed as percent litter body weight obtained after 30 minutes nursing following 10 hours isolation gave a mean of 3.8 ± .19. Results of the present study obtained under the same standard conditions indicated .05 USP/kg oxytocin was necessary to evoke let-down of same mean quantity of milk whereas .02 USP/kg was insufficient. Since in the previous study (14) 12 of 40 normal rats showed milk let-down permitting removal of 2.0-3.0%, 15 rats of 3.7% and 12 others a removal of 4.6-5.6%, it is suggested, on the basis of present results, the amount of oxytocin released by those animals was approximately .02, .05 and .1 USP/kg. It is concluded that normal 14 day postpartum lactating rats vary in amount of release of oxytocin following nursing stimuli from less than .02 to .1 USP/kg. It is of interest to note injection of .1 USP kg oxytocin elicited a much greater withdrawal of milk which would seem to indicate more milk is present in mammary glands of lactating rats than a litter of 6 normally obtains during 30 minutes nursing, although previous observations(14) indicated young were satiated by this time.

That complete anaesthesia blocks milk let-down reflex and that posterior pituitary principles are able to cause discharge of milk in the anaesthetized animal has been determined for species other than the rat(6-8,10,15). These observations have been extended in the present work to include the rat. Extensive experiments on the lactating sow have led Whittlestone(11) to estimate 0.5-1.0 I.U. oxytocin released normally at a nursing. Cross(8) found .05 I.U. similarly needed for the lactating rabbit. Results of this study indicate about .014 USP oxytocin is released per lactating rat in response to nursing stimuli which, on a body weight basis, is approximately 3 and 10 times the amount released by the rabbit and sow, respectively.

Summary. We estimated amount of oxytocin required for normal milk let-down in lactating rats. This was determined by in-

jecting various doses of oxytocin i.v. into anaesthetized mothers while young were actively sucking and comparing weight of milk obtained by young expressed as percent litter body weight with normal values. Nembutal (3 mg/100 g) blocked milk let-down reflex and prevented removal of milk by the young. .05 USP/kg oxytocin restored normal milk withdrawal; .02 USP/kg did not. Much greater yields were obtained with .1 USP/kg. Milk flow commenced with all doses after latent period of 5-10 seconds and lasted 4-5 minutes. It is concluded that normal 14 day postpartum lactating rats vary in amount of release of oxytocin following nursing stimuli from less than .02 to .1 USP/kg.

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Antigenicity of Egg White Proteins in the White Mouse.* (23143)

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It has been well demonstrated by a variety of technics(1-3) that diverse antibodies other than anti-ovalbumin are present in "specific" antisera directed against crystalline ovalbumin. Indeed, Vaughan and Kabat(4) have demonstrated that, in addition to anti-conalbumin, anti-ovomucoid and anti-lysozyme, antibodies exist in such sera as a result of the antigenic stimulation of hitherto unidentified egg white impurities in ovalbumin. In attempting to relate serum antibody titers to fatal anaphylactic shock in the ovalbumin-sensitized mouse the question of antibody identity arose. Having previously noted(5) that all proteins do not manifest the same ability to sensitize the white mouse to the same degree, the sensitizing capacities and cross-reactivities of the available protein contaminants of crystalline ovalbumin were characterized by the pertussis-technic(6).

Materials and methods. Thrice recrystallized egg albumin (Ea) was prepared according to the procedure of Kekwick and Cannan (7) as described by Kabat and Meyer(8). Conalbumin (Ca), as the crystalline iron complex, and ovomucoid (Ovo) were prepared(9) and generously supplied by Dr. Robert C. Warner of New York University. Crystalline lysozyme (Lyso) was purchased from Nutritional Biochemicals Corp. Female white mice of the Swiss-Webster strain weighing about 20 g were obtained from Tumblebrook Farms. Groups of animals were sensitized by a single intraperitoneal injection of a given amount of the egg white protein contained in 0.5 ml of a suspension of 2 x 10^{10}

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