Release and Restoration of Pituitary Lactogen in Response to Nursing Stimuli in Lactating Rats.* (23588)

CLARK E. GROSVENOR† AND CHARLES W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

Lactogenic hormone is thought to be reflexly released from the hypophysis in response to the sucking stimulus, regular application of which seems to be essential for the maintenance of milk secretion(1). In light of this concept, the influence of 3 hours nursing upon lactogen discharge has been studied in the rat(2), rabbit and guinea pig(3). We thought it desirable to reinvestigate this phenomenon in the lactating rat in an effort to obtain more complete data which could serve as a basis for future work concerning neurohumoral mechanisms involved in lactogen release. In the present study, we have shown release of lactogen following nursing in the lactating rat occurs much more rapidly than previously determined, but subsequent restoration is a much slower process. We have also shown differences exist in pituitary lactogen levels which are reflected in total lactational performance in two strains of rats studied.

Materials and methods. Forty primiparous lactating rats of the Wistar-Missouri strain and 60 of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages and fed Purina Lab Chow and water *at libitum.* Shortly after birth each litter was reduced to 6 young and when 14 days old was isolated from their mother for 10 hours. Maternal and litter weights were recorded at this time. Fifteen rats of each strain then were immediately killed. The remaining animals were reunited with their litters and allowed to nurse for exactly 30 minutes, then killed either immediately, 2½ or 9½ hours after nursing. After death of the mothers, their pituitaries were removed, weighed individually, and frozen until assayed for lactogenic hormone. Five glands were pooled for each assay. These were crushed in a small agate mortar, suspended in a measured amount of distilled water and assayed in adult common pigeons by the Reece-Turner intradermal method(2).

Results. Although the Wistar lactating rats were much smaller, their litters at the 14th day postpartum were almost as heavy as those of Sprague-Dawley mothers (Table I). However, there was 15% more pituitary gland/100 g in the Wistar animals and, after 10 hours of isolation without nursing, assayed 30% more hormone than pituitaries of Sprague-Dawley mothers (3.50 and 2.44 Reece-Turner units/100 g, respectively) (Fig. 1.) Following 30 minutes nursing the pituitary lactogen level dropped 33% (1.16 units/100 g) in Wistar and 32% (0.78 unit/100 g) in Sprague-Dawley rats. About one-half the prenursing level was restored in both strains within 2½ hours postnursing, but in the one strain tested (Sprague-Dawley) pituitary restoration to original level was not accomplished even by 9½ hours postnursing.

Discussion. The rapidity in which the act of nursing elicited a drop in pituitary lactogenic hormone in the present study would seem to substantiate the view such release is mediated through nervous reflex. Previous workers(2,3) allowed nursing to continue at least 3 hours before killing the mothers, though it has been our experience young of lactating rats become satiated within 30 minutes and stop sucking(4). Restoration of lactogen to prenursing levels was not accomplished even by 9½ hours postnursing, *i.e.,* the same time allowed for determination of prenursing levels. Lactating rats, of course, nurse more frequently than once every 20 hours on the 14th day of lactation(5) so pituitary lactogen values probably do not represent those found in normal nursing situations. The evidence obtained in both strains that pituitary lactogen had returned

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TABLE I. Comparison of Lactational Performance of 2 Strains of Albino Rats, Each with a Litter of 6 Young on 14th Day Postpartum.

	No. of rats	Avg wt of Litters (g)	Avg wt of Mothers (g)	Avg wt of Pituitary glands (mg)	Avg wt of Pituitary glands (mg/100 g)	Avg prenursing pituitary lactogen (Reece-Turner units/100 g)
Wistar-Missouri	40	140.3	214.9	8.8	4.1	3.50
Sprague-Dawley-Rolfsmeyer	60	145.8	290.2	10.2	3.5	2.44

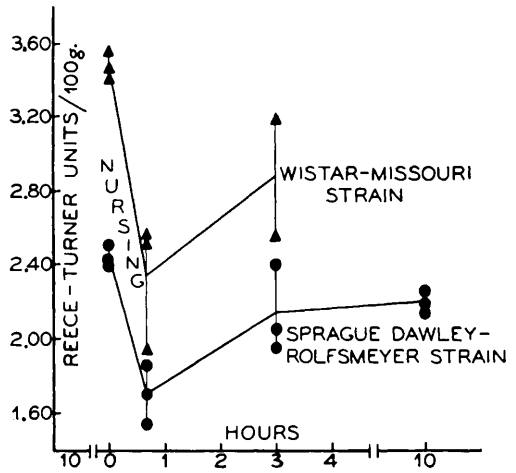

FIG. 1. Strain difference in release and restoration of pituitary lactogen in response to nursing stimuli after 10 hr isolation of mother and young in 14 day postpartum lactating rats. Each point represents assay of 5 pituitary glands.

in 2½ hours to about one-half that determined before nursing suggests enough hormone is restored to insure continuance of milk secretion following the next nursing. As lactation progresses it is possible there is a gradual diminution in amount of hormone restored after nursing to the point when milk secretion is no longer possible.

The observation that small Wistar rats are capable of raising litters as heavy as the larger Sprague-Dawley animals suggests more milk is produced in relation to body size in the Wistar rat. This may be due to the relative greater size of their pituitary glands and subsequent relative higher lactogen content. Also, the amount of hormone released in response to sucking was 33% greater in Wistar lactating rats. However, as pointed out by Folley and Young(6), maintenance of milk secretion is probably a response to the coordinated actions of a complex of pituitary hormones with lactogen as an essential element so it is difficult, therefore, to compare milk secretory activity on the basis of lactogenic hormone alone.

Summary. 1) The effects of nursing stimuli upon release and subsequent restoration of lactogenic hormone from the hypophysis has been studied in 2 strains of lactating rats on 14th day postpartum. 2) Wistar lactating rats produced litters which, when 14 days old, were almost as heavy as those of the much larger Sprague-Dawley mothers. There was 15% more pituitary gland/100 g in Wistar rats which, after 10 hours isolation of mother and litter assayed 30% more lactogen than pituitaries of Sprague-Dawley mothers (3.50 and 2.44 units/100 g, respectively). The Reece-Turner pigeon intradermal method of assay was used. Following 30 minutes nursing there was a greater discharge of lactogen (33%) in Wistar (1.16 units/100 g) than in Sprague-Dawley rats (0.78 unit/100 g). About one-half prenursing level was restored in both strains within 2½ hours postnursing but in one strain tested (Sprague-Dawley) restoration to prenursing level did not occur even 9½ hours postnursing. The influence of amounts of lactogen released in the two strains is discussed in relation to lactational performance.

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The Effects of Diamox® on the Uterine Response of Estrogen Treated Rats.* (23589)

H. MAURICE GOODMAN,† RENATA EINSTEIN AND IRA RINGLER‡
(Introduced by Frederick L. Hisaw)
Biological Laboratories, Harvard University, Cambridge, Mass.

The earliest detectable response of the rat uterus to estrogen administration is a rapid imbibition of water, followed by a period of cellular proliferation and growth. The tissues of the uterus become edematous and the entire organ enlarges as a result of luminal fluid accumulation.

The drug, Diamox® (2-acetylamide-1,-3,4-thiadiazole-5-sulfonamide) is a powerful inhibitor of the enzyme carbonic anhydrase (1,2). Such inhibition in animals results in the excretion of alkaline urine, diuresis and lowered plasma bicarbonate(3). Perlmutt and Olewine(4) reported that acetazolamide increased the excretion of water about one and one-half times in normal rats. This greatly increased water excretion suggested that Diamox® may also affect the water imbibition of the uterus following administration of estrogen.

Materials and methods. The effects of Diamox® on uterine response to estrogen were studied in both sexually immature and adult rats. The effects on the immature rat uterus were determined by means of the bioassay method of Astwood(5). Animals 21-23 days old and weighing 40 to 50 g were given a single subcutaneous injection of 0.1 μg of estradiol-17β and graded doses of the sodium salt of Diamox®. The uteri were removed at various time intervals, stripped of fat and weighed immediately. Adult female rats 100 days old were used to determine changes in wet weight, dry weight and volumes of luminal fluid induced by Diamox®. Forty-eight animals were castrated and allowed to remain untreated for 7 days, at which time they were divided into 5 groups and were treated as follows: the first group received no treatment; the second was given 5 μg estrone daily for 3 days; the remaining 3 groups received 5 μg of estrone per day for 3 days and a daily injection of 40, 60 and 80 mg of Diamox®, respectively. The amount of luminal fluid was measured by withdrawing the fluid into a glass syringe fitted with a No. 20 needle. The exact amount of fluid could not be measured by this method, but a fair indication of the relative amount could be ascertained. *Water content* was determined by oven-drying uteri for 24 hours at 90-100°C. Nitrogen content of the dried uteri was measured by Nesslerization after digestion in a selenium-sulfuric acid mixture. The *sodium salt* of Diamox® (Lederle lot No. 7-6015)§ was dissolved in physiological saline at a concentration of 200 mg per ml. pH of this solution was 9.1. Estradiol-17β and estrone were dissolved in sesame oil. Rats were maintained on a normal diet and given water *ad libitum*. All injections were made subcutaneously either in the scruff of the neck or the skin of the back. The volume never exceeded 0.5 ml.

Results. The experiments in which the

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† Present address: Harvard Medical School, Boston, Mass.

‡ Present address: Research Division, American Cyanamid Co., Pearl River, N. Y.

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