Postcopulatory Mammary Gland Secretion in Rats* (33469)

WILLIAM G. DILLEY AND NORMAN T. ADLER[1] (Introduced by Howard A. Bern)

Department of Zoology and its Cancer Research Genetics Laboratory, and Department of Psychology, University of California, Berkeley, California 94720

Hormonal changes occur in female mammals as a consequence of stimuli derived from mating. In rats, a rise in the plasma concentration of LH occurs within 5–10 min after coitus, and a slight rise in the FSH content of the pituitary is seen 1 hr after copulation (1). Pituitary LTH falls within 30 min after cervical stimulation (2), whereas plasma progesterone rises 24 hr after mating (3).

Despite the potentially mammotropic effects of these hormones (4), no changes in rat mammary tissue in the period shortly following mating have been reported. The present study was undertaken to investigate the effects of various levels of copulatory activity on mammary secretion in female rats during the first 5 days following coitus.

Materials and Methods. Adult virgin Sprague-Dawley rats (at least 90 and less than 150 days old) were purchased from Berkeley Pacific Laboratories, Berkeley, California. The females were housed in individual cages, and the colony was maintained on a reversed light–dark cycle, in a windowless room, with the temperature averaging 78°F. The photoperiod was 12 hr on and 12 hr off; the midpoint of the light segment was at 3 p.m.

There were four groups of females: (i) no copulatory stimulation; (ii) low stimulation —females received three or fewer intromissions; (iii) medium stimulation—females received 4–20 intromissions; (iv) high stimulation—females received more than 40 intromissions. Females in groups 2, 3, and 4 all received sperm and showed a vaginal plug (5).

One to 5 days after mating, the females were sacrificed. All the mammary glands from each animal were fixed in 15% formalin and stained as wholemounts with hematoxylin. Pieces of tissue adjacent to the nipple of the no. 2 thoracic glands were taken from the wholemounts for preparation of paraffin sections which were stained with hematoxylin and eosin. Secretion, as judged by size of alveolar lumina and by intensity of staining of luminal contents, was graded on a scale from 0–5. Slides were coded so as to eliminate subjective bias in the assessment of secretion. Results of these ratings were analyzed by the Kruskall-Wallis one-way analysis of variance (6).

TABLE I. Effect of Intromissions on Mammary Secretion.[a]

	Copulatory stimulation (no. of intromissions)			
	None (0)	Low (1–3)	Medium (4–20)	High (>40)
Mammary secretion ratings	2	4	5	5
	0	4	5	5
	0	4	4	5
	0	3	4	5
	0	1	2	4
	0	0	2	4
		0	0	4
		0	0	4
		0	0	
		0	0	
		0	0	
		0		

[a] Each number represents the secretory rating assigned to the mammary tissues from one rat. This table represents pooled data from the 5 termination days.

Results. Since there was not any correlation of secretion with day of termination, the data for all termination dates was pooled within each stimulation group. One to 5 days after coitus, there was stainable secretion in the mammary glands of nulliparous Sprague-Dawley rats. Moreover, the amount of secretion varied with the amount of copulatory stimulation. Median values for the four groups were: group i, 0; group ii, 0; group iii, 2; group iv, 4 (Table I). The differ-

* Supported by USPHS Grants Nos. MH-04000, CA-05388, and CA-05045.

[1] Present address: Department of Psychology, University of Pennsylvania, Philadelphia, Pennsylvania.

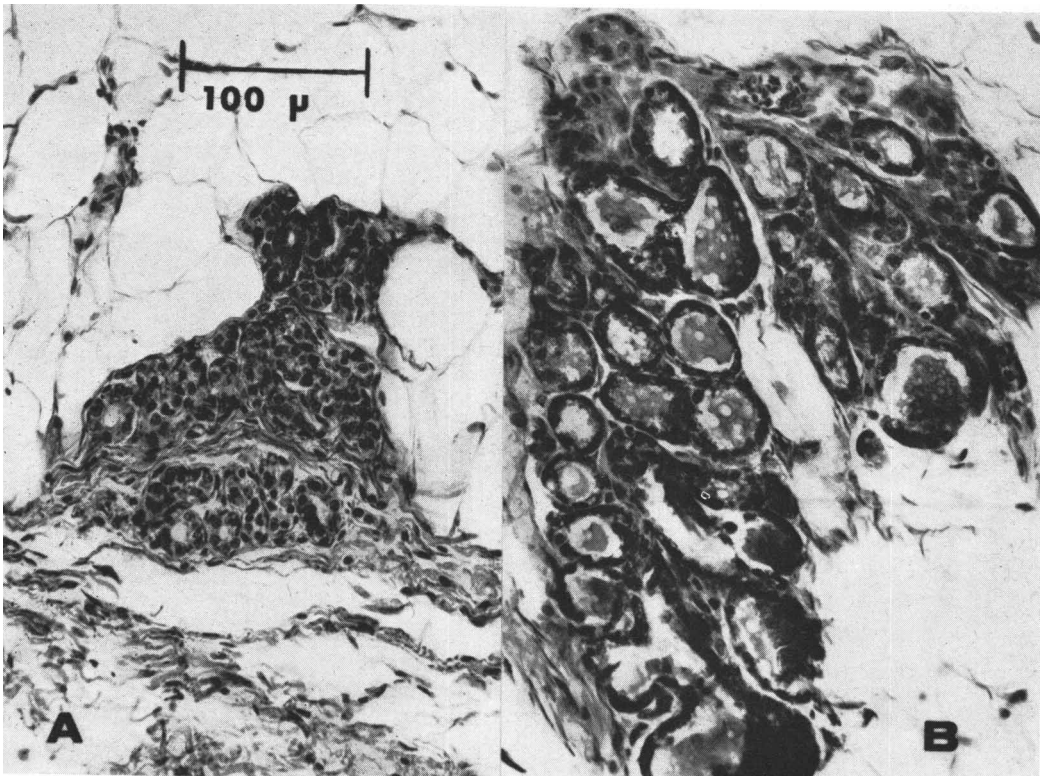

FIG. 1. Tissues were excised from wholemounts, sectioned, and stained with hematoxylin and eosin. (A) Tissue from rat receiving low (1–3 intromissions) copulatory stimulation. Alveoli are compact with small or absent lumina. (B) Tissue from rat receiving medium (4–20 intromissions) copulatory stimulation. Alveoli are dilated and filled with secretion (grade "5" response).

ences among groups were statistically significant ($p<.001$). Figure 1 illustrates maximally and minimally stimulated tissues. The results obtained from assessment of the slides were confirmed by assessment of alveolar distension in the wholemounts.

Discussion. The female rat normally does not secrete enough gestagen to permit implantation and pregnancy unless a certain amount of cervical stimulation has been provided (7). Normally, the male rat provides this stimulation through the multiple intromissions he delivers during copulation (5). Since the increase in progesterone following the male rat's multiple intromissions (3) may be produced by the release of pituitary LTH, this gonadotropin may have stimulated alveolar secretion. Indeed, when Meites *et al.* (8) found that cervical stimulation led to the development of mammary secretion in estrogen-primed rats, they postulated release of LTH. In their review of the literature on prolactin (9), however, Meites and Nicoll summarized the support for the theory that changes in the level of adrenocortical hormones rather than of LTH are primarily responsible for initiation of lactation in rats at parturition. Accordingly, ACTH in addition to or instead of LTH may have been responsible for the mammary response reported here. However, release of ACTH has not been observed after copulation in rats, although it does occur during coitus in rabbits (10). In fact, it has been reported that adrenocortical activity in rats during the first five days of pregnancy is not higher than in virgin animals (11).

We wish to thank Barbara Parker, Barbara Lucas and John Underhill for technical assistance. We are grateful to Professors H. A. Bern and S. Nandi for their critical advice.

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Received July 8, 1968. P.S.E.B.M., 1968, Vol. 129.

The Effect of Glutathione on Survival of Endotoxin Treated Mice (33470)

DENNIS C. SZYMANSKI AND CHARLES D. JEFFRIES

Department of Microbiology, School of Medicine, Wayne State University, Detroit, Michigan 48207

The nonprotein sulfhydryl (NPSH) level in the liver of mice administered endotoxin has been shown to decrease by nearly 25% within 2 hr and to fall to about 45% of normal in 18 hr (1, 2). A similar rapid decrease in the liver NPSH has been reported following a number of other stress conditions, such as, scalding or ligature of hind legs (2). However, stress induced by single or repeated doses of ACTH, or prolonged administration of deoxycorticosterone failed to decrease the NPSH (3). In the liver, glutathione (GSH) has been reported (4) to comprise at least 90% of the NPSH. The role of the NPSH, and therefore the GSH, in tissues has not been clearly established but it is known to be a cofactor for enzymes such as phosphoglyceraldehyde dehydrogenase (5) and a substrate in the detoxification of halogenated aromatic compounds (6, 7). Because of the importance of the known functions of GSH and the unexplained, rapid decrease of this substance following endotoxin treatment, we determined the effect of exogenous glutathione, given before or after endotoxin, on the development of endotoxin shock in mice.

Supported in part by Grant No. AI-06767 from the National Institute of Allergy and Infectious Diseases.

Male, ICR mice, weighing 20–25 g, were obtained from Rawley Farms (Plymouth, Michigan) and held in the laboratory for at least 1 week before use. Water and food were freely available at all times. The lyophilized endotoxin prepared from *Salmonella enteritidis* by the method of Ribi *et al* (8) was rehydrated in nonpyrogenic saline (Sherman Labs., Detroit) for 3 hr before use. The test mice were injected intraperitoneally with one LD_{50} dose (225 μg) of the endotoxin in 0.2 ml.

The GSH (Sodium glutathione, Schwarz BioResearch, Inc. or Glutathione, Sigma Chemical Co.) was dissolved in pyrogen-free saline at 150 mg/ml. The GSH was administered in a single dose of 0.2 ml at either 1, 2, or 4 hr before the endotoxin, or the endotoxin was given first and those animals received six doses of GSH, three doses at 3-hr intervals beginning at 18 hr and again at 42 hr after the endotoxin. Groups of endotoxin treated animals injected in the same ways with nonpyrogenic saline served as controls for injection trauma.

The administration of exogenous GSH results in an unphysiologic situation, because this compound rarely occurs extracellularly, especially in the blood where it is a com-