(such as rate of absorption and total body water) and thus the contribution of body weight is cancelled. In any event, the applicability of the results reported here may not be limited to this particular experimental model since it has been concluded(1) that for several pharmacological agents the lethal dose for small animals is usually a little larger than for large animals when both are expressed on a unit weight basis.

The above considerations are not intended to imply that the same relationship exists with other drugs or species or even with the same drug when a different pharmacological effect is evaluated. However they do emphasize that dependence of the dose on body weight can not be implicitly assumed in the absence of data showing that a relationship actually exists. Such a dependence may be practically zero, as in the present case, or it may be represented by any fraction of unity. In the later case it is best established by covariance analysis, which has been reported(2). It is noteworthy that a similar lack of a relation between body weight and toxicity has been shown to exist in the case of the lethal effects of botulinal toxins on mice(3) and alpha-naphthyl-thiourea in rats(4).

Summary. Data from 360 mice are presented which show that the toxicity of intraperitoneal histamine is independent of body weight. Adjustment for body weight in the range of 15 to 25 g increased the variation so that smaller animals appeared to be less sensitive to histamine than larger mice. Possible mechanisms are discussed.

1. Gaddum, J. H., *Pharmacology,* Oxford Univ. Press, 1953, p508.

2. Deutsch, S., Angelakos, E. T., Loew, E. R., *Endocrinology,* 1956, v58, 33.

3. Lamanna, C., Jensen, W. I., Bross, I. D. J., *Am. J. Hyg.,* 1955, v62, 21.

4. Rall, D. P., North, W. C., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 825.

Received October 19, 1959. P.S.E.B.M., 1960, v103.

Initiation of Lactation in Rats with Hypothalamic or Cerebral Tissue.*† (25495)

JOSEPH MEITES, P. K. TALWALKER AND C. S. NICOLL
Dept. of Physiology and Pharmacology, Michigan State University, East Lansing

Recently we demonstrated that epinephrine, acetylcholine, serotonin and reserpine can initiate lactation in estrogen-primed virgin rats, and maintain mammary secretion in postpartum rats after litter removal(1,2). Numerous other drugs and a variety of stressful stimuli can also initiate mammary secretion in rats (unpublished). These drugs and stressful stimuli are believed to induce release of prolactin and probably ACTH from the anterior hypophysis, since administration of prolactin alone is ineffective in initiating lactation in intact rats and requires the addition of ACTH or glucocorticoids(3). Large doses of ACTH or glucocorticoids alone may induce mammary secretion in intact rats, presumably by stimulating pituitary prolactin release(4). Harris(5) and others have suggested that the hypothalamus and perhaps other portions of the brain produce neurohormones which reach the anterior pituitary *via* the hypophyseal portal vessels and induce release of its hormones. Thus hypothalamic extracts have been shown to elicit ACTH discharge(6,7) and pineal extracts to evoke aldosterone release(8). It was of interest, therefore, to determine whether hypothalamic or cerebral tissue from rats could induce release of sufficient prolactin and probably ACTH to initiate lactation in rats.

Methods. Brain tissue was taken from postpartum or estrogen-treated mature female rats (Carworth strain). They were killed with

* Published with approval of Mich. Agr. Exp. Sta. as Jour. Art. No. 2516.

† This work was supported in part by NIH grant.

TABLE I. Effects of Different Rat Tissues on Initiation of Mammary Secretion in Rats.

Treatment (5 rats/group)			No. rats with secretion
Tissue containing hypothalamus	29.4 mg,	½ twice daily	4
	29.4	1 × daily	4
	65.6	*Idem*	4
Cerebrum	29.4	"	2
	64.7	"	1
	217.6	"	1
Kidney	64.0	"	0
Liver	64.0	"	0
.85% saline		"	0

ether anesthesia and the whole brain was carefully lifted from the skull at the same time the connection to the pituitary stalk was severed. The tissue between the optic chiasma and mammillary bodies containing the hypothalamus was carefully excised, weighed, dropped into a vial and immediately placed in a freezer at a temperature of −15°C. When a sufficient number of such tissues were collected, they were macerated with a small mortar and pestle and suspended in .85% saline. Average wet weight of this tissue/rat was about 25 mg. Equivalent amounts of rat cerebrum, kidney or liver were similarly prepared for injection. A control group of 5 rats was injected subcutaneously with .85% saline daily. The tissues were injected subcutaneously in .2 cc volumes daily into groups of 5 mature rats each for 5 days after the rats had been injected subcutaneously for 10 days with 10 μg estradiol daily to develop their mammary glands. On the 6th day the rats were killed and their right inguinal mammary glands were removed for histological examination. To check on possible prolactin contamination, 29.2 mg of hypothalamic tissue was injected intradermally over one crop sac of each of 5 mature White Carneau pigeons for 5 days and saline was injected over the other crop sac. On 6th day the pigeons were killed, and the crop sacs were removed and examined macroscopically for evidence of stimulation.

Results. The amount of tissue injected/rat and their lactational responses are shown in Table I. It can be seen that tissue containing the hypothalamus induced mammary secretion in 4 out of 5 rats in each of the 3 groups injected, irrespective of the dose or schedule of administration. Mammary maintenance was also noted in the 3 rats not showing secretion, indicating at least a partial response to the tissue containing the hypothalamus. In the 3 groups given cerebral tissue, 2 out of 5 rats showed lactational responses to the lowest dose and 1 out of 5 rats in each of the 2 groups given the higher doses of this tissue. No mammary secretion was observed in rats when liver, kidney or saline was injected, and the 5 pigeons given suspensions of tissues containing the hypothalamus did not show a crop gland response. Representative histological sections of mammary glands from saline and hypothalamic-injected rats are shown in Fig. 1-2.

Discussion. These results show that rat hypothalamus and possibly cerebral tissue, can initiate mammary secretion in estrogen-primed rats. The tissue containing the hypothalamus induced lactation in 12 out of 15 rats and at least maintained the mammary structure in the other 3 rats, whereas cerebral tissue elicited mammary responses in only 4 out of 15 rats. Inability of the tissue containing the hypothalamus to elicit pigeon crop milk secretion by the sensitive intradermal test indicates that the active principle is not prolactin. Presumably this tissue induced discharge of prolactin and probably ACTH from the anterior pituitary. It is not clear why the higher doses of cerebral tissue failed to elicit more responses than the lower doses, although its effects may not be as specific as those of the hypothalamus. Failure of liver or kidney tissue to induce mammary secretion suggests that in amounts equivalent to the 2

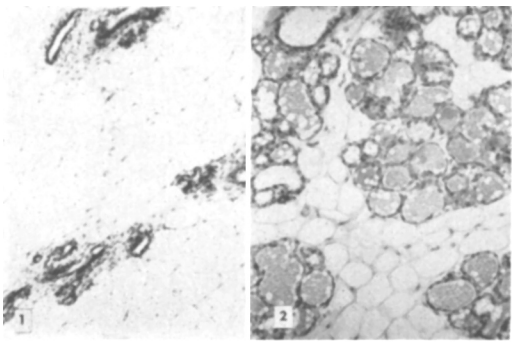

FIG. 1-2. Histological sections of mammary glands from rats inj. with (1) saline (2) tissue containing hypothalamus. ×63.

brain tissues, they do not exhibit prolactin and ACTH releasing ability possessed by the brain tissues. It is not known whether these brain tissues acted directly on the anterior hypophysis or indirectly through other centers. Preliminary assays of bovine hypothalamic and cerebral cortical tissues‡ show that these may also induce mammary secretion in rats.

Summary. Rat tissue containing the hypothalamus, cerebral tissue, kidney or liver were injected subcutaneously in saline suspension into rats for 5 days, after they had previously been injected with estradiol for 10 days to develop their mammary glands. Saline alone was injected into control rats. The tissue containing the hypothalamus initiated lactation in 12 out of 15 rats; cerebrum in 4 out of 15 rats; and liver, kidney or saline in none out of 5 rats each. Tissue containing the hypothalamus was injected intradermally over crop sacs of 5 pigeons for 5 days and failed to induce a response, indicating that the active principle is not prolactin. It is suggested that rat hypothalamus and possibly cerebral tissue produce release of prolactin and probably ACTH from the anterior pituitary in amounts sufficient to initiate lactation.

‡ Actone-dried preparations of these tissues were kindly supplied by Dr. C. E. Graham, Wilson Labs., Chicago.

1. Meites, J., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 750.
2. Meites, J., Nicoll, C. S., Talwalker, P. K., *ibid.*, 1959, v101, 563.
3. Reece, R. P., *ibid.*, 1939, v40, 25.
4. Johnson, R. M., Meites, J., *ibid.*, 1955, v89, 455.
5. Harris, G. W., *Neural Control of the Pituitary Gland*, 1955, Edward Arnold Ltd., London.
6. Slusher, M. A., Roberts, S., *Endocrinol.*, 1954, v55, 245.
7. Guillemin, R., Hearn, W. R., Cheek, W. R., Householder, D. H., *ibid.*, 1957, v60, 488.
8. Farrell, G., *ibid.*, 1959, v65, 239.

Received October 19, 1959. P.S.E.B.M., 1960, v103.

Pituitary ACTH Levels During Adrenal Involution Following Thiouracil.* (25496)

E. A. LAZO-WASEM
Wilson Laboratories, Chicago, Ill.

Adrenal atrophy following administration of goitrogen thiouracil has been amply demonstrated(1,2,3,4) but the causal mechanism has not been fully elucidated. Atrophy of the adrenal cortex after thiouracil and inability to obtain adrenal hypertrophy following exposure to cold in thiouracil fed rats led Zarrow and Money(5) to conclude that adrenal involution was due either to decreased ACTH production by the pituitary or decreased sensitivity of the adrenal to ACTH. Subsequent studies by Zarrow and Zarrow(6) showing that the atrophic adrenal gland of thiouracil treated rat retains its sensitivity to ACTH were interpreted to indicate that adrenal involution in thiouracil fed rats is caused by decreased ACTH secretion. In the present experiment levels of pituitary ACTH were measured in rats showing adrenal involution induced by feeding thiouracil.

Materials and methods. Normal male albino rats, descendants from Sprague-Dawley strain weighing 150 ± 10 g were separated into 2 groups. Ten rats were kept as untreated controls and were fed Rockland rat

TABLE I. Mean Body and Fresh Organ Weights of Male Rats Fed a Diet Containing 0.3% Thiouracil for 3 Weeks.

Group	Body wt, g	Fresh organ wt, mg/100 g body wt		
		Thyroid	Adrenals	Pituitary
Thiouracil	196 ± 6*	44.7 ±2.8	13.1 ± .2	4.6 ±.3
Controls	246 ±17	7.2 ± .7	18.3 ±1.2	2.8 ±.3

* Stand. error of mean. All differences significant at P .05.

* The author gratefully acknowledges technical assistance of William Watson and Joan Michalski.