INITIATION OF LACTATION IN RATS BY NONSPECIFIC STRESSES

Thesis for the Degree of M. S.

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Charles Samuel Nicoll

1960

INITIATION OF LACTATION IN RATS

BY NONSPECIFIC STRESSES

BY

CHARLES SAMUEL NICOLL

AN ABSTRACT

Submitted to the college of Science and Arts,
Michigan State University of Agriculture and
Applied Science, in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

Department of Physiology & Pharmacology

1960

Approved Joseph Meites

ABSTRACT

1. Release of ACTH and luteotropin or prolactin from the anterior pituitary may be induced by numerous factors such as stresses, adrenalectomy, suckling, thyroxin, acetylcholine, serotonin, chlorpromazine, morphine, epinephrine and reserpine. Since several of these factors are capable of initiating lactation in rats and other species, it was of interest to determine whether several nonspecific stresses could promote release of sufficient prolactin and ACTH to initiate lactation in the rat.

2. Sixty mature virgin female rats of the Carworth strain were divided into 6 groups of 10 each and injected subcutaneously for 10 days with 10 µgm. estradiol in 0.1 cc corn oil. For the subsequent 5 days the groups were treated as follows: 1) controls, 0.1 cc saline injected subcutaneously once daily; 2) severe cold (0° C) 24 hours/day; 3) intense light and heat (35°) for 12 hours/day; 4) restraint, 12 hours/day; 5) starvation, no food or water for 5 days; 6) subcutaneous injection of 0.1 cc or 0.2 cc of 10% formaldehyde once daily. On the 6th day the rats were killed and the mammary tissue was removed from each rat for histological examination. The adrenals, ovaries, uterus and thymus of each rat were weighed.

3. The mammary glands of the control rats regressed from a lobulo-alveolar system to a bare duct system during the period of saline injections. In the stressed groups lactation was initiated in 8 of the cold exposed, 7 of the light and heat treated, 9 of the restrained, 3 of the starved and 5 of the formalin injected rats. Thymic weight was significantly reduced in all of the stressed groups and the adrenals were significantly enlarged in the groups exposed to cold, starvation and restraint. This indicates adrenal cortical stimulation in response to the stresses. Ovarian and

ABSTRACT

uterine weights were significantly reduced in all stressed groups with the exception of those exposed to cold.

4. It is concluded that certain nonspecific stresses can induce the release of prolactin and ACTH from the anterior pituitary in amounts sufficient to initiate lactation in the estrogen-primed rat. These observations suggest that the stress of parturition may be involved in the initiation of lactation which normally accompanies delivery.

The ability of numerous factors to promote release of prolactin and ACTH from the anterior pituitary suggests that a common mechanism may be involved in regulation of the secretion of both hormones. Such a mechanism may involve a common neural regulatory center or centers which are functionally associated.

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Dedicated

to

my wife

Note

The text of this thesis is a verbatim copy of a manuscript which was submitted for publication to the American Journal of Physiology. Notification of acceptance of the manuscript was received on January 3, 1960.

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INTRODUCTION

Release of ACTH from the anterior pituitary of the rat may be induced by numerous factors such as various stresses (1), adrenalectomy (2), the suckling stimulus (3), thyroxine administration (4), and injections of numerous drugs including acetylcholine (5), serotonin (6), chlorpromazine (7), morphine (8), epinephrine and reserpine (9). All these factors have also been reported to produce physiological manifestations in the rat which are interpreted as indicative of increased secretion of luteotropin or prolactin from the anterior pituitary. Thus adrenalectomy (10), stresses (11), epinephrine (11), thyroxine (12), suckling (13), reserpine and chlorpromazine (14) induced pseudopregnancy in the rat. Lactation may be initiated in rats by the suckling stimulus (13), and injections of morphine (15), epinephrine, acetylcholine, serotonin and reserpine (16, 17) and numerous other agents which have previously been reported to promote ACTH secretion (unpublished). Since several of the factors which apparently elicit release of both ACTH and prolactin are capable of initiating lactation in rats, it was of interest to determine whether several nonspecific stresses could promote release of ACTH and prolactin in amounts adequate to initiate lactation in rats.

METHODS

Sixty virgin female rats (Carworth) weighing 200 - 250 gms were divided into 6 groups of 10 each and injected subcutaneously once daily for 10 days with 10 µgm estradiol in 0.1 cc corn oil. The estradiol induces a variable degree of mammary lobulo-alveolar development, in the rat, and is necessary to render the mammary tissue responsive to hormones which stimulate lactation. For the following 5 days the rats were treated as follows: 1) controls, 0.1 cc physiological saline injected subcutaneously once daily; 2) severe cold (0° C) 24 hrs/day; 3) intense light and heat (35° C) produced by placing two 150 watt reflector floodlights over the cage containing the rats, for 12 hrs/day; 4) restraint produced by wrapping the tails or hind limbs of the rat with several turns of masking tape, and then taping the tails or hind limbs of 5 of the animals together for 12 hours per day. This procedure greatly hindered the movements of each animal and resulted in considerable fighting among the rats. Preliminary trials with simple restraint, produced by securing the forelimbs of the rats to their thorax by several turns of masking tape, showed that this was not a severe enough stress to initiate lactation: therefore, the more severe method was adopted; 5) starvation, with no food or water for 5 days; 6) subcutaneous injection of 0.1 or 0.2 cc 10% neutral formaldehyde to 5 rats each. On the 16th day of the experiment the rats were killed with nembutal and the right inguinal mammary gland was removed and prepared for histological examination by standard techniques (17).

The ovaries, uterus, adrenals and thymus were also excised from all animals and weighed on a Roller - Smith balance.

RESULTS

The experimental data are summarized in Table I. When saline was injected for 5 days following estrogen treatment (group 1), the mammary glands regressed from a lobulo-alveolar to an almost bare duct system in all cases. All the groups subjected to nonspecific stresses after estrogen-priming showed maintenance of the mammary lobulo-alveolar structure and lactation in varying degrees (Fig. 1 - 6).

The control rats (group 1) and those exposed to cold (group 2) or light and heat (group 3) showed no change in body weight during the experimental period. The starved rats (group 5) lost an average of 58 grams each and those subjected to restraint (group 4) lost an average of 10 grams each. The rats given 0.1 cc formaldehyde (group 6A) gained an average of 30 grams but showed no weight change when given 0.2 cc (group 6B). Formaldehyde produced large edematous pockets at the site of injection. The 0.2 cc dose of formaldehyde produced a harsher stress than the 0.1 cc dose as indicated by adrenal and thymus weights. The adrenals were significantly enlarged in the groups exposed to cold, starvation and restraint while the rats treated with 0.1 cc formalin showed a decrease in adrenal weight per 100 gms body weight. This is probably due to the relatively large body weight gain in this group. All the stressed rats showed reduced thymus weights which is indicative of increased adrenal cortical secretion. Ovarian and uterine weights were significantly decreased in all stressed rats with the exception of

those exposed to cold; this can be considered indicative of decreased gonadotropic secretion, previously reported to occur under stress (18).

TABLE I. EFFECT OF STRESSES ON MAMMARY GLANDS AND BODY AND ORGAN WEIGHTS.

Group No.	5-day treatment (after injection of 10 µgm Estradiol for 10 days)	No. of Rats with secretion	Av. Body Wt. in gm.		Organ Wgts. in mg per 100 gm Body Wgt.			
			Initial	Final	Adrenal	Thymus	Ovaries	Uterus
1 (10)*	Controls, Saline	0	215.0±4.8+	218.0±5.3	25.8±1.5	99.9±8.8	26.1±1.8	207.1±19.0
2 (10)	Cold (0°C)	8	203.2±2.2	200.6±4.5	33.9±2.2	69.2±2.9	24.3±0.5	187.6±6.5
3 (10)	Light & Heat (35°C)	7	224.7±5.5	226.4±6.2	27.8±1.2	67.0±3.0	19.2±1.5	115.4±6.2
4 (10)	Restraint	9	211.9±2.1	202.3±2.6	34.8±1.1	45.6±2.9	22.2±1.1	134.1±7.2
5 (10)	Starvation	3	233.2±2.0	175.2±6.4	37.1±1.4	80.5±7.2	16.4±0.9	148.2±4.0
6A (5)	0.1cc of 10% Form.	2	213.6±1.2	243.8±3.6	20.6±0.3	59.4±3.1	21.3±1.5	105.9±30.5
6B (5)	0.2cc of 10% Form.	3	227.8±4.4	232.8±4.1	26.3±4.8	47.5±7.0	17.3±0.3	123.6±4.5

* No. of rats

Form = Formaldehyde

$$S.E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

Figure 1. Photomicrograph of control mammary tissue from a rat injected with saline for 5 days after 10 days of estradiol treatment.
X 130.

Figure 2. Photomicrograph of mammary tissue from a rat exposed to severe cold stress for 5 days after 10 days of estrodiol treatment.
X 130.

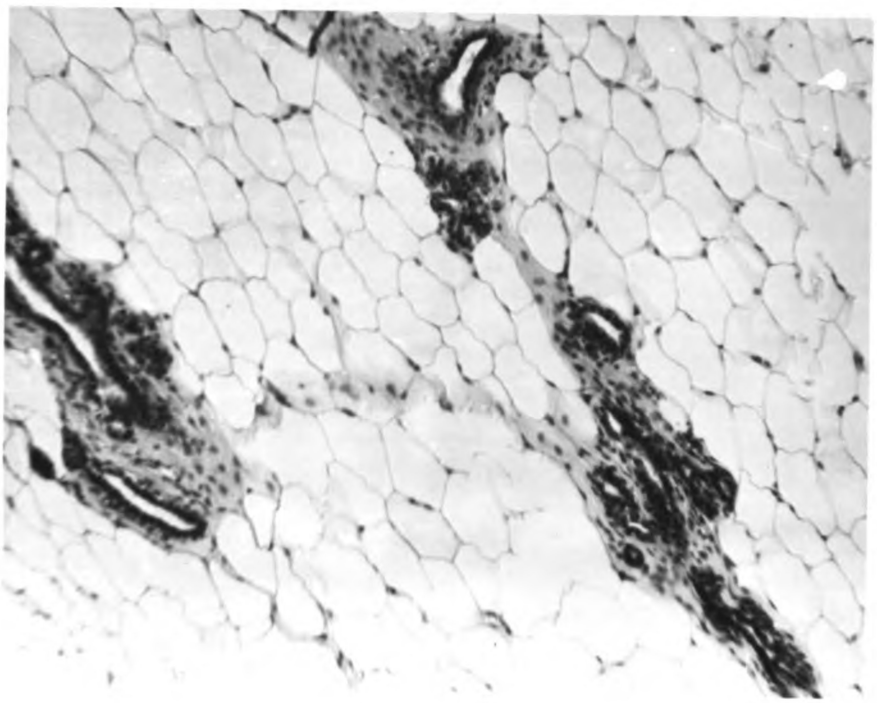

Figure 1

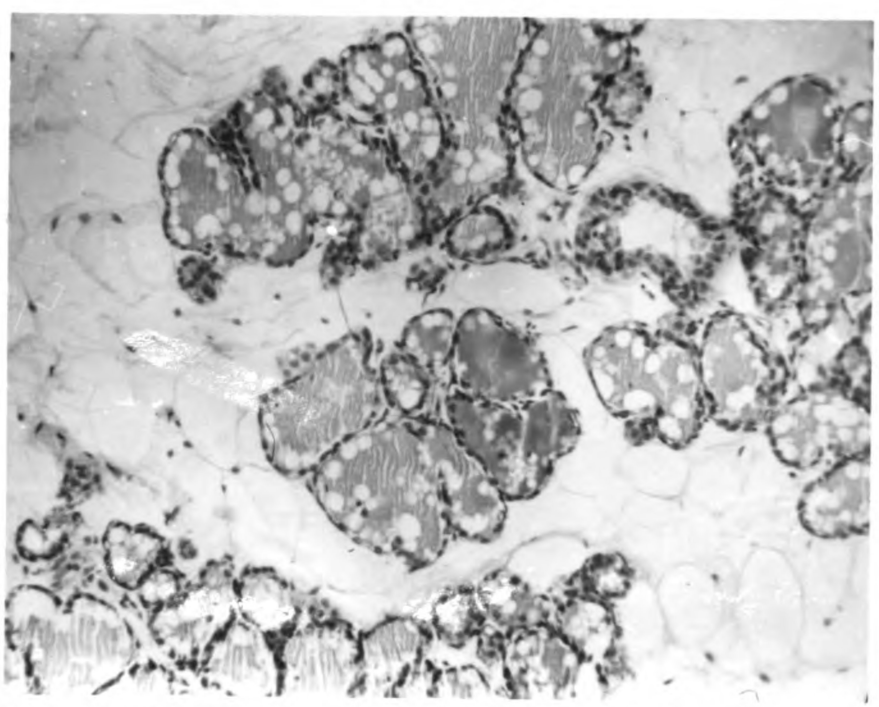

Figure 2

Figure 3. Photomicrograph of rat mammary tissue from a rat exposed to heat and light stress for 5 days after 10 days of estradiol treatment.

Figure 4. Photomicrograph of rat mammary tissue from a rat exposed to restraint stress for 5 days after 10 days of estradiol treatment.

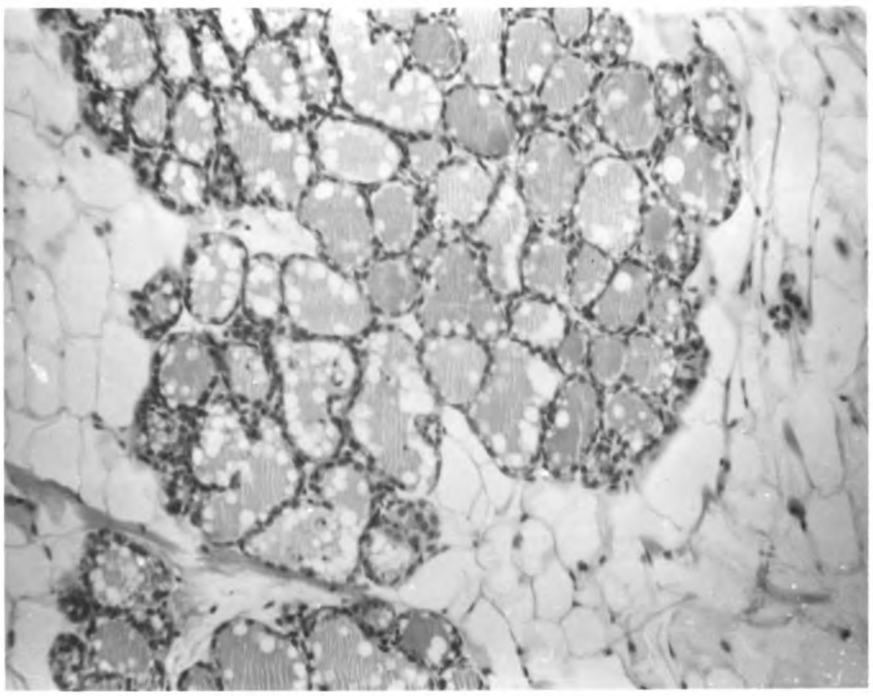

Figure 3

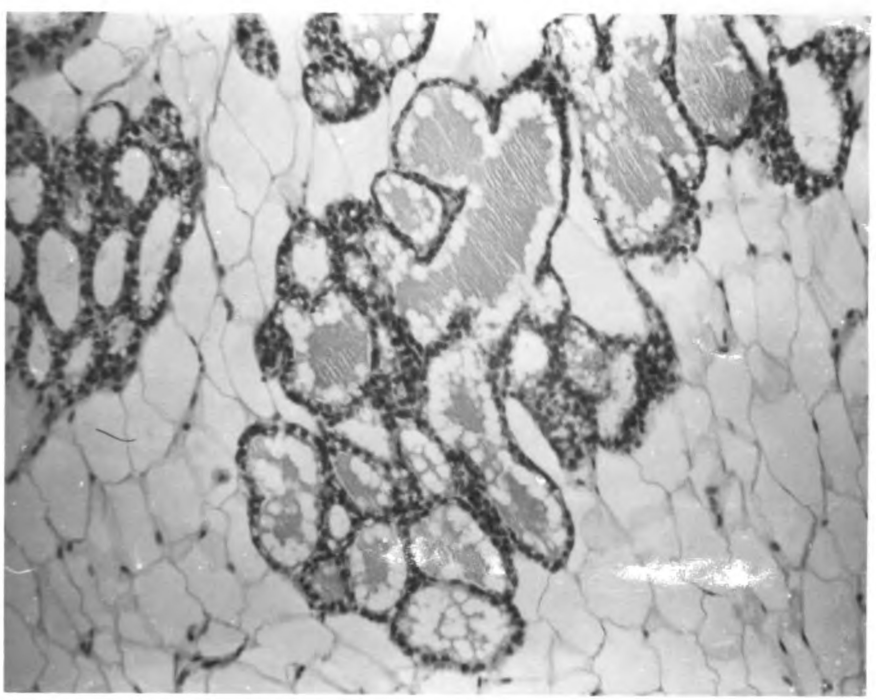

Figure 4

Figure 5. Photomicrograph of mammary tissue from a rat exposed to starvation stress for 5 days after 10 days of estradiol treatment.

X 130.

Figure 6. Photomicrograph of mammary tissue from a rat injected with formaldehyde for 5 days after 10 days of estradiol treatment.

X 130.

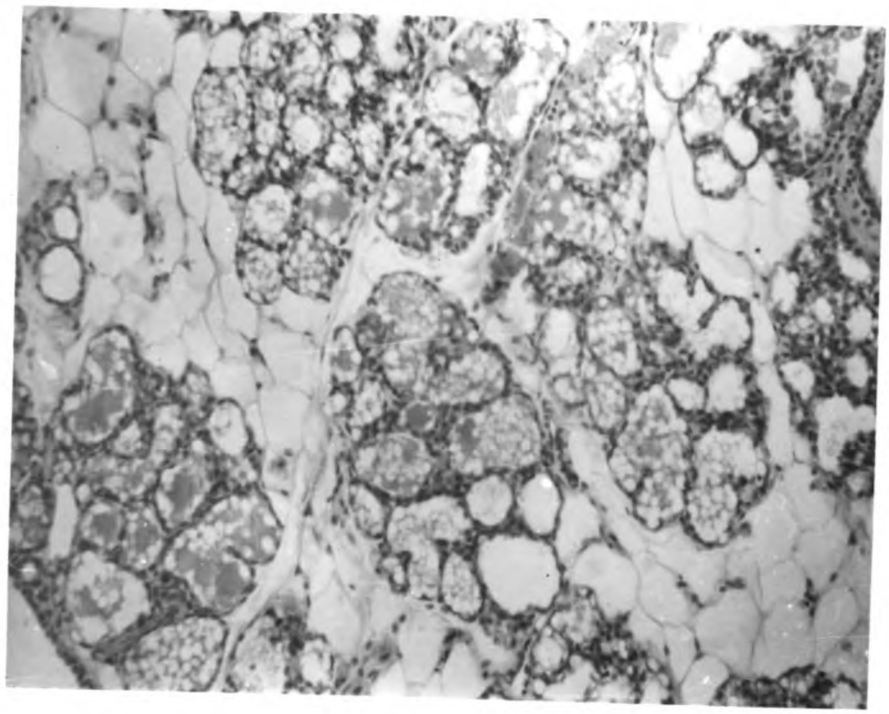

Figure 5

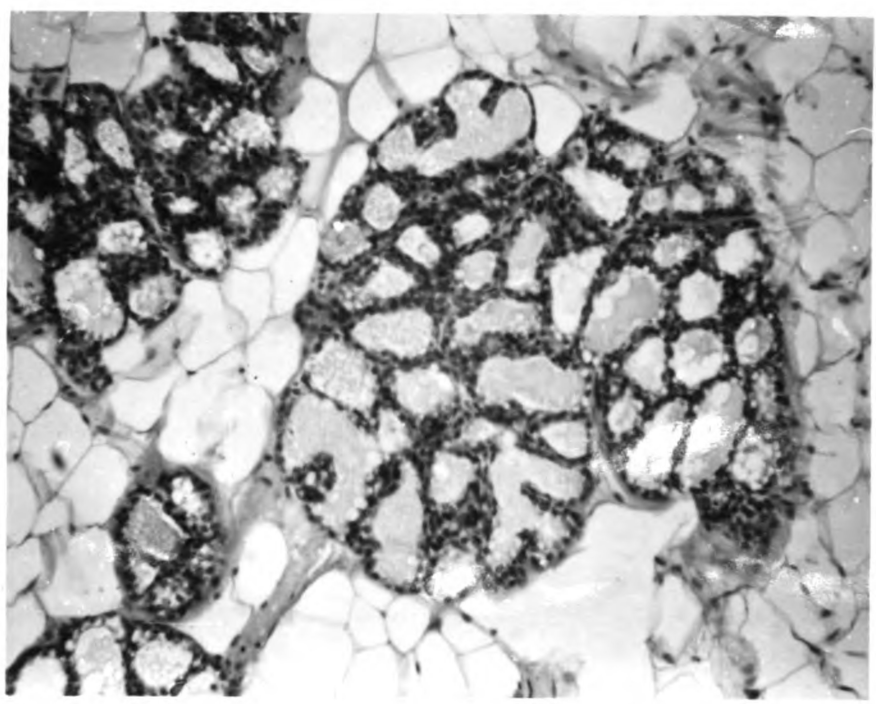

Figure 6

DISCUSSION

It is evident that certain nonspecific stresses can induce the release of prolactin and ACTH from the anterior pituitary in amounts sufficient to initiate secretory activity in the mammary glands of the estrogen-primed rat. This is of interest when one considers previous reports that stresses inhibit nonessential functions such as growth, reproduction and lactation (19). Administration of large doses of ACTH or glucocorticoids for 10 days has been observed to initiate slight lactation in untreated virgin rats (20). We have recently observed, however, that daily injection of 2 I.U. or 4 I.U. of ACTH for 5 days following estrogen priming failed to induce lactation in rats despite obvious stimulation of the adrenal cortex. Injection of 2 mg. or 4 mg. of prolactin (20 I.U. per mg) per day after estrogen priming also failed to initiate secretion in the mammary glands of these rats. However, daily injections of 2 I.U. ACTH and 2 mg prolactin together was effective in inducing secretion in these rats (unpublished)*. We believe these observations indicate that the induction of lactation in rats with nonspecific stresses is not merely the result of enhanced adrenal cortical activity, but also of augmented secretion of prolactin from the anterior pituitary.

Selye (21) has previously reported that lactation was initiated

(* See Appendix)

in spayed rats when estradiol was administered with cortisol or large doses of ACTH for 8 days, but kaolin or formaldehyde injections in conjunction with estradiol failed to induce mammary secretion. It is difficult to rationalize Selye's observation with those of the present study since the experimental conditions varied considerably and the type and severity of the stresses employed are not strictly comparable. Possibly the use of intact rats and sequence of treatment in the present study accounted for the differences. Initiation of lactation by stresses in the estrogen-primed rat may be related to clinical reports of induction of lactation in women after thoracoplasty (22), simple appendectomy (23), severe burns, starvation and other harsh trauma (24). Ovariectomy has also been reported to induce lactation in pseudopregnant rats (25) and in women (26) and has been attributed to the hormonal withdrawal phenomena. However, surgical trauma during ovarectomy may also be partially responsible for initiating lactation in these cases.

Recent theories concerned with the initiation of lactation at parturition have emphasized the changing levels of circulating ovarian hormones at term (27). High blood levels of estrogen and progesterone during gestation are believed to render the mammary gland relatively refractory to prolactin stimulation, and the predominance of progesterone over estrogen is believed to prevent the latter from increasing prolactin secretion. At the end of pregnancy, the inhibition of the two hormones on the mammary glands is decreased, and estrogen is believed to become sufficiently predominant to increase prolactin secretion and initiate lactation. The ability of nonspecific stresses to initiate lactation suggests that the stress of parturition may also

have a role in promoting the copious milk flow which normally accompanies delivery.

In view of the apparent ability of nonspecific stresses and numerous other factors to promote secretion of prolactin and ACTH, it is possible that a common mechanism can influence the secretion of both hormones. There is evidence that the CNS normally inhibits prolactin secretion, as indicated by persistent secretion of prolactin from rat pituitary autografts (28). There is also evidence that the midbrain of the rat contains inhibitory and stimulatory centers for ACTH secretion (29). If the neural inhibition of prolactin and ACTH involves the same CNS center, or centers which are functionally associated, the numerous factors which promote the secretion of these hormones may operate by suppressing the inhibitory center(s). In accordance with this view, the autotransplanted pituitary would continue to elaborate prolactin since the neural inhibition would be removed, and the secretion of ACTH would diminish because the CNS stimulatory center would no longer be effective. Such a concept is compatible with the results of the present study and with other circumstances were prolactin and ACTH are released concomitantly.

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APPENDIX

In order to test the hypothesis that stress induced lactation in the estrogen-primed rat is due to augmented secretion of prolactin and ACTH from the anterior pituitary, and not merely the result of enhanced adrenal cortical activity, ACTH and prolactin were administered singularly and in combination to estrogen primed rats.

Twenty-five mature virgin female rats were divided into 5 groups of 5 each and injected subcutaneously for 10 days with 10 µgm of estradiol in 0.1 cc of corn oil. For the subsequent 5 days the respective groups were injected twice daily with the following; 1) 1 mg of prolactin; 2) 2 mg of prolactin; 3) 1 I.U. of ACTH; 4) 2 I.U. of ACTH; 5) 1 mg of prolactin and 1 I.U. of ACTH. All injections were given subcutaneously. On the 16th day of the experiment the right inguinal mammary glands were removed from each rat for histological examination and the adrenals, thymus, ovaries and uterus were excised from groups 2 - 5 and weighed on a Roller-Smith balance. The uteri from group 3 were not weighed.

As may be seen from the accompanying table (Table 1A), neither prolactin nor ACTH were effective in initiating lactation in the estrogen primed rat when given alone. However, when ACTH and prolactin were administered together (Fig. 1A) lactation was initiated in 4 of the 5 rats, thus providing support to the hypothesis that stress induced lactation in the estrogen-primed rat is the result of enhanced

prolactin and ACTH secretion and not to increased ACTH secretion alone. Although neither dose level of prolactin was effective in initiating lactation in the estrogen-primed rat, the 4 mg per day level retarded mammary involution in all of the 5 rats treated (Fig. 2A).

Both dose levels of ACTH effectively stimulated the adrenal cortex of the rats as judged by the thymolytic effects which are comparable to those observed in the stressed rats. The 2 I.U. per day level of ACTH resulted in a 40.5% reduction and the 4 I.U. per day level resulted in a 48.1% reduction in thymic weight in comparison with the control weight. The stressed rats exhibited a range of thymic weight reduction from 19.6% for the starved rats to 54.9% for the restrained group.

TABLE IA. EFFECT OF PROLACTIN AND ACTH ON MAMMARY GLANDS AND BODY AND ORGAN WEIGHTS.

Group No.	5-day treatment following estradiol priming	No. of Rats with Mammary secretion	Av. Body Wgt. in gm. Initial	Av. Body Wgt. in gm. Final	Organ Wgts. in mg. per 100 gm Body Wt Adrenals	Thymus	Ovaries	Uterus
1 (10)*	Controls, Saline.	0	215.0±4.8	218.0±5.3	25.8±1.5	99.9±8.8	26.1±1.8	207.1±19.0
2 (5)	Prol.** 1 mg.2x/Day.	0	-	-	-	-	-	-
3 (5)	Prol., 2 mg., 2x/Day.	0	224.8±8.8	239.0±8.0	26.1±2.8	71.2±4.2	18.6±4.1	174.9±13.4
4 (5)	ACTH, 1 I.U. 2x/Day.	0	212.8±7.0	220.2±7.2	29.1±1.1	59.3±6.3	21.5±1.5	-
5 (5)	ACTH, 2 I.U. 2x/Day.	0	196.6±9.5	207.0±9.1	31.5±6.4	51.8±5.4	22.4±2.5	175.0±16.6
6 (5)	Prol** 1 mg. 2x/Day, plus ACTH, 1 I.U. 2x/Day.	4	218.0±6.8	228.0±12.2	25.1±4.4	73.0±10.9	16.8±6.0	131.1±14.0

*No of rats

** Prol. = Prolactin (20 I.U./MG.)

ACTH, DEPO GEL. (Upjohn)

$$S.E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

Figure 1A. Photomicrograph of mammary tissue from a rat treated with 2 I. U. of ACTH and 2mg. of prolactin for 5 days following 10 days of estradiol injection. X 130.

Figure 2A. Photomicrograph of mammary tissue from a rat treated with 4mg. of prolactin per day for 5 days following 10 days of estradiol injection. X 250.

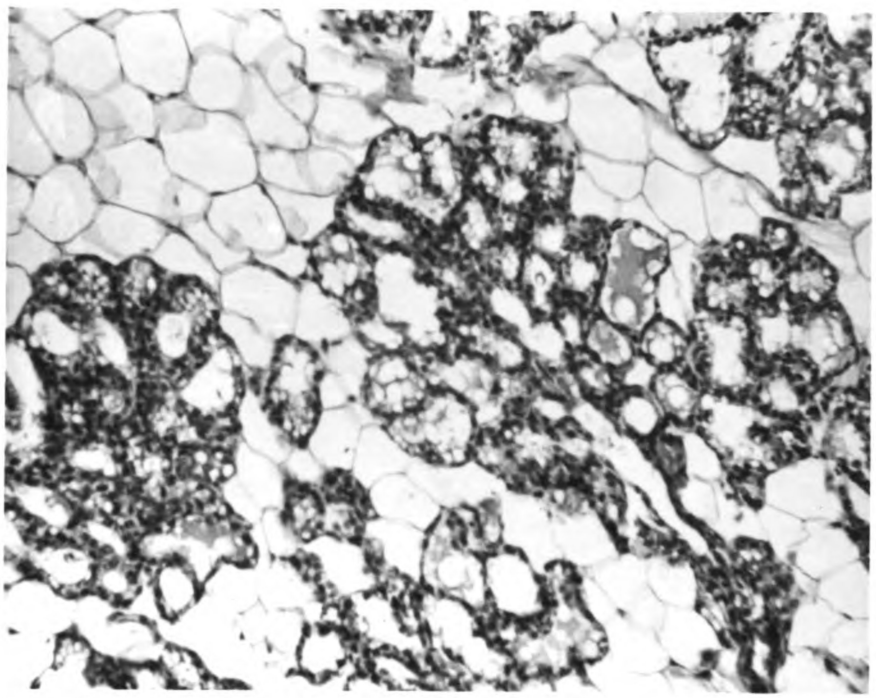

Figure 1A.

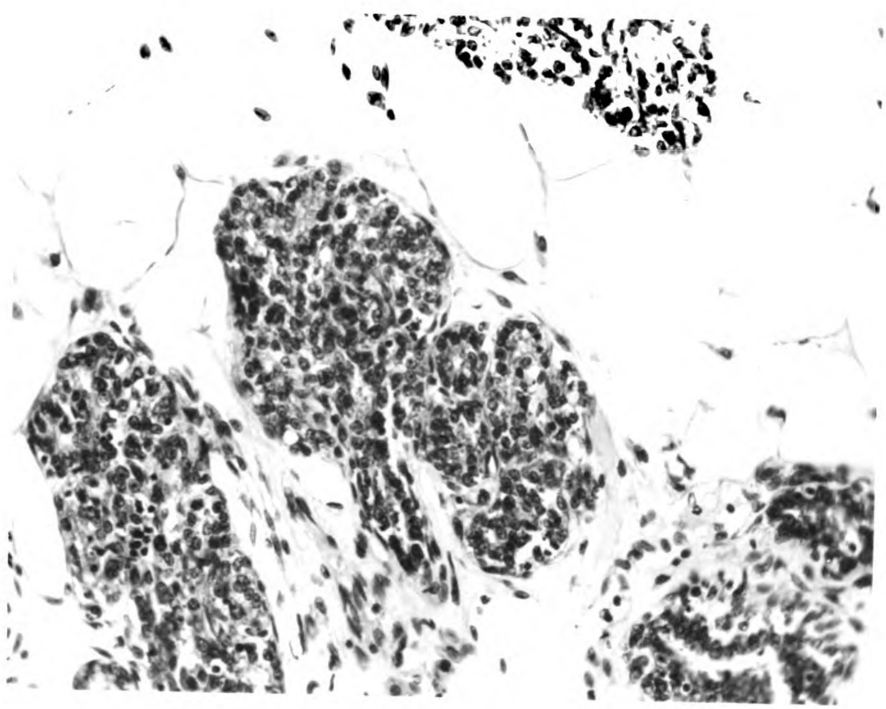

Figure 2A.