

I. EFFECTS OF SUCKLING ON PITUITARY
ACTH RELEASE AND PLASMA
CORTICOSTERONE LEVELS IN
POSTPARTUM LACTATING RATS
II. EFFECTS OF A PROLACTIN IMPLANT
INTO THE HYPOTHALAMUS OF
IMMATURE FEMALE RATS ON
PITUITARY FSH RELEASE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JAMES LEONARD VOOGT
1968

THESIS

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ABSTRACT

- I. EFFECTS OF SUCKLING ON PITUITARY ACTH RELEASE AND PLASMA CORTICOSTERONE LEVELS IN POSTPARTUM LACTATING RATS
- II. EFFECTS OF A PROLACTIN IMPLANT INTO THE HYPOTHALAMUS OF IMMATURE FEMALE RATS ON PITUITARY FSH RELEASE

By James Leonard Voogt

1. Plasma corticosterone levels were determined in female rats during various stages of pregnancy, parturition, lactation and in cycling controls utilizing a fluorometric method developed by Silber et al. (1958) and modified by Guillemin et al. (1959). Low resting levels of plasma corticosterone were found in cycling controls and in pregnant rats on the 10th and 20th day of pregnancy (12-16.8 ug/100 ml). Plasma corticosterone levels increased about threefold during parturition (40.2 ug/100 ml) and remained high during lactation. Thymus weights were found to be greatest during pregnancy, and showed a significant decrease at parturition and during lactation. These findings lend support to the view that adrenal cortical hormones are important in the initiation and maintenance of lactation in the rat.

2. The effects of suckling on pituitary ACTH and plasma corticosterone levels were determined in postpartum lactating rats. ACTH was assayed in hypophysectomized rats by the method of Guillemin et al. (1958). Twelve hours of non-suckling followed by 3 hours

suckling increased pituitary ACTH concentration about 85% and increased plasma corticosterone levels about fourfold. When pups were allowed to suckle for a half hour, pituitary ACTH levels decreased about 60% and plasma corticosterone levels increased about fourfold. It was concluded that the suckling stimulus increased ACTH release and plasma corticosterone levels, adding support to the view that suckling stimulates release of the pituitary hormones needed to maintain lactation in the rat.

3. The effects of implanting prolactin into the median eminence area of the hypothalamus in 21 day old female rats was studied. Prolactin implanted rats when killed 5 or 8 days after implantation, had a significantly reduced pituitary FSH content compared to cholesterol implanted controls, as determined by the Steelman-Pohley assay method (1953) for FSH. Examination of the ovarian and uterine histology indicated that prolactin implantation resulted in follicular stimulation of the ovaries and endometrial stimulation of the uterus. These findings suggest that prolactin implantation into the median eminence stimulates FSH release in prepubertal rats, and thus may play a role in the onset of puberty in the rat.

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James Leonard Voogt

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1968

ACKNOWLEDGMENTS

The author wishes to express sincere gratitude to Dr. Joseph Meites, Professor of Physiology, for his guidance and interest throughout the course of this study. His help was necessary to make this manuscript possible. An expression of thanks are also extended to the members of the guidance committee, Drs. P.O. Fromm, G. Riegler and H.A. Tucker for their help in the preparation of this manuscript.

The author is indebted to the National Institutes of Health, Michigan State University and Dr. J. Meites for the financial assistance during this project. Purified pituitary hormone (FSH and prolactin) were kindly supplied by the National Institutes of Health and ACTH was generously given by The Upjohn Company.

Special thanks are offered to the author's wife, Mary Jane, for her inspiration, understanding and patience shown during the time spent in this study.

Dedicated

to

my Mother

and

my Father

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INTRODUCTION

The role of the pituitary and adrenals in the initiation and maintenance of lactation has been well established in many species. Prolactin and adrenocorticotrophic hormone (ACTH) or adrenal cortical hormones are the minimal requirements for maintaining lactation, while other hormones including growth hormone (GH), thyroid-stimulating hormone (TSH), insulin and parathormone are needed for optimal milk production.

The control of synthesis and release of anterior pituitary hormones involved in lactation resides in the hypothalamus where specific neurohumors are produced. These neurohumors are carried to the anterior pituitary by the portal vessels where they stimulate or inhibit the secretion of anterior pituitary hormones. Both internal and external environmental factors have been shown to act through the central nervous system (CNS) to cause the release or inhibition of release of many pituitary hormones. The suckling stimulus is one external factor necessary for the secretion of hormones by the pituitary to maintain lactation. This stimulus to nerves in the nipples elicits impulses which travel via the spinal cord to the brain. It is believed that these nerve impulses reach discrete areas of the hypothalamus to alter the release of appropriate neurohumors into the portal blood. Since the effect of the suckling stimulus on pituitary ACTH and plasma corticosterone concentration has not been reported in

the rat, it was the purpose of the present study to measure these hormones in lactating rats following various periods of suckling. Changes in glucocorticoid activity during pregnancy, parturition and lactation were also determined in the rat, and an attempt was made to relate these findings to the corticosterone requirements of the rat during these stages.

This thesis is also concerned with some aspects of the onset of puberty in rats. The observation that lesions in the anterior hypothalamus significantly hastened the onset of puberty in rats was the first experimental evidence that the brain had an important function in the control of puberty. Since the brain, especially the hypothalamus, greatly influences the secretion of pituitary hormones, and since an intact pituitary-gonadal relationship is necessary for the onset of puberty, it was of interest to study some aspects of brain control of puberty.

REVIEW OF THE LITERATURE

I. Hormonal Requirements for Lactation

The necessity for adrenal cortical hormones as well as prolactin for initiation and maintenance of lactation has been well established in many species. Prolactin and adrenal cortex extracts or ACTH were both required to induce lactation in hypophysectomized guinea pigs (Nelson et al., 1943; Folley and Young, 1941). Hypophysectomized, ovariectomized and adrenalectomized (triply operated) mice injected with cortisol and prolactin or GH showed lactogenesis (Nandi and Bern, 1961). Pregnant rats injected with hydrocortisone acetate at midpregnancy lactated shortly thereafter, whereas, prolactin or oxytocin, each given alone or in combination did not cause lactation (Talwalker et al., 1961). Thus it seems clear, at least for the rat, that insufficient glucocorticoids is the limiting factor for initiating lactation during gestation. A large dose of ACTH caused systemic lactation in pseudopregnant rabbits (Chadwick and Folley, 1962), and either prolactin or cortisol acetate induced lactation in the pregnant rabbit (Meites et al., 1963).

Studies on mammary tissue cultures (Elias, 1957; Rivera, 1964) showed that both prolactin and adrenal corticoids are necessary for mammary secretion. It was of interest in the present study to determine the plasma level of corticosterone during pregnancy, parturition and lactation in the rat and relate these findings to the

initiation and maintenance of lactation.

II. Adrenal Cortical Activity during Pregnancy, Parturition and Lactation

Evidence for insufficient adrenal glucocorticoids to initiate lactation during pregnancy is mostly indirect. Adrenal cortical activity, determined by measuring adrenal cholesterol concentration, showed a peak on the 15th day of pregnancy in the rat, and then a decline until parturition (Poulton and Reece, 1957). It was also observed (Gregoire, 1947) that thymus weight decreased as pregnancy progressed, implying increased corticoid activity as parturition approached. By using a fluorometric method for directly measuring corticosterone in the plasma it was observed (Gala and Westphal, 1965), that during early and midpregnancy in the rat resting levels were lower than in virgin female rats, but in late pregnancy there was an increase in corticosterone levels. This same trend has also been shown for the guinea pig, rabbit and mouse (Gala and Westphal, 1967; Solem, 1966).

Many clinical investigators have found that adrenal function in women increases during pregnancy (Venning, 1946; Gemzell, 1953; Daughaday et al., 1962; Robinson et al., 1955). This does not necessarily mean there is a rise in biological glucocorticoid activity; on the contrary there probably is a decrease. The blood contains certain proteins which bind to adrenal corticoids rendering them biologically inactive (Slaunwhite and Sandberg, 1959). It has been shown (for refs. see Seal and Doe, 1963) that the concentration of

corticoid binding proteins (transcortin) is elevated during pregnancy. It has been suggested that this elevation in binding is due to the elevated estrogen level during pregnancy (Slaunwhite and Sandberg, 1959). This increased binding is probably not present in the rat. Corticosterone-binding activity (CBA) in pregnant rats is the same as in virgins, even though estrogen levels are higher during pregnancy (Gala and Westphal, 1965). These investigators (unpublished observations) claim that estrogen injection into female rats does not alter CBA. They suggest the difference between the 2 species may be that the normal estrogen concentration is not sufficient for maximal CBA in the human, whereas, in the virgin female rat the estrogen level may be optimal for CBA.

Parturition has been shown to greatly increase blood levels of 17-hydroxycorticosteroids in humans (McKay et al., 1957). In the rat, adrenal cortical activity is increased during parturition as measured by adrenal cholesterol changes (Poulton and Reece, 1957).

Glucocorticoid activity is high during lactation in most species studied. The peak activity in the rat was observed to be about the 10th day postpartum (Poulton and Reece, 1957; Gala and Westphal, 1965). Resting levels of glucocorticoids were also high during lactation in the mouse, rabbit and guinea pig (Gala and Westphal, 1967). In the present study direct measurement of plasma corticosterone levels during pregnancy, parturition and lactation were made in an attempt to confirm previous findings and further understand the role of glucocorticoids in the initiation and maintenance of lactation in the rat.

III. Effect of Suckling on Pituitary Hormones

The importance of suckling in the maintenance of lactation is well established. Suckling stimulates nerve endings in the nipples which cause nerve impulses to travel to the brain via the spinal cord (Eayrs and Baddeley, 1956). These workers found that interruption of these sensory pathways results in severe or total impairment of lactation. Edwardson and Eayrs (1967) postulated that the relationship between the neural stimulus of suckling and the subsequent endocrine response is quantitative; partial denervation of the nipple results in reduced lactational performance due to reduced endocrine stimulation. The precise pathway of these sensory impulses to the hypothalamus is not known but it is believed that the nerve impulses end in discrete areas of the hypothalamus to elicit or inhibit the release of specific neurohumors. These neurohumors enter the portal vessels and exert their effect on the anterior pituitary (Harris, 1948).

Several anterior pituitary hormones as well as oxytocin and vasopressin (ADH) from the posterior pituitary and melanocyte-stimulating hormone (MSH) from the intermediate lobe have been reported to be released in response to suckling. Taleisnik and Orias (1966) reported a drop in pituitary MSH content after 1 hour of suckling in rats; however the physiological significance of this observation has not been established.

Oxytocin and ADH are synthesized in the paraventricular and supraoptic nuclei of the hypothalamus. These 2 hormones travel via the supraoptico-hypophyseal tract to the posterior pituitary where

they are stored. Ely and Petersen (1941) demonstrated that suckling evoked a release of oxytocin, the hormone necessary for milk ejection. Cross and Harris (1952) showed that lesions of the supraoptico-hypophyseal tract in the median eminence area abolished the milk ejection response to suckling. ADH is also released in response to suckling but in quantities not large enough to elicit milk ejection (Cross, 1961).

Suckling for a half hour (Grosvenor, 1964 a) or 3 hours (Sar, 1968) has been shown to deplete the anterior pituitary of GH in the rat. Three hours of suckling in the rat (Sar, 1968) failed to affect the pituitary content of TSH. Grosvenor (1964 b) observed that the nursing stimulus elevates thyroid secretion in the lactating rat and suggested that the increased thyroid secretion is due to milk removal rather than CNS excitation.

It has been demonstrated that suckling reduces prolactin concentration in the pituitary of the rat (Reece and Turner, 1937; Grosvenor and Turner, 1957; Grosvenor et al., 1967 a). It has been shown that suckling acts through the hypothalamus to reduce prolactin inhibiting factor (PIF), which results in increased prolactin release. A comparison of suckled lactating rats with cycling control rats revealed a significant decrease of PIF in the suckled rats (Ratner and Meites, 1964; Minaguchi and Meites, 1967). In the latter report pituitary prolactin concentration was shown to be significantly higher in the lactating rat. It was observed that immediately after suckling, lactating rats had a lower PIF content in the hypothalamus and

a lower pituitary prolactin concentration than non-suckled lactating controls (Sar, 1968). The nerve impulses triggered by suckling caused a reduction in PIF resulting in more prolactin release from the pituitary to act on the mammary gland.

Many studies have shown that gonadotropin secretion is depressed during suckling (Desclin, 1947; Rothchild and Dickey, 1960; McCann et al., 1961). Evidence that depression of gonadotropins is mediated through the hypothalamus is indicated by a reduction in luteinizing-hormone releasing factor (LRF) concentration in the hypothalamus as well as a reduction in LH concentration in the pituitary of suckled postpartum rats as compared to cycling controls (Minaguchi and Meites, 1967).

There is some evidence, mostly indirect, which indicates that ACTH is released in response to suckling. The maintenance of thymus regression in suckled rats (Gregoire, 1947) suggested that suckling caused ACTH release. An increase in suckling intensity also resulted in a decrease in thymus weight. Removal of the suckling stimulus from lactating rats induced mammary involution; however ACTH or cortisone administration prevented this involution (Johnson and Meites, 1957). Pituitary ACTH levels, as measured by an indirect assay method, were unchanged with increased suckling frequency (Tucker et al., 1967 a). The same group of workers also reported that rats suckling 2 pups had 104% more ACTH than rats suckling 4 pups and 24% more ACTH than rats suckling 6 pups (Tucker et al., 1967 b). However these results were not statistically significant. Mother rats which had their young

removed for 8 hours and then suckled for 1 hour had the same ACTH concentration in the pituitary as non-suckled mothers (Taleisnik and Orias, 1966). They concluded that ACTH is not released in response to suckling. However Denamur et al. (1965) showed in the goat and sheep that suckling for 30 minutes reduced pituitary ACTH. After 1 hour of suckling the level of ACTH was the same as before suckling. The reasons for these differences between rats and goats or sheep are not clear.

A drop in circulating eosinophils has been shown to be connected with increased ACTH release (Recant et al., 1950). Lactating women showed a maximum drop of 29% in eosinophil levels 2 hours after suckling began (Bromberg et al., 1952). A reduction in adrenal ascorbic acid level was observed 1 hour after suckling in mice, constituting further indirect evidence of ACTH release by suckling (Tabachnick and Trentin, 1951).

Unfortunately, all of the above work cited as evidence for ACTH release in response to suckling utilized methods for measuring ACTH release which are insensitive, indirect or nonspecific (Mangili et al., 1966). It was the purpose of this work to determine the effects of suckling on pituitary ACTH and plasma corticosterone levels in the rat utilizing a more direct and accurate method.

IV. Factors Affecting the Onset of Puberty

A. Environmental Factors

Many environmental factors, internal and external, play a role

in the onset of puberty. These factors are often reflected in a change in the hormonal balance of the organism, and it is these hormonal changes which are thought to precipitate puberty. Many of the early researchers attempted to understand the mechanisms of puberty by studying only the pituitary-gonadal axis. The observation by many clinicians that hypothalamic and cerebral abnormalities were coincident with precocious or delayed puberty changed the focus of research to the brain as the primary controller of the onset of puberty.

Exposure of immature rats to constant light was shown to hasten the onset of puberty (Jochle, 1956; Luce-Clausen and Brown, 1939), probably by increasing FSH release. Gentle handling of immature rats every day hastened puberty several days (Morton et al., 1963). Rats repeatedly placed in cold temperature for several days had vaginal opening a few days earlier than the controls (Mandl and Zuckerman, 1952). Electrical stimulation of the uterine cervix in prepubertal rats hastened prepubertal development as evidenced by enlarged uteri and developed corpora lutea (Swingle et al., 1951). Dibenamine, an antiadrenergic agent, blocked this effect. Thus various external environmental stimuli, seemingly unrelated to one another, were shown to influence the onset of puberty.

The first experimental demonstration that the brain was closely involved in puberty was a report by Donovan and van der Werff ten Bosch (1959), showing that lesions of the anterior hypothalamus but not the preoptic area caused an advancement of puberty

of about 1 week in rats. Since then other workers have confirmed these observations and further explored the brain in an attempt to understand the processes of puberty. Anterior and posterior hypothalamic lesions hastened vaginal opening in Wistar female rats (Schiavi, 1964). However lesions from the mammillary bodies to the ventromedial nucleus delayed puberty in another group of rats (Corbin and Schottelius, 1960). Gellert and Ganong (1960) found puberty hastened by lesions in the arcuate nucleus of the posterior tuberal region, whereas, lesions in the anterior hypothalamus, hippocampus, cerebral cortex and thalamus had no effect. Precocious ovarian stimulation in rats was observed with anterior hypothalamic or amygdaloid lesions (Elwers and Critchlow, 1960). Electrical stimulation of the corticomедial portion of the amygdala delayed puberty in the rat (Bar-Sela and Critchlow, 1962), implying that the amygdala exerts some inhibitory effect on puberty. The significance of the above findings is still uncertain but it may involve an effect on steroid feedback centers.

Exogenous hormones and drugs have also been used to gain further insight into the mechanisms of puberty. Oxytocin injection into intact immature rats accelerated vaginal canalization, whereas in hypophysectomized rats oxytocin was ineffective (Corbin and Schottelius, 1961). ADH or epinephrine injections had no effect on puberty but serotonin injections delayed puberty several days. Serotonin was also shown to delay puberty in mice (Robson and Botros, 1961). Reserpine, an agent which depletes the brain of catecholamines (Lippman et al., 1967), retarded growth of the interstitial

tissue of the testis and reduced spermatogenesis in immature male mice, implying a decrease in LH and FSH release into the blood (Adams and Fudge, 1959).

Iproniazid, a monoamine oxidase inhibitor which may delay the destruction of serotonin and other catecholamines, delayed puberty in female rats (Setnikar et al., 1960) and in mice (Robson and Botros, 1961). Thus agents which increase or deplete the catecholamines of the brain appeared to delay puberty. The significance of these rather contradictory observations are as yet unexplained.

Injection or implantation of estrogen, the hormone responsible for vaginal opening, into the hypothalamus resulted in precocious puberty in the rat. Ramirez and Sawyer (1965) found that very small doses of estradiol benzoate hastened puberty more than 1 week and postulated that estrogen may be the limiting factor in the natural onset of puberty. Recently it was demonstrated that acute implants of estrogen into the anterior hypothalamicpreoptic area will hasten puberty (Smith and Davidson, 1968).

B. Hormonal Factors

Many investigators have demonstrated large changes in gonadotropin secretion as an animal approaches puberty. The early workers used crude techniques for measuring gonadotropin concentration in the anterior pituitary, but reported a much higher content of FSH and LH in prepubertal rats than in mature rats (Clark, 1935; Lauson et al., 1939; Hoogstra and Paesi, 1955). LH, FSH, GH and TSH were found to

be in higher concentration in the pituitary of immature male rats than in mature male rats (de Jongh and Paesi, 1958). More recently Kragt and Ganong (1967) and Corbin and Daniels (1967) using more precise and sensitive bioassay techniques, showed that FSH concentration in the pituitary of the rat was highest at 21 days of age and then decreased as puberty approached. Ramirez and Sawyer (1965) showed that LH concentration in the pituitary decreased just prior to puberty with a concurrent increase of LH in the blood. It is now believed that FSH is first necessary to cause full maturation of the ovarian follicles and to stimulate estrogen secretion, and that LH is needed for follicular ovulation.

Most recently Clemens et al. (manuscript in preparation) have shown that subcutaneous prolactin injections as well as prolactin implants into the median eminence of the hypothalamus of immature female rats hasten puberty about 1 week. It was the intention of the present investigator to determine the mechanism whereby these prolactin implants hasten puberty.

MATERIALS AND METHODS

I. Animals

A. Experimental Animals

All rats were of the Sprague-Dawley strain obtained from the following 2 sources: Spartan Research Labs., (Haslett, Michigan) and Holtzman Company (Madison, Wisconsin). Female rats (used for pregnancy and lactation studies) were housed 5 to a cage with 1 male rat for breeding purposes. After pregnancy was determined by finding sperm in the vagina, the females were housed individually. Female rats used in puberty studies were housed 10 to a cage. All animals were fed Wayne Lab Blox (Allied Mills, Chicago, Illinois) ad libitum. The temperature of the animal room was maintained at $25 \pm 2^{\circ}\text{C}$ with a lighting schedule of 14 hours of light and 10 hours of darkness (7:00 A.M. to 9:00 P.M.).

B. Bioassay Animals

Animals for FSH and ACTH bioassays were of the Sprague-Dawley strain obtained from the following 2 sources: Holtzman Company (Madison, Wisconsin) and Hormone Assay Labs. (Chicago, Illinois). Animals used for FSH bioassay were delivered at 23 days of age and used at 24 or 25 days of age. Rats for ACTH bioassay were hypophysectomized male rats (125-150 g) used within 24 hours after hypophysectomy. All bioassay rats were kept in the same conditions as experimental rats, except that the hypophysectomized rats

received a supplement of fresh oranges and sugar cubes in their diet.

II. Preparation of Pituitary Tissue for Bioassay

Animals assayed for pituitary ACTH were killed very rapidly by guillotine within 1 minute after the animal room was entered. The pituitaries were quickly removed, weighed and stored at -20°C . On the day of the ACTH assay the pituitaries were thawed and ground with a glass homogenizer in acid-saline ($\text{pH}=2.0$) medium. This material was used within 2 hours after preparation for ACTH bioassay.

Animals used for FSH bioassay were killed by guillotine, and the pituitaries were quickly removed, weighed and stored at -20°C . On the day when bioassay began, the pooled pituitary tissue was homogenized in cold physiological saline and kept at 4°C .

III. Bioassays

A. Adrenocorticotrophic Hormone (ACTH)

Pituitary ACTH was assayed by the method of Guillemin et al. (1958). Hypophysectomized rats were injected intravenously with the assay material and killed by decapitation 15 minutes later. The systemic blood was collected in heparinized beakers, centrifuged and the plasma was frozen at -20°C within one hour of collection. This plasma was assayed for corticosterone by the method of Silber et al. (1958) as modified by Guillemin et al. (1959). All ACTH assays included one dose level of each unknown and 3 doses of standard ACTH.

The reference standard for ACTH assays was Upjohn ACTH with a stated potency of 40 USP/ml or 1.14 U/mg Second USP Corticotropin

Reference Standard. Three standard doses were used for each assay, ranging from 0.2-1.8 mU ACTH. This standard was diluted in physiological saline immediately prior to use. ACTH was kindly supplied by The Upjohn Company, Kalamazoo, Michigan.

B. Follicle Stimulating Hormone (FSH)

Pituitary FSH content was assayed by the Steelman and Pohley method (1953) as modified by Parlow and Reichert (1963). Materials were assayed at either two dilutions with a fourfold difference in concentration or at one concentration.

The reference standard hormone used for FSH assay was NIH-FSH-S4, supplied by the Endocrinology Study Section, National Institutes of Health. It was dissolved in physiological saline just prior to use. Two or three standard doses were used ranging from 60-140 ug.

IV. Histological Preparations

All tissues were fixed in Bouin's fluid, sectioned and stained with eosin and hematoxylin, and examined microscopically.

V. Statistical Treatment

One FSH bioassay was analyzed according to the method of Bliss (1952). All other bioassays were analyzed by Student's "t" test (Steel and Torrie, 1960), using a two-tailed test. One-way analysis of variance was used to test for significance among a large number of means. Duncan's New Multiple Range test was employed to statistically separate these means.

EXPERIMENTAL

I. EFFECT OF PREGNANCY, PARTURITION AND LACTATING ON PLASMA CORTICOSTERONE LEVELS IN THE RAT

A. Objective

The purpose of this experiment was to determine the plasma corticosterone level in the rat during pregnancy, parturition and postpartum lactation, and to relate the findings to the needs of the rat for initiation and maintenance of lactation.

B. Procedure

Adult female rats were mated and day 1 of pregnancy was considered as the day sperm were detected in the vagina. The pregnant rats were placed into individual cages and killed by rapid decapitation between 8 and 9 A.M. on different days of pregnancy or lactation. During lactation all litters were adjusted to 8 pups on day 2. Eight rats were also killed during parturition, which occurred at various times during the day. The pituitary and thymus were also removed and weighed. Systemic blood was collected in heparinized beakers and the separated plasma was assayed for corticosterone.

C. Results

Plasma corticosterone levels during pregnancy were not significantly different from cycling control rats (Table 1). During parturition corticosterone levels were much higher than during pregnancy

Table 1. Effects of pregnancy, parturition and lactation on thymus weight and plasma corticosterone concentration in the rat

Treatment	No. of rats	Avg body wt (g) ¹	Avg AP ² wt (mg)	Avg thymus wt (mg)	ug cortico-sterone/100 ml plasma
Cycling	8	249.3 ± 4.1	10.4 ± 0.4	292.0 ± 15.4	16.8 ± 8.1
10th day of pregnancy	8	249.1 ± 5.4	10.4 ± 0.3	364.7 ± 24.3	12.5 ± 2.5
20th day of pregnancy	8	308.3 ± 4.4	10.1 ± 0.4	230.7 ± 24.5	16.8 ± 1.8
Parturition day	8	314.3 ± 9.8	10.2 ± 0.2	163.7 ± 8.3	40.2 ± 9.8
1st day of lactation	8	265.0 ± 3.8	10.6 ± 0.6	169.9 ± 7.4	26.7 ± 6.5
6th day of lactation	8	263.8 ± 2.4	11.4 ± 0.6	170.2 ± 22.0	18.2 ± 3.7
12th day of lactation	8	275.0 ± 2.6	9.5 ± 0.4	155.0 ± 18.0	38.0 ± 6.7
18th day of lactation	8	285.0 ± 2.9	10.2 ± 0.3	168.4 ± 20.9	24.3 ± 5.5

¹Mean ± SE

²AP = Anterior Pituitary

($p < .05$). The corticosterone level remained high during most of lactation with a peak of 38.0 ug/100 ml plasma on day 12 and a minimum level of 18.2 ug/100 ml plasma on day 6.

Thymus weights were highest during early pregnancy and decreased as pregnancy neared completion. During lactation thymus weights were lower and corticosterone levels were higher than at any other period (Fig. 1). Anterior pituitary weight did not vary significantly during these several stages.

D. Discussion

This study demonstrates that on the day of parturition and during lactation in rats adrenal glucocorticoid activity is significantly increased as compared with pregnant or cycling rats. The significantly lower weights of the thymus on the day of parturition and during lactation as compared with pregnancy and cycling, can be attributed to the higher corticosterone levels. These data provide further evidence that the adrenal cortical hormones play an essential role in the initiation and maintenance of lactation in the rat.

The data on adrenal corticoid activity are in good agreement with related work in this area. Gala and Westphal (1965) reported that corticosteroid-binding globulin (CBG) in the rat decreased during late pregnancy and remained low during lactation. These workers calculated the levels of unbound corticosterone in the plasma by using the resting corticosteroid levels and the CBG values. The calculations suggested that unbound corticosteroids were low during early pregnancy but increased in late pregnancy and lactation, in

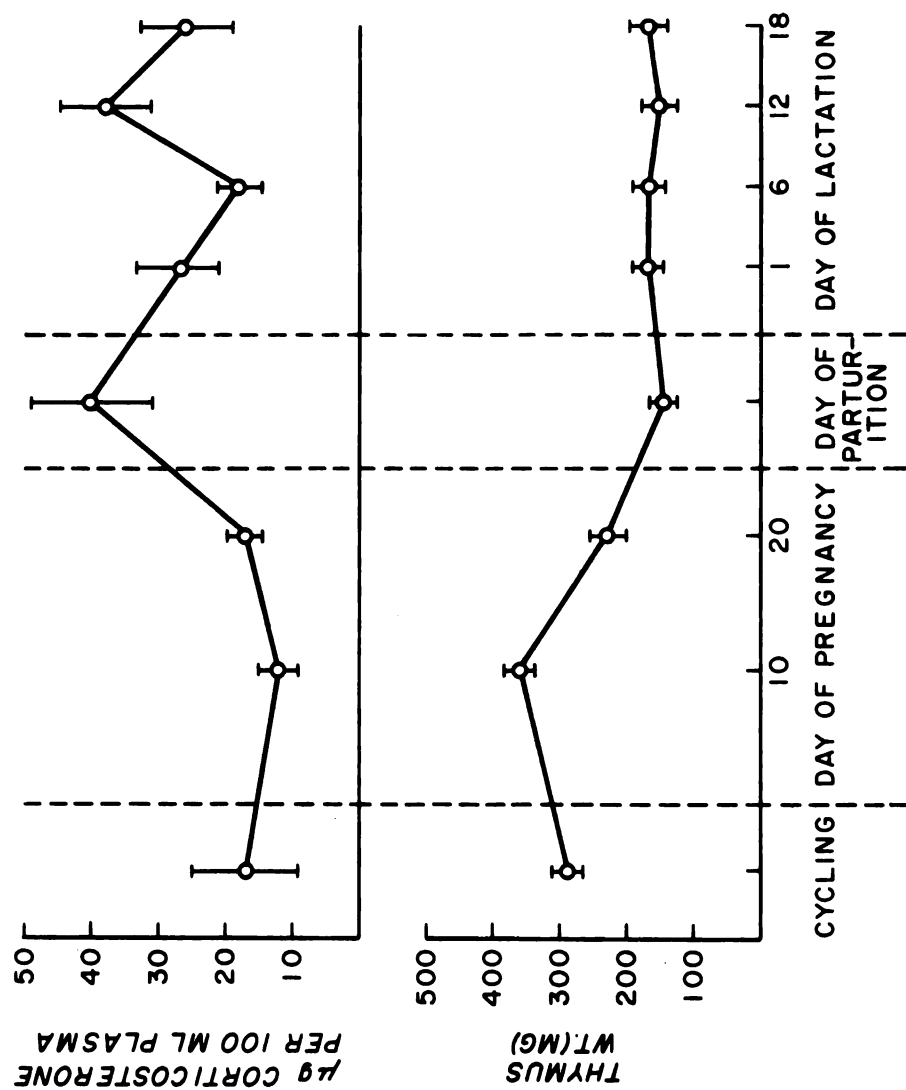


Figure 1. Influence of pregnancy, parturition and lactation on plasma corticosterone and thymus weight in the rat

agreement with the results presented here. Other species including the mouse and rabbit have shown a similar trend in adrenal corticoid activity during pregnancy and lactation (Gala and Westphal, 1967).

Using an indirect method (adrenal cholesterol depletion) for adrenal cortical activity, Poulton and Reece (1957) found higher activity on the 10th day of lactation in rats than during pregnancy. Thymus weight has often been used as an indirect measure of corticoid activity. As corticoid activity increases, thymus weight decreases. Gregoire (1947) found that the thymus involuted during late pregnancy and remained involuted during lactation if suckling was maintained. This is in agreement with the results obtained in the present study.

It is worthy of note (Figure 1) that as the levels of plasma corticosterone increased, thymus weights decreased. This indicates that the method used for measuring corticosterone measured biologically active corticoids. Guillemin et al. (1958) showed that the fluorometric method used in this study to measure plasma corticosterone is reasonably specific for measuring corticosterone in the plasma of the rat. Thus it can be assumed that the changes reported for plasma corticosterone levels in this study are indicative of actual biologically active glucocorticoid changes. Interference from other glucocorticoids is not likely since corticosterone is the main glucocorticoid (> 95%) in rat blood.

Previous workers (cited in the Review of Literature) found that administration of ACTH or adrenal steroids to the pregnant rat

or rabbit initiates lactation. Also both prolactin and adrenal hormones were found to be necessary for initiation of lactation in hypophysectomized guinea pigs (Nelson et al., 1943), and in hypophysectomized, ovariectomized and adrenalectomized mice (Nandi and Bern, 1961) and in rats (Lyons et al., 1958). These studies indicated that neither prolactin nor adrenal cortical hormones were present in amounts large enough during pregnancy to initiate lactation. The present study supports this view since corticosterone levels during pregnancy were much lower than during parturition and lactation in the rat.

II. EFFECTS OF SUCKLING ON PITUITARY ACTH AND PLASMA CORTICOSTERONE LEVELS IN THE LACTATING RAT

A. Objective

Since suckling has been shown to cause release of several pituitary hormones involved in lactation in the rat, it was of interest to determine the effect of suckling on the pituitary-adrenal axis. Experiments were designed to measure pituitary ACTH and plasma corticosterone levels following different periods of suckling.

B. Procedure

Adult female rats were bred and placed in individual cages. On day 2 postpartum, litter size was adjusted to 8 pups. On the evening of day 3 the pups were separated from their mothers and placed in other cages. At 8:00 A.M. the next morning the pups were either replaced with their mothers (experimental group) or not replaced (con-

trol group). To rule out the possibility that any disturbance in the cage of the suckled mother as a result of returning the pups might stimulate the ACTH-adrenal cortical axis, the cage of the control non-suckled mother was also disturbed but the pups were not returned. After either one-half or 3 hours of suckling the mothers were quickly killed by guillotine, the pituitaries were removed and later assayed for ACTH content, and the plasma was collected in heparinized beakers and later assayed for corticosterone.

C. Results

The effects of 3 hours suckling on pituitary ACTH and plasma corticosterone levels in Experiments 1 and 2 are shown in Table 2. When the pups were allowed to suckle for 3 hours following a 12 hour non-suckling period, plasma corticosterone levels of the mother rats increased 3-4 times as compared with controls not suckled for 15 hours. This increase is highly significant. In the animals of Experiment 1, pituitary ACTH concentration of the suckled rats was 7.4 mu/mg tissue as compared with 4.0 mu/mg in the non-suckled rats, a difference significant at the 2% level. Anterior pituitary weight was the same in each group.

The effects of a half hour of suckling on pituitary ACTH and plasma corticosterone levels are given in Table 3. Mother rats non-suckled for 12 hours followed by a half hour of suckling had corticosterone levels about 4 times above that of control mother rats not suckled for 12.5 hours, a highly significant increase (Experiments 1 and 2). These same suckled rats showed a significant decrease in

Table 2. Effects of 3 hours suckling on pituitary ACTH and plasma corticosterone concentration in postpartum lactating rats

Exp. no.	No. of rats	Treatment	Avg body wt (g) ¹	Avg AP ² wt (mg)	ug cortico-sterone/100 ml plasma	ACTH concentration ³ mu/mg AP
1	6	15 hrs non-suckling	269.5 ± 7.3	10.6 ± 0.5	6.3 ± 1.3	4.0 ± 0.7
	6	12 hrs non-suckling, 3 hrs suckling	283.3 ± 8.9	10.8 ± 0.6	26.2 ± 5.4**	7.4 ± 0.9*
2	10	15 hrs non-suckling	267.0 ± 7.4	9.7 ± 0.5	10.8 ± 3.1	--
	16	12 hrs non-suckling 3 hrs suckling	288.9 ± 8.7	10.4 ± 0.4	31.7 ± 5.0***	--

¹Mean ± SE

²AP = Anterior Pituitary

³Upjohn ACTH (Kalamazoo, Michigan) was used as the reference standard and had a stated potency of 40 USP/ml

*P < .02

**P < .01

***P < .005

Table 3. Effects of suckling for a half hour on pituitary ACTH and plasma corticosterone levels in postpartum lactating rats

Exp. no.	No. of rats	Treatment	Avg body wt (g) ¹	Avg AP ² wt (mg)	ug cortico-sterone/100 ml plasma	ACTH concentration mu/mg AP ³
1	5	12.5 hrs non-suckling	256.0 ± 7.1	11.1 ± 0.5	10.4 ± 1.1	4.9 ± 0.9
	5	12.0 hrs non-suckling, .5 hrs suckling	256.0 ± 5.4	10.9 ± 0.5	40.0 ± 7.3***	1.2 ± 0.1***
2	8	12.5 hrs non-suckling	251.9 ± 4.9	10.8 ± 0.4	8.4 ± 1.5	3.6 ± 0.6
	8	12.0 hrs non-suckling, .5 hrs suckling	254.3 ± 3.2	10.4 ± 0.3	39.8 ± 7.8**	2.1 ± 0.1*

¹Mean ± SE

²AP = Anterior Pituitary

³Upjohn ACTH was used as the reference standard and had a stated potency of 40 USP/ml

*p < .05

**p < .002

***p < .005

pituitary ACTH levels as compared to the non-suckled rats. Comparison of pituitary ACTH concentration in rats not suckled for 15 hours (Table 2) with those not suckled for 12.5 hours (Table 3) revealed no difference, whereas this same comparison in suckled rats showed that after a half hour of suckling there was a marked decrease in ACTH concentration, whereas after 3 hours of suckling there was a significant increase in pituitary ACTH concentration.

In a separate experiment pups were placed within seeing, hearing or smelling distance of the mothers for a half hour but were not allowed to suckle. This apparently had some stimulatory effect on corticosterone levels in the plasma of 9 mother rats treated in this manner, who showed a mean corticosterone level of 21.1 ± 8.4 ug/100 ml plasma, as compared to 8.4 ± 1.5 ug/100 ml plasma for a non-suckled group (8 mother rats) with no pups nearby. This was not a statistically significant difference.

D. Discussion

The results of these experiments provide the first direct evidence that the suckling stimulus in the rat causes a release of pituitary ACTH and an increase in plasma corticosterone levels. These results strongly support previous work reported on this subject. Suckled lactating rats had involuted thymus glands as compared to non-suckled lactating rats (Gregoire, 1947), suggesting that suckling caused release of ACTH and subsequent increases in glucocorticoid levels.

Using the indirect and less exact adrenal ascorbic acid depletion method for assaying ACTH, Taleisnik and Orias (1966) reported that the pituitary ACTH potency of 3 rats suckled for 1 hour was not different from non-suckled controls. They concluded that suckling did not induce ACTH release. However, the results reported in this present study indicate that pituitary ACTH concentration is related to the length of time the suckling stimulus is applied. Pituitary ACTH concentration after a half hour of suckling averaged 1.65 mu/mg tissue, and after 3 hours suckling averaged 7.40 mu/mg tissue. Pituitary ACTH levels for both the 12.5 and 15 hour non-suckled groups averaged 4.17 mu/mg tissue, about 2.5 times the concentration of the half-hour suckled group and half the concentration of the 3-hour suckled group. The increase in pituitary ACTH after 3 hours of suckling may reflect a greater increase in synthesis of ACTH than in release. On the other hand the decrease in pituitary ACTH after a half hour of suckling may reflect a greater release of ACTH than of synthesis during this brief period. It is possible that there is a suckling interval between these 2 periods of a half hour and 3 hours when the pituitary ACTH concentration is the same in the suckled and non-suckled groups. This may be the reason that Taleisnik and Orias (1966) found no difference in pituitary ACTH between rats suckled for one hour and non-suckled rats. Observations in the goat and sheep (Denamur et al., 1965) support this possibility. These workers found that suckling reduced pituitary ACTH by 29% by the end of the first 5 minutes, and reached the lowest concentration

after 30 minutes of suckling in the goat and sheep. Thereafter pituitary ACTH increased. After 1 hour of suckling the ACTH level was the same in suckled as in non-suckled controls.

Further support for the view that suckling stimulates increased synthesis and release of ACTH is found in the 3-5 fold increase in plasma corticosterone levels observed after either 3 or a half hour suckling. This increased activity of the adrenal cortex can only be a reflection of increased stimulation by ACTH from the pituitary.

There are at least 2 explanations why pituitary ACTH content of rats suckled for a half hour is lower than in non-suckled controls, whereas pituitary ACTH content of 3 hour suckled rats is higher than in non-suckled controls. The first explanation concerns the rate of synthesis of ACTH in the pituitary. Presumably the suckling stimulus induces ACTH secretion by increasing hypothalamic corticotropin-releasing factor (CRF) release, although this has never been determined. CRF probably increases both the synthesis and release of ACTH (Vernikos-Danellis, 1965; Hokin et al., 1958). The immediate action of CRF has been shown to cause a release of ACTH (Vernikos-Danellis, 1965). This investigator injected median eminence extracts intracarotidly and observed a marked decrease in pituitary ACTH reaching a minimum 40 minutes later. Thereafter pituitary ACTH concentration rose to near preinjection levels. In agreement with this are the findings of Rochefort et al. (1959) who reported a decrease in adeno-hypophysial ACTH content after 30, 60 and 120 minutes following neurohypophysial injections of CRF. These workers also found a

decrease in anterior pituitary ACTH content after 30 and 60 minutes following loud sound or histamine injection stresses. After 2 hours following these stresses ACTH levels were near those of preinjection time.

Another explanation for the differences in pituitary ACTH and corticosterone levels after suckling involves the intensity of the suckling stimulus. When pups had been removed from their mother for 12 hours they suckled vigorously for the first 30-60 minutes upon their return. After an hour or 2 the suckling was more sporadic, often only 2 or 3 pups were suckling after being with their mother for 3 hours. Thus stimulation of the nerve endings in the nipple is much less after the pups have been with their mothers for 3 hours than after a half hour. This may help explain why the plasma corticosterone level of the 3-hour suckled group was only 28.9 ug/100 ml plasma as compared with 39.9 ug/100 ml plasma for the half-hour suckled group. This decreased suckling intensity may also explain why pituitary ACTH levels after 3 hours suckling returned to and even went above pre- or non-suckled levels.

The finding that corticosterone levels were slightly increased following placement of the pups near the mother but not allowing them to suckle indicates that exteroceptive stimulation of CRF may also be a factor in the mechanism of suckling induced release of ACTH. Grosvenor (1965) and Grosvenor et al. (1967 b) reported that prolactin release during lactation may also be influenced by exteroceptive stimulation. As a separate experiment, it was found that

when pups from 6 mother rats were removed during the 11th day of lactation and the mothers were killed 12 hours later, plasma corticosterone levels were 14.1 ± 7.9 ug/100 ml plasma as compared with 38.0 ± 6.7 ug/100 ml plasma for mother rats who were with their pups constantly. This further supports the view that the suckling stimulus increases ACTH release.

III. EFFECTS OF IMPLANT OF PROLACTIN IN MEDIAN EMINENCE ON PITUITARY FOLLICLE-STIMULATING HORMONE CONTENT IN PREPUBERTAL RATS

A. Objective

Since median eminence implants of prolactin appear to advance puberty in female rats (Clemens et al., unpublished work), it was of interest to determine the effect of prolactin implants on follicle-stimulating hormone (FSH) content of the anterior pituitary of prepubertal rats.

B. Procedure

A total of 114 immature female rats were implanted with either a prolactin (NIH-P-S8)^{*}-cholesterol mixture, cholesterol alone or cholesterol pellets containing maximal amounts of anterior pituitary hormone contaminants calculated to be present in NIH-P-S8 prolactin. A mixture of equal quantities of prolactin and cholesterol was used to form pellets each weighing approximately 500 ug; thus each pellet contained about 250 ug prolactin. A second type of pellet weighing 500 ug contained the NIH-P-S8 contaminants⁺ and was prepared as follows:

^{*}28 IU/mg

⁺Contaminants of other hormones in NIH-P-S8 were stated to be as follows: GH, 0.0033 IU/mg; FSH, 0.008 IU/mg; LH, 0.003 IU/mg; TSH, 0.0005 USP/mg.

1.044 ug NIH-GH-S8, 1.818 ug NIH-FSH-S3, 0.102 ug NIH-LH-S8, 0.045 ug NIH-TSH-B3 and cholesterol. Another pellet was used as a control and contained cholesterol alone.

The various mixtures were each packed into 21 gauge stainless steel hypodermic tubes slightly flared at the tip. A Stoelting stereotaxic instrument with an attached electrode carrier was used to lower the tube into the rat brain, from which the pellet was slowly ejected into the anterior median eminence. The tube was then withdrawn. All rats were 21 days of age when implanted and were killed either 5 or 8 days later. The ventral surface of each rat brain was examined under a dissecting scope to determine the site of implantation. Only those rats which had implants in the median eminence were used in this experiment. Anterior pituitaries were removed and assayed for FSH by the Steelman-Pohley method. Ovarian and uterine weights were recorded and the organs were then fixed in Bouin's fluid for histological examination. Results were analyzed by Bliss statistics for bioassay (Bliss, 1952) and the "t" test.

C. Results

The effect of prolactin implants on rats killed 26 days of age, 5 days after implantation, is shown in Table 4. The results of Experiment 1 indicate that pituitary FSH content of prolactin implanted rats was so low as compared to cholesterol implanted controls that it could not be measured. Injections of 1.5 mg and 3.0 mg of pituitary tissue from the prolactin implanted group into the assay rats did not increase the ovarian weight whereas the same amount of

Table 4. Effect of a prolactin implant in the median eminence on pituitary FSH concentration in prepubertal rats

Exp. no.	Treatment	No. of rats	Organ Weights (mg) ³			FSH content ⁴ ug/mg gland	λ
			Anterior Pituitary	Ovaries	Uterus		
1	Cholesterol ¹	14	2.7	18.1 $\pm$ 1.7	--	50.6(35.9-71.3)	0.136
	Prolactin	14	2.7	23.6 $\pm$ 2.4	--	None detectable	
2	Cholesterol ¹	11	2.6	18.5 $\pm$ 0.6	26.1 $\pm$ 2.1	--	
	Impurities	13	2.2	15.4 $\pm$ 0.7	27.5 $\pm$ 1.5	--	
	Prolactin	9	2.8	19.0 $\pm$ 1.0	61.1 $\pm$ 4.8***	--	
3	Impurities ²	12	2.1	15.2 $\pm$ 1.1	26.7 $\pm$ 1.7	42.0 $\pm$ 4.0	
	Prolactin	13	2.4	18.1 $\pm$ 1.2	59.7 $\pm$ 9.9**	25.9 $\pm$ 1.9**	
4	Impurities ²	17	2.3 $\pm$ 0.1	18.1 $\pm$ 0.5	32.1 $\pm$ 1.7	44.3 $\pm$ 6.1	
	Prolactin	11	2.3 $\pm$ 0.1	19.1 $\pm$ 0.5	80.7 $\pm$ 5.9	28.5 $\pm$ 1.9*	

¹Implanted at 21 days and killed at 26 days of age

²Implanted at 21 days and killed at 29 days of age

³Mean $\pm$ SE

⁴Calculated from mean ovarian weight of assay rats $\pm$ SE of mean

λ = Index of precision

* P < .05

** P < .002

*** P < .005

Figure 2. Photomicrograph of uterus of prolactin-implanted rat (X25)

Figure 3. Photomicrograph of uterus of impurities-implanted rat (X25)

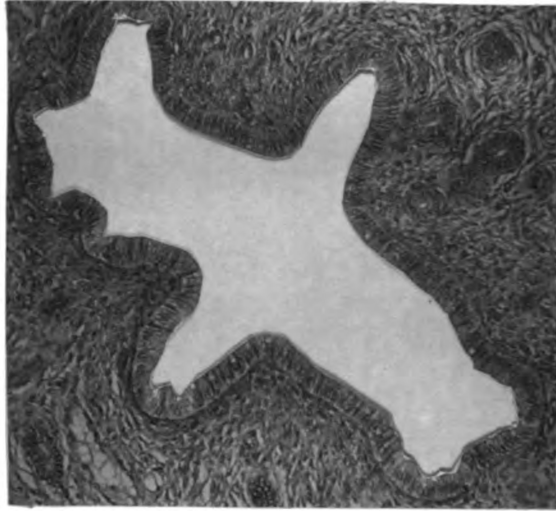


Figure 2

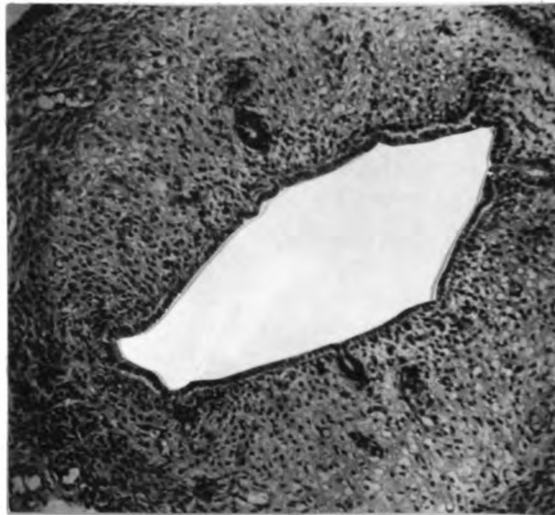


Figure 3

Figure 4. Photomicrograph of ovary of prolactin-implanted rat
(X9)

Figure 5. Photomicrograph of ovary of impurities-implanted rat
(X9)

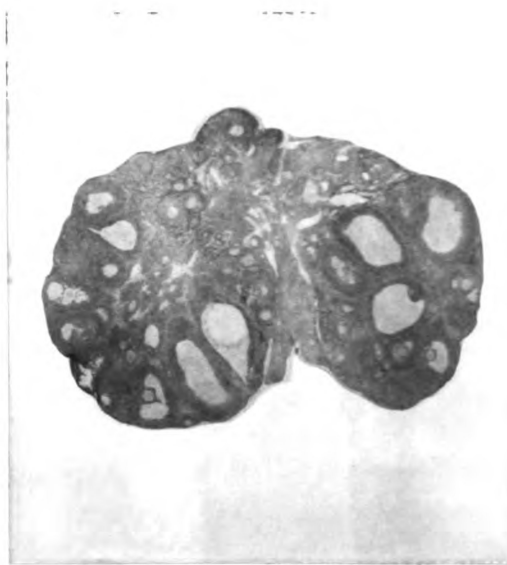


Figure 4



Figure 5

pituitary tissue from cholesterol implanted group injected into assay rats elicited good ovarian responses. The results are given of assays for pituitary FSH content of rats killed 8 days after implantation. Prolactin implants resulted in a 38.4% decrease in FSH content in Experiment 3, and a 35.6% decrease in Experiment 4, significant at the 0.002% and 0.05% levels, respectively. Data on pituitary FSH content in Experiment 2 could not be obtained because sufficient amounts of pituitary tissue were not available.

The uterine weights of prolactin implanted rats (Experiment 2) were significantly increased ($p < 0.005$) as compared with cholesterol or impurities-implanted controls. The uterine weights of prolactin implanted rats were increased 123%, ($p < 0.002$) in Experiment 3 and 151% ($p < 0.005$) in Experiment 4. Microscopic examination of the uteri (Figure 2) of the prolactin implanted group revealed a distinct increase in development of the endometrial layer and epithelium in comparison to the uteri of the impurities-implanted group (Figure 3). Ovarian weights were not significantly different (Experiments 1-4). However microscopic examination indicated increased stimulation of the follicles in the prolactin implanted rats as compared with the control rats (Figures 4 and 5).

D. Discussion

The results of the present study indicate that a median eminence implant of prolactin into immature rats increased release of pituitary FSH as shown by a decrease in pituitary FSH concentration, an increase in follicular tissue in the ovaries and an increase in

uterine weight. FSH acts directly on the ovaries to increase follicular growth, and at the same time to increase estrogen secretion by the ovary. Estrogen acts on the uterus to increase development of the endometrial cell layer.

A role for FSH in the onset of puberty has been implicated by several workers. A peak in pituitary gonadotropin potency was observed during the 3rd week of life in rats (Clark, 1935; Lauson et al., 1939), and thereafter a drop in potency occurred. Recently pituitary FSH measurements in female rats at various ages indicated FSH release was maximal during the few days prior to vaginal opening (Kragt and Ganong, 1967; Corbin and Daniels, 1967). Follicle stimulating hormone-releasing factor (FSH-RF) content in the hypothalamus was observed to fall prior to puberty and to increase after puberty (Corbin and Daniels, 1967). These findings suggest that the central nervous system (CNS), in its regulation of hypothalamic FSH-RF release and subsequent FSH release from the pituitary, plays an important role in the onset of puberty.

Recent findings by Clemens et al. (manuscript in preparation) indicates that prolactin implants in the median eminence as well as injections of prolactin significantly hastened puberty. This suggests that prolactin may be involved in the initiation of puberty, acting through the CNS. However, no change in pituitary prolactin content was found in the pre-pubertal rat (Minaguchi et al., 1968). The findings in the present study suggest that prolactin implants in the median eminence caused release of FSH from the pituitary, possibly

by stimulating increased FSH-RF release. Implants of the maximal amounts of anterior pituitary hormone contaminants stated to exist in NIH-P-S8 prolactin had no significant effects on pituitary FSH, ovaries or uterus. This eliminated the possibility that hormone contaminants in the prolactin implants caused the observed effect on FSH release.

The evidence that anterior pituitary hormones themselves feedback on the hypothalamo-hypophyseal axis to influence further secretion of anterior pituitary hormones is impressive. Median eminence implants of ACTH (Legori et al., 1965) decreased plasma corticosterone concentrations. Adrenalectomy increased corticotropin releasing factor (CRF), but adrenalectomy and ACTH injections decreased CRF, suggesting that ACTH acted back on the hypothalamus to stop synthesis of CRF, and consequently ACTH release was decreased. Growth hormone (GH) implants in the median eminence have been shown to decrease pituitary GH concentration in rats (Katz et al., 1967). Corbin and Cohen (1966), Corbin (1966) and David et al. (1966) demonstrated that median eminence implants of luteinizing hormone (LH) decreased pituitary LH, but FSH implants had little or no effect on pituitary LH. FSH implants in the median eminence decreased pituitary FSH and median eminence FSH-RF stores (Corbin and Story, 1967), whereas LH implants had no effect on pituitary FSH. This suggested that each pituitary hormone feeds back only on itself, not influencing other pituitary hormones.

Not all possible combinations of hormone implants affecting

pituitary hormones have been tried. There is some indication that some pituitary hormones may influence other pituitary hormones. Transplants of pituitary tumors which secrete prolactin, GH and ACTH resulted in significant decrease in thyroid and an increase in mammary gland uptake of I^{131} , suggesting an inhibitory effect on secretion of TSH by the animal's pituitary gland (MacLeod, 1967). It was also found that rats bearing a prolactin secreting tumor (7315a) decreased pituitary prolactin and GH as indicated on disc electrophoresis (MacLeod et al., 1966). The present study provides evidence that prolactin implants in the median eminence can alter pituitary FSH release. Other studies (Clemens and Meites, 1967; Chen et al., 1967) have shown that prolactin, acting through the CNS to increase prolactin inhibiting factor (PIF), decreased pituitary prolactin release.

In addition to the possibility that prolactin implants in the median eminence caused FSH release by stimulating FSH-RF release, it is conceivable that prolactin implants reduced pituitary prolactin (Clemens and Meites, 1967) and thereby increased the number of pituitary cells available for synthesis and release of FSH. There may be other explanations for the results of this experiment.

Two excellent reviews of the control and physiology of puberty (Donovan and van der Werff ten Bosch, 1965; Critchlow and Bar-Sela, 1967) summarized the increasing evidence that hypothalamic and amygdaloid lesions result in precocious puberty in the rat. Their

evidence implies that the amygdala and its projections to the hypothalamus exerts some inhibitory influence on the onset of puberty. It is possible that implants of prolactin in the hypothalamus overcame this inhibitory influence, resulting in precocious puberty.

The findings in the present experiments do not explain the neural mechanism responsible for increased FSH secretion at the time of puberty. However, further research in this area may provide a basis for a theory to explain the control of the onset of puberty. The next logical step is to measure the effect of median eminence implants of prolactin on hypothalamic content of FSH-RF.

CONCLUSIONS

The importance of the nervous system in stimulating hormone release from the anterior pituitary was emphasized by these experiments. The suckling stimulus was shown to elicit increased release of ACTH from the pituitary and increased plasma corticosterone levels. Implantation of prolactin into the median eminence area of the hypothalamus of prepubertal female rats resulted in increased FSH release.

Probably the most important factor responsible for increased ACTH and adrenal cortical activity during lactation is the suckling stimulus by the nursing pups. Further research is needed to determine the effects of suckling on the hypothalamic content of corticotropin releasing factor (CRF). This would give valuable information on the mechanism of increased ACTH release in response to suckling.

The increased release of FSH from the anterior pituitary observed following implantation of prolactin into the median eminence of immature rats may help explain the mechanism whereby similar prolactin implants advanced puberty in female rats (Clemens et al., manuscript in preparation). These workers reported that prolactin implants increase hypothalamic prolactin inhibiting factor (PIF) content, and thereby reduced prolactin secretion. It is of interest that implantation of one pituitary hormone, prolactin, can influence secretion of another pituitary hormone FSH. Whether prolactin

implants stimulated FSH release by causing release of follicle stimulating hormone-releasing factor (FSH-RF) into the portal vessels, or by some other mechanism remains to be determined.

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APPENDIX

To establish the validity of the plasma corticosterone assay used in this thesis, the data presented below in the form of a table were collected. Plasma used for the recovery study was obtained from unstressed female rats with the results given below. The reproducibility of the corticosterone assay of 9 plasma samples from a plasma pool of ether-stressed female rats are also given in the table.

<u>Recovery</u>			
Plasma (ml)	Corticosterone added (ug)	Corticosterone found (ug)	% Recovery
0.5	--	0.046	
0.5	0.1	0.149	102.0
0.5	0.2	0.250	101.0

<u>Reproducibility</u>		
		Corticosterone (ug/100 ml)
Plasma (ml)		
0.5		112
0.5		108
0.5		114
0.5		106
0.5		105
0.5		110
0.5		113
0.5		112
0.5		114
Mean = 0.5		Mean $\pm$ SE = 110.4 $\pm$ 1.1