STUDIES ON THE RELATION OF PROLACTIN-INHIBITING ACTIVITY OF THE HYPOTHALAMUS TO PITUITARY PROLACTIN RELEASE IN THE RAT

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ABSTRACT

STUDIES ON THE RELATION OF PROLACTIN-INHIBITING ACTIVITY OF THE HYPOTHALAMUS TO PITUITARY PROLACTIN RELEASE IN THE RAT

by Albert Ratner

An acid extract of rat hypothalamus inhibits the release of prolactin by the anterior pituitary (AP) *in vitro*. These studies were undertaken to determine whether agents which stimulate or inhibit pituitary prolactin release *in vivo* do so by altering the concentration of "prolactin inhibiting factor" (PIF) in the hypothalamus.

1. APs incubated with HE from estradiol-treated rats (50 μg/day for 10 days) released 73-153% more prolactin than APs incubated with HE from control rats. HE from estradiol-treated rats had no effect on prolactin release by incubated APs, whereas HE from control rats inhibited prolactin release. These results indicate that estradiol can deplete the hypothalamus of prolactin-inhibiting activity, thereby increasing AP prolactin release.

2. The amount of prolactin released by APs incubated with HE from ovariectomized rats was not different from the amount released by APs incubated with HE from control rats. APs incubated with HE from ovariectomized rats released 56-61% less prolactin than APs incubated withcut

HE. These results suggest that ovariectomy does not affect prolactin-inhibiting activity of the hypothalamus.

3. The amount of prolactin released by APs incubated with HE from male rats was not different from the amount released by APs incubated with HE from female rats. APs incubated with HE from male rats released 44-49% less prolactin than APs incubated without HE. These results indicate that hypothalamus from male rats has prolactin-inhibiting activity.

4. The amount of prolactin released by APs incubated with HE from progesterone-treated rats (4 mg/day for 10 days) was not different from the amount released by APs incubated with HE from control rats. APs incubated with HE from progesterone-treated rats released 50-60% less prolactin than APs incubated without HE. These results suggest that progesterone does not affect prolactin-inhibiting activity of the hypothalamus.

5. The amount of prolactin released by APs incubated with HE from tapazole-treated rats (5 mg/day for 30 days) was not different from the amount released by APs incubated with HE from control rats. APs incubated with HE from tapazole-treated rats released 44-63% less prolactin than APs incubated without HE. These results suggest that tapazole does not affect prolactin-inhibiting activity of the hypothalamus.

6. APs incubated with HE from suckled rats (12-18 days postpartum) released 88-175% more prolactin than APs

incubated with HE from control rats. HE from suckled rats had no effect on prolactin release by incubated AP, whereas HE from control rats inhibited prolactin release. These results indicate that the suckling stimulus can deplete the hypothalamus of prolactin-inhibiting activity, thereby increasing AP prolactin release.

7. APs incubated with HE from reserpine-treated rats (50 μg/day for 10 days) released 113-181% more prolactin than APs incubated with HE from control rats. HE from reserpine-treated rats had no effect on prolactin release by incubated APs, whereas HE from control rats inhibited prolactin release. These results indicate that reserpine can deplete the hypothalamus of prolactin-inhibiting activity, thereby increasing AP prolactin release. When reserpine was added to an incubation medium (10 μg/ml) containing APs and hypothalamus pieces, 83-92% more prolactin was released than by corresponding APs and hypothalamic pieces incubated in the absence of reserpine. Reserpine had no effect on prolactin release when incubated with APs alone. This indicates that reserpine acted directly on the hypothalamic tissue to depress PIF production.

8. APs incubated with HE from cervically stimulated rats (200 millivolts for 30 seconds) released 133-184% more prolactin than APs incubated with HE from control rats. APs incubated with HE from cervix-stimulated rats released 327-427% more prolactin than APs incubated without HE.

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3. The amount of prolactin released by APs incubated with HE from male rats was not different from the amount released by APs incubated with HE from female rats. APs incubated with HE from male rats released 44-49% less prolactin than APs incubated without HE. These results indicate that hypothalamus from male rats has prolactin-inhibiting activity.

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These results suggest that the hypothalamus from the cervically stimulated rats increased AP prolactin release _in vitro_.

9. Incubation of a corticotropin hormone-releasing factor (CRF) preparation with rat AP did not alter prolactin release. This indicates that CRF does not affect pituitary prolactin release and is different from PIF.

10. Incubation of a luteinizing hormone-releasing factor (LRF) preparation with rat AP did not alter prolactin release. This indicates that LRF does not affect pituitary prolactin release and is different from PIF.

11. An intracarotid injection of HE (2 or 4 hypothalami/rat) did not alter the prolactin content of the rat AP.

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By

Albert Ratner

A THESIS

Submitted to
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in partial fulfillment of the requirements
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DOCTOR OF PHILOSOPHY

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Dedicated

to my

Mother and Sister

With Love and Gratitude

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INTRODUCTION

Recent studies have emphasized that the anterior pituitary (AP) is regulated mainly by the central nervous system (CNS). The hypothalamus is believed to be most intimately associated with the control of AP function (Harris, 1955). The development of modern ideas of hypothalamic control of AP function can perhaps be considered to begin with the work of Marshall (1936, 1942), in which he emphasized the importance of environmental stimuli acting through the CNS on the AP, in regulating reproductive rhythms and cycles. The supposition emerged from Marshall's observations that the CNS exerted a controlling influence over the secretion of gonadotropic hormones by the AP. Harris (1955) has probably done more than anyone else in recent times to systematize the knowledge of CNS regulation of AP function, and to emphasize its importance.

Investigators of hypothalamic regulation of the AP have employed a variety of procedures to disrupt hypothalamic control of the AP, such as transplantation of the AP to non-cranial sites, transection of the pituitary stalk, placement of hypothalamic lesions, use of certain CNS-depressant drugs, and *in vitro* culture of AP tissue. Under such conditions there is a marked reduction in the secretion of ACTH, TSH, STH, FSH and LH but not of prolactin.

Prolactin release is actually increased, as indicated by prolonged maintenance of the corpora lutea in rats, induction of mammary growth and lactation in several species and increased secretion of prolactin by the pituitary <u>in vitro</u> (see Meites et al., 1963). These observations suggest that the hypothalamus is essential for the normal secretion of AP hormones other than prolactin. The hypothalamic mechanism controlling prolactin secretion is therefore believed to be inhibitory in nature, whereas all the other AP hormones require hypothalamic stimulation.

The hypothesis that a neurohumoral mechanism controls AP function was emphasized by Green and Harris (1947). They proposed that nerve fibers from the hypothalamus liberate humoral substances into the capillaries of the portal vessels in the median eminence, and that these vessels carry the substances from the pituitary stalk down into the sinusoids of the AP, to excite or inhibit the secretory activity of the gland cells.

Since prolactin secretion is favored by measures which remove hypothalamic control from the AP, the hypothalamic neurohumoral mechanism controlling prolactin secretion was considered to be inhibitory in nature. Demonstration of prolactin-inhibiting activity in hypothalamic tissue was recently reported by Talwalker et al. (1963). Acid extract of rat hypothalamus was shown to depress prolactin synthesis and release by the rat AP during a two hour incubation period. Acid extract of cerebral cortex, acetylcholine, epinephrine, norepinephrine, serotonin, histamine, substance P, oxytocin, and arginine or lysine vasopressin had no effect on AP prolactin release. These observations indicate that the hypothalamus contains a factor(s) which inhibits synthesis and release of prolactin, and this factor(s) does not appear to be any of the known neuropharmacological substances which are normally present in the hypothalamus. The active agent was tentatively named "prolactin-inhibiting factor" (PIF). Cultures of AP with hypothalamic extract (HE) or pieces has provided additional evidence for PIF in the hypothalamus (Pasteels, 1962; Danon et al., 1963; Gala and Reece, 1964b). Recently, Grosvenor et al. (1964) reported <u>in vivo</u> evidence for prolactin-inhibiting activity by hypothalamus. The chemical nature of PIF has not yet been determined, but it appears to be a small molecule (Talwalker, 1964).

It was of interest to attempt to correlate physiclogical alterations in prolactin secretion with the amount of PIF present in the hypothalamus. Studies were undertaken to determine whether agents which are known to increase or decrease prolactin release <u>in vivo</u> could do so by altering hypothalamic production of PIF. Thus, the effects of such agents as suckling, reserpine, estradiol, progesterone, cervical stimulation of the uterine cervix, etc., on hypothalamic PIF content and AP concentration and release of prolactin were tested. Demonstration of a correlation between AP release of prolactin and the concentration of PIF in the hypothalamus should provide evidence for the physiological significance of this factor. In addition, attempts were made to determine whether PIF was different from other hypothalamic factors which regulate AP function.

MATERIALS AND METHODS

Animals

Mature female rats of the Wistar strain (Wilson and son, Acton, Indiana) 3-5 months old, were used in these experiments. The animals were maintained in a temperature-controlled (75 ± 1° F) and artificially illuminated (14 hr/day) room. They were fed Wayne Lab Blox pellets *ad libitum*. White King pigeons (Cascade Squab Farm, Grand Rapids, Michigan), 4-8 weeks old were used for prolactin assays. Male rats of the same strain and age were utilized in one experiment.

Incubation Procedure

The rats were decapitated and the pituitaries were removed as quickly as possible and placed in a Petri dish over moistened filter paper. The posterior lobe was removed and the anterior pituitary (AP) was hemisected. A total of 3 APs (6 halves) were weighed and placed into a 25 ml Erlenmeyer flask containing 2 ml of synthetic protein-free medium 199 (Difco Laboratories, Detroit, Michigan). The medium contains all the known essential metabolites for cellular nutrition and maintenance *in vitro*.

In vitro release of prolactin by the AP from 2 different groups was studied by placing 3 APs (6 halves) from 3 control rats into each control flask, and 3 APs (6 halves) from 3 experimental rats into each experimental flask.

The effect of hypothalamic extract (HE) or of any other agent on in vitro prolactin release was studied by placing half of each AP into a control flask and the other half into an experimental flask. A total of 3 APs (6 halves), from the same 6 APs were placed into each flask. Preliminary studies showed, that under these conditions, the incubated APs released the same amounts of prolactin into both flasks. This provided each control and experimental flask with equivalent AP tissue.

All incubations were carried out at pH 7.4 in a Dubnoff metabolic shaker (60 cycles/min) under constant gassing with humidified 95% O_2 - 5% CO_2 at 37° C for 2 hours. The medium from each flask was collected and stored in a freezer until used for assay.

Preparation of Hypothalamic Extract

Each hypothalamus, including the median eminence, was removed and placed in cold 0.1N HCl (10 hypothalami/ml), homogenized with a ground glass homogenizer and centrifuged at 20,000 g for 30 minutes at 4° C. The supernatant was decanted and stored overnight at 4° C. The following day the supernatant was added to medium 199 and the pH was adjusted to 7.4 by addition of 5.6% $NaHCO_3$ solution. The final volume was made up so that each ml of medium contained extract equivalent to 3 rat hypothalami. When hypothalami from two different groups of rats were compared, the hypothalami were removed at the same time and prepared in a similar manner.

The Assay Procedure

The culture medium was assayed for prolactin activity by the intradermal crop method of Lyons (1937), as modified by Reece and Turner (1937). A dose of 0.1 ml of undiluted medium was injected daily for 4 days into each bird. The medium sample from each control and experimental flask was assayed separately or the media from 2 control flasks were pooled and assayed against the pooled media from 2 experimental flasks. The number of birds utilized for each assay are indicated in the tables.

Prolactin activity of the samples was determined by using a paired assay procedure. The samples were assayed in the same bird, by injecting the control sample over one side of the crop sac and the experimental sample over the other side. This permitted each bird to serve as its own control and provided a direct comparison between the 2 samples.

The prolactin responses from each bird were converted into International Units by use of a standard dose-response curve established in the same breed of pigeons with NIH prolactin (Nicoll and Meites, 1963). During the period in which the present experiments were performed, a number of additional dose-response curves were obtained with NIH prolactin. The slope of the log-dose response curve and index of precision in each case remained essentially similar. The same standard curve can therefore be used with reasonable certainty for converting Reece-Turner Units into International Units.

Statistical Treatment

In each experiment, regardless of the number of assays performed, the total number of responses were combined and treated as one group. The total responses, from the assays of the control and experimental samples in each experiment were then analyzed, using the "t" test for paired experiments (Batson, 1961). Analysis of this type is appropriate, since the control and experimental samples were assayed in the same animals. The percentage difference in prolactin content between the control and experimental samples was determined as follows:

$$\% \text{ Difference} = \frac{\text{Experimental-Control}}{\text{Control}} \times 100$$

Assay results from different experiments are not strictly comparable, since the amount of prolactin released into the medium by pituitary tissue from different rats varies from one experiment to another. The most accurate comparison's are between the amounts of prolactin released by equivalent AP halves from the same rats, incubated at the same time and assayed in the same pigeons, by a paired incubation and assay procedure. A schematic representation of the paired incubation and assay procedure is shown in Figure 1.

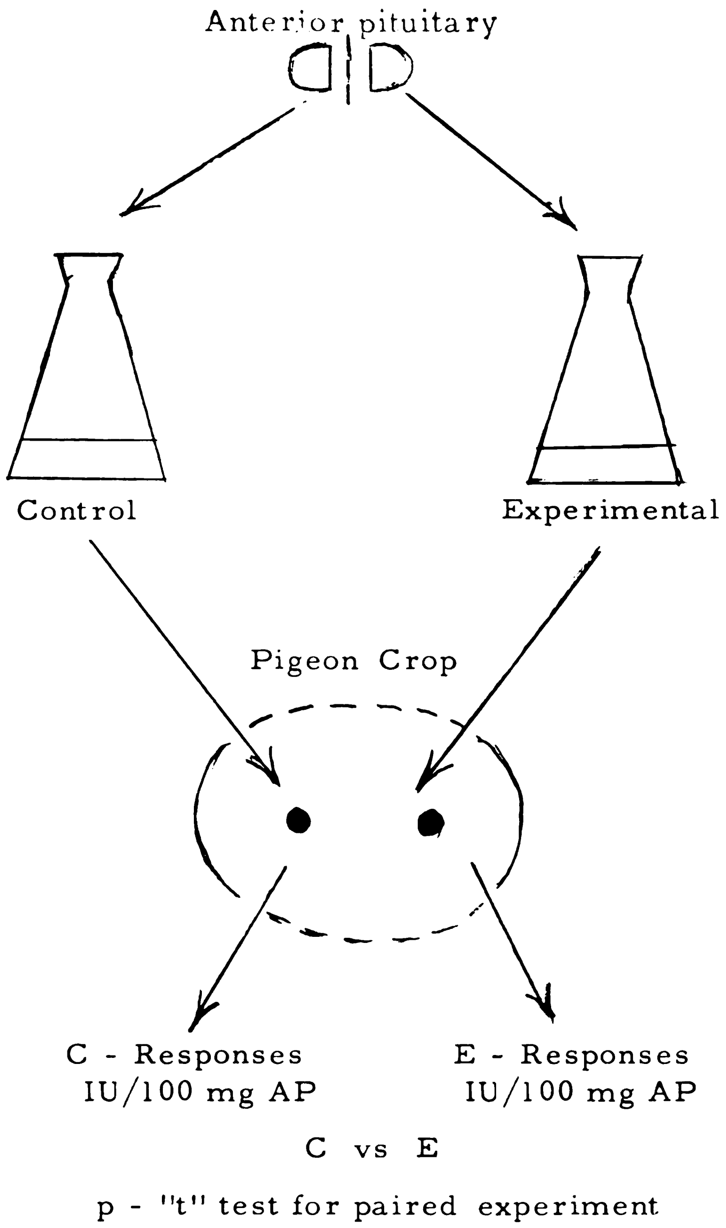

Figure 1. Schematic representation of the paired culture and assay procedure.

EXPERIMENTAL

Estrogen and Pituitary Prolactin Release

Effect of Estradiol Injections on Pituitary Prolactin Content and Prolactin Release In Vitro

The experimental animals were injected subcutaneously with 50 μg estradiol benzoate daily for 6 days, while the control animals were injected with an equal volume of the solvent. The estradiol was diluted with sesame oil and the daily injection volume was 0.1 ml. The rats were killed on the 7th day and the pituitaries were removed for assay. The data show that administration of estradiol increased AP prolactin content by 187% when expressed on a per 100 mg pituitary weight basis (Table 1, Expt. 1). The APs from the estradiol-treated rats weighed 45% more than APs from control cycling rats. When the APs were incubated for 2 hours, APs from the estradiol-treated rats released considerably greater amounts of prolactin (43 and 57%) into the medium per 100 mg of pituitary tissue incubated than APs from control rats (Table 1, Expts. 2, 3). These results indicate that estradiol administration *in vivo* enhances the capacity of the pituitary to release prolactin *in vitro*. Whether the increased release of prolactin demonstrated is due merely to the greater initial content

Table 1. Effect of estradiol injections on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
				AP PROLACTIN CONTENT			
1	-	1	9	3.24	9.21	+187	.01
				PROLACTIN RELEASED INTO MEDIUM			
2	5	5	18	0.49	0.70	+ 43	.05
3	4	4	15	0.94	1.48	+ 57	.01

AP = Anterior pituitary

remains to be determined. Most *in vivo* studies suggest that an increased pituitary prolactin content is associated with increased prolactin release (Meites, 1959).

Effect of Hypothalamic Extract from Estradiol-treated Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from the estradiol-treated rats was added to each experimental flask and an equivalent amount of HE from control rats to each control flask. APs incubated with HE from estradiol-treated rats released an average of 114% more prolactin into the medium than APs incubated with HE from control rats (Table 2, Expts. 1, 2, 3). These results suggest that HE from the estradiol-treated rats has less capacity to inhibit prolactin release than the HE from control rats.

In another series of experiments, medium containing HE from estradiol-treated rats was added to each experimental flask and medium containing no HE was added to each control flask. No significant difference was found in the amount of prolactin released by the APs incubated with HE from estradiol-treated rats as compared to APs incubated in medium containing no HE (Table 2, Expts. 4, 5, 6). When APs were incubated in medium containing HE from control cycling rats, 54% less prolactin was released into the medium than corresponding APs incubated in medium containing no HE (Table 2, Expt. 7). These results indicated that the HE from the estradiol-treated rats had no ability to inhibit prolactin release *in vitro*.

Table 2. Effect of hypothalamic extract from estradiol-injected rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin 1U/100 mg AP Control	Prolactin 1U/100 mg AP Experimental	% Difference	P C vs E
HE FROM CONTROL CYCLING RATS vs HE FROM ESTRADIOL-TREATED RATS							
1	5	3	19	1.32	3.36	+153	.01
2	4	2	13	1.51	2.64	+ 73	.05
3	4	2	15	2.52	5.43	+116	.01
NO HE vs HE FROM ESTRADIOL-TREATED RATS							
4	4	2	15	3.74	3.82	+ 3	NS
5	4	2	16	1.15	1.31	+ 18	NS
6	4	2	14	2.44	2.38	- 4	NS
NO HE vs HE FROM CONTROL CYCLING RATS							
7	4	2	14	2.64	1.21	- 54	.01

AP = Anterior pituitary
HE = Hypothalamic extract
NS = Not Significant

Prolactin Release in Pituitary-Hypothalamus Incubation

Effect of Hypothalamic Pieces on Pituitary Prolactin release *In Vitro*

Hypothalami from control cycling rats were removed and hemisected. Six hypothalami (12 halves) were placed into each experimental flask containing 2 ml of medium 199. No hypothalamic pieces were added to the corresponding control flask. The results (Table 3) do not demonstrate inhibition of prolactin release by addition of hypothalamic pieces, although the findings are highly suggestive of such an effect. Several workers have shown that culturing pituitary tissue with hypothalamic tissue for several days does result in diminished release of prolactin. Cerebral tissue was ineffective. Apparently a factor is released by the hypothalamic tissue *in vitro* which inhibits prolactin release (Pasteels, 1961b; Donan et al., 1963; Gala and Reece, 1964b).

Effect of Adding Estradiol To Pituitary-hypothalamus Incubation on Prolactin Release

Half of each hypothalamus from rats was placed into a control flask and the other half was placed into a similar experimental flask. A total of 6 hypothalami (12 halves) were placed into each flask. Estradiol was added to the medium of the experimental flask from a stock solution of the steriod in absolute ethanol. The final concentration in the medium was 1 μg estradiol per ml in 0.5% ethanol.

Table 3. Effect of hypothalamic pieces on anterior pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
				No HP	HP		
1	2	2	8	1.86	1.59	- 15	NS
2	2	2	8	1.46	1.15	- 21	NS
3	2	2	7	2.12	1.70	- 19	NS

AP = Anterior pituitary
NS = Not Significant
HP = Hypothalamic pieces

The control medium contained an equivalent concentration of ethanol. The data show that the amount of prolactin released by the APs incubated with hypothalamic pieces and estradiol was not significantly different from the amount released by the APs incubated with hypothalamic pieces alone (Table 4, Expts. 1, 2, 3). These results indicate that the dose of estradiol used did not produce an effect on either the hypothalamic or pituitary tissue during a 2-hour incubation.

In order to determine whether estradiol acted directly on the AP, medium containing 1 μg estradiol per ml was added to each experimental flask, and medium containing no estradiol was added to the corresponding control flasks. The results show (Table 4, Expts. 4, 5, 6) that estradiol had no effect on prolactin release into the medium, indicating that the particular dose of estradiol used had no measurable effect on the AP during a 2-hour incubation.

Discussion

In vivo experiments have shown that estrogen can increase pituitary prolactin content and induce mammary growth and lactation in a variety of species (see Meites, 1959). An increase in plasma prolactin activity following estradiol treatment in cycling rats was reported by Wolthuis (1963), using a sensitive prolactin assay method. Other studies have provided evidence that estrogen exerts a

Table 4. Effect of incubating estradiol with anterior pituitary and hypothalamus on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
			AP + HP vs AP + HP + E				
1	4	2	12	1.36	1.50	+ 10	NS
2	5	3	15	1.24	1.11	- 11	NS
3	6	3	18	1.48	1.20	- 19	NS
			AP vs AP + E				
4	4	2	11	1.51	1.67	+ 11	NS
5	4	2	13	2.40	2.00	- 17	NS
6	4	2	13	1.76	1.90	+ 8	NS

AP = Anterior pituitary
HP = Hypothalamic pieces
E = Estradiol
NS = Not Significant

stimulating action on the acidophils of the pituitary (Baker and Everett, 1947; Meyer et al., 1946). Hymer et al. (1961), using the electron microscope, observed dynamic changes in the endoplasmic reticulum of the acidophils during estrogen administration. These morphological findings indicate that estrogen acts in some manner upon these cells to increase prolactin secretion.

Desclin (1950), noted that estrogen promoted acidophil degranulation in pituitary transplants located in the kidney capsule of hypophysectomized rats and induced more intensive luteinization of the ovaries. These results suggested that estrogen acted directly on the AP cells. Kim et al. (1963) examined the effect of estradiol on prolactin production by grafted "mammotropic" pituitary tumor in hypophysectomized rats, and reported that estradiol increased the plasma prolactin level of the tumor-bearing rats. They attributed this to direct stimulation of pituitary tumor prolactin secretion. Similar results were reported by Mizuno et al. (1964) using intact pituitary "mammotropic" tumor grafted rats. Nicoll and Meites (1962a, 1964) demonstrated that estradiol incorporated into a tissue culture medium increased prolactin production by the cultured AP. This study provided further evidence that estrogen can act directly on the pituitary. Although these results were recently confirmed by Ben-David et al. (1964), they were not confirmed by Gala and Reece (1964a).

Kanematsu and Sawyer (1963a) examined the effects of intrahypothalamic and pituitary implants of estradiol on prolactin secretion in rabbits. Implants in the posterior median eminence area of the hypothalamus elevated the AP content but did not initiate lactation. Implants into the AP was followed by activation of the mammary gland and initiation of milk secretion; the pituitary prolactin content was lowered. These workers interpreted their results as indicating that estrogen acted on the hypothalamus to promote synthesis and storage of prolactin, whereas estrogen acted directly on the AP to promote release of prolactin. In rats Ramirez et al. (1963) found that estradiol implants into either the hypothalamus or AP promoted prolactin release.

The results of the present study indicate that estradiol administered *in vivo* suppressed hypothalamic inhibition of prolactin release by depleting the hypothalamus of PIF. A direct effect of estradiol upon hypothalamic tissue *in vitro* was not demonstrated. Possibly a different dose of estradiol or a longer incubation period would produce such a direct effect.

Michael (1962) reported that some systemically administered radioactive hexestrol collects in the hypothalamus. It is therefore possible that some of estradiol injected into our rats entered the hypothalamus. If the estradiol remained in the hypothalamic acid extract, it could have acted directly on the incubated pituitary tissue.

However, when AP was incubated with estradiol, no significant stimulation of prolactin release occurred. This suggests, that under our experimental conditions, estradiol has no measurable effect on AP prolactin release.

Whether the major effect of estrogen is at the hypothalamic or pituitary level, in promoting prolactin release, cannot be ascertained from these experiments. At present, the evidence suggests that estrogen can promote prolactin release by acting both at the hypothalamic and pituitary levels.

Ovariectomy and Pituitary Prolactin Release

Effect of Ovariectomy on Pituitary Prolactin Content and Prolactin Release In Vitro

The experimental animals were ovariectomized and not treated further for 4 weeks. The pituitary prolactin content of these animals was found to be less (26 and 21%) than that of the intact controls (Table 5, Expts. 1, 2). Although these differences were not statistically different, they suggest that ovariectomy results in a reduction in pituitary prolactin content, which is in agreement with the work of Reece and Turner (1937). Ovariectomy did not affect pituitary weight. When APs from ovariectomized and control rats were incubated, it was found that there was no significant difference in the amount of prolactin released into the medium (Table 5, Expts. 3, 4).

Table 5. Effect of ovariectomy on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
				AP PROLACTIN CONTENT			
1	-	1	7	1.37	1.01	- 26	NS
2	-	1	9	1.78	1.39	- 21	NS
				PROLACTIN RELEASED INTO MEDIUM			
3	4	2	16	1.76	1.43	- 19	NS
4	4	2	12	1.43	1.37	- 4	NS

AP = Anterior pituitary
NS = Not Significant

Effect of Hypothalamic Extract from Ovariectomized Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from ovariectomized rats was added to each experimental flask and an equivalent amount of HE from control rats was added to each control flask. The amount of prolactin released by the APs incubated with HE from ovariectomized rats, was found not to be significantly different from the amount released by the APs incubated with HE from control rats (Table 6, Expts. 1, 2, 3). These results suggest that the HE from ovariectomized rats and control rats have similar effects upon pituitary prolactin release *in vitro*.

In another series of experiments, medium containing HE from ovariectomized rats was added to each experimental flask and medium containing no HE was added to each control flask. APs incubated with HE from ovariectomized rats released considerably less prolactin (61 and 56%) than APs incubated without HE (Table 6, Expts. 4, 5). HE from control rats inhibited prolactin release by 45% (Table 6, Expt. 6). These results suggest that ovariectomy did not alter the ability of the hypothalamus to inhibit prolactin release *in vitro*.

Discussion

Ratner and Meites (1964) reported that estrogen administration depleted the hypothalamus of PIF, thus removing hypothalamic inhibition to pituitary prolactin

Table 6. Effect of hypothalamic extract from ovariectomized and intact cycling rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
			HE FROM INTACT CYCLING RATS vs HE FROM OVARIECTOMIZED RATS				
1	3	3	10	1.85	2.31	+ 29	NS
2	4	2	16	1.52	1.40	- 8	NS
3	4	2	14	1.08	0.91	- 16	NS
			NO HE vs HE FROM OVARIECTOMIZED RATS				
4	4	2	15	2.41	0.90	- 61	.01
5	4	2	13	2.83	1.34	- 56	.01
			NO HE vs HE FROM INTACT CYCLING RATS				
6	4	2	11	2.41	1.34	- 45	.05

AP = Anterior pituitary
HE = Hypothalamic extract

NS = Not Significant

release. These results suggested that ovariectomy, which has been shown to decrease pituitary prolactin content (Reece and Turner, 1937; Meites and Turner, 1948; Wolthuis, 1963), may act to reduce prolactin secretion by increasing hypothalamic PIF production. However, the results of the present study provide no evidence that ovariectomy can alter the PIF content of the hypothalamus. It is possible that PIF synthesis and release were increased at an equal rate, and hence elicited no change in hypothalamic PIF content. Further work is necessary to establish more definitely whether ovariectomy can alter PIF synthesis or release.

Pituitary Prolactin Release by Male Rats

Pituitary Prolactin Content and Prolactin Release In Vitro by Anterior Pituitaries from Male and Female Rats

The male and female rats used in this experiment were of the same age (3 months old) and strain (Carworth). The results show that the prolactin content of the male rats was less (40 and 44%) than that of the female rats when expressed on a per 100 mg pituitary weight basis (Table 7, Expts. 1, 2). APs from the male rats weighed 50% less than APs from the female rats. When *in vitro* prolactin release by APs from the male and female rats was compared, it was found that the APs from the male rats released significantly less prolactin per 100 mg AP

Table 7. Pituitary prolaction content and prolactin release *in vitro* by anterior pituitaries from male and female rats.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Female	Prolactin IU/100 mg AP Male	% Difference	P C vs E
				AP PROLACTIN CONTENT			
1	-	1	9	1.46	0.87	- 40	.01
2	-	1	8	1.28	0.72	- 44	.05
				PROLACTIN RELEASED INTO MEDIUM			
3	4	2	15	2.35	0.81	- 66	.01
4	3	3	12	2.04	0.98	- 52	.01

AP = Anterior pituitary

incubated (66 and 52%) than APs from the female rats (Table 7, Expts. 3, 4). These results indicate that AP tissue from male rats has less capacity to release prolactin *in vitro* than AP from female rats. Whether the decreased release of prolactin by the male AP is due merely to the lower initial content remains to be determined.

Effect of Hypothalamic Extract From Male and Female Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from male rats was added to each experimental flask and an equivalent amount of HE from control female rats to each control flask. No significant difference was observed in the amount of prolactin released by APs incubated with HE from male rats as compared to corresponding APs incubated with HE from cycling female rats (Table 8, Expts. 1, 2, 3). These results suggest that the HE from male and female rats have similar effects upon pituitary prolactin release *in vitro*.

In another series of experiments, medium containing HE from male rats was added to each experimental flask and medium without HE was added to each control flask. APs incubated with HE from the male rats released an average of 46% less prolactin than APs incubated without HE (Table 8, Expts. 4, 5). HE from cycling female rats was found to inhibit prolactin release by 42% (Table 8, Expt. 6). These results indicate that HE from male rats can inhibit prolactin release by the AP *in vitro* to the same extent as HE from cycling female rats.

Table 8. Effect of hypothalamic extract from male and female rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
			HE FROM CONTROL CYCLING FEMALE RATS vs HE FROM MALE RATS				
1	4	4	14	1.42	1.51	+ 6	NS
2	4	2	13	1.10	1.21	+ 10	NS
3	3	3	10	1.76	1.40	- 21	NS
			NO HE vs HE FROM MALE RATS				
4	4	2	13	1.65	0.92	- 44	.05
5	3	3	10	2.14	1.12	- 49	.01
			NO HE vs HE FROM FEMALE CYCLING RATS				
6	3	3	10	1.42	0.83	- 42	.05

AP = Anterior pituitary
HE = Hypothalamic extract
NS = Not Significant

Discussion

Prolactin release from heterotopic pituitary grafts in male rats has been reported as indicated by their growth stimulating effects on mammary tissue after estrogen priming (Ahren, 1961) and by a luteotrophic action on transplanted ovaries (Zeilmaker, 1962, 1963). Nicoll and Meites (1962c) reported that APs from male mice and monkeys released considerable quantities of prolactin *in vitro*. These results indicate that the AP from male rats can actively secrete prolactin in the absence of hypothalamic influence and suggest that the hypothalamus may participate in the control of pituitary prolactin secretion in the male. This is of considerable interest since no definite function for prolactin has been established in the male.

The results of the present study indicate that the male hypothalamus contains PIF and that the amount present is not significantly different from that found in the hypothalamus of the female. This is so despite the finding that the male AP contains less prolactin and releases less prolactin *in vitro* than the female rat. This suggests that the hypothalamic content of PIF may not necessarily reflect the amount synthesized or released under all conditions. The differences in pituitary prolactin content between the male and female rat may also reflect differences in the capacity of the AP of the two sexes to secrete this hormone.

Progesterone and Pituitary Prolactin Release

Effect of Progesterone Injections on Pituitary Prolactin Content and Prolactin Release In Vitro

Experimental animals were injected subcutaneously with 4 mg progesterone daily for 10 days, while the control rats were injected with an equal volume of the solvent. The progesterone was diluted with sesame oil and the daily injection volume was 0.1 ml. The results show that progesterone had no significant effect on AP prolactin content (Table 9, Expt. 1). Progesterone administration was also shown not to affect the weight of the pituitary. When APs from progesterone-treated rats and untreated control rats were incubated, it was found that there was no significant difference in the amount of prolactin released into the medium (Table 9, Expts. 2, 3).

Effect of Hypothalamic Extract From Progesterone-treated Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from the progesterone-treated rats was added to each experimental flask and an equivalent amount of HE from control rats to each control flask. The amount of prolactin released by the APs incubated with HE from the progesterone-treated rats was not significantly different from the amount released by the APs incubated with HE from control rats (Table 10, Expts. 1, 2). These results suggest that the HE from progesterone-treated rats

Table 9. Effect of progesterone injections on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
			AP PROLACTIN CONTENT				
1	-	1	9	1.54	1.38	- 10	NS
			PROLACTIN RELEASED INTO MEDIUM				
2	4	2	10	2.36	2.14	- 9	NS
3	4	2	11	1.96	1.88	- 4	NS

AP = Anterior pituitary
NS = Not Significant

Table 10. Effect of hypothalamic extract from progesterone-injected rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
	HE FROM CONTROL CYCLING RATS vs HE FROM PROGESTERONE-INJECTED RATS						
1	4	2	12	3.04	3.24	+ 6	NS
2	4	2	13	1.76	1.54	+ 13	NS
		NO HE vs HE FROM PROGESTERONE-INJECTED RATS					
3	4	2	10	1.89	0.76	- 60	.01
4	4	2	14	1.34	0.66	- 50	.01
		NO HE vs HE FROM CONTROL CYCLING RATS					
5	4	2	12	2.24	1.08	- 54	.01

AP = Anterior pituitary
HE = Hypothalamic extract
NS = Not Significant

and control rats have similar effects upon pituitary prolactin release in vitro.

In another series of experiments, medium containing HE from the progesterone-treated rats was added to each experimental flask and medium containing no HE was added to each control flask. APs incubated with HE from the progesterone-treated rats released considerably less prolactin (60 and 50%) than the APs incubated without HE (Table 10, Expts. 3, 4). Similar results were obtained with HE from control cycling rats (Table 10, Expt. 5). These results indicate that the HE from progesterone-treated rats retained its ability to inhibit pituitary prolactin release in vitro.

Discussion

Progesterone is reported to moderately stimulate prolactin secretion in rats in vivo. Administration of large doses of progesterone increased prolactin levels in the pituitary (Meites and Turner, 1948) and initiated mammary secretion in rats (Reece and Bivens, 1942). Rothchild (1960b) reported that daily injection of progesterone in amounts large enough to suppress estrus, results in the persistence of large corpora lutea whose size and appearance indicated functional status. Rothchild (1960b) proposed that the progesterone-prolactin relationship in the rat is that of a positive feedback mechanism, which is unlike the feedback mechanism operative between the AP and other target organ hormones.

A single injection of 10 mg progesterone on the day of estrus (Rothchild, 1963), or daily injections of 2 mg beginning on the day of estrus (Everett, 1963), induced pseudopregnancy of the usual 2 weeks duration. Similar results followed daily treatment with prolactin for 4 days beginning on the day of estrus (Alloiteau and Vignal, 1958). These results suggest that pseudopregnancy is self-sustaining, theoretically because intrinsic progesterone from the corpora lutea continues to stimulate prolactin through a positive feedback system. Whether this was accomplished by a direct action of progesterone on the pituitary or through a hypothalamic mechanism has not been determined.

Nicoll and Meites (1964) cultured rat AP with progesterone in an attempt to determine if it had any direct effect on the AP. Progesterone did not influence prolactin production. They suggested that a hypothalamic mediated effect on the AP may explain the action of progesterone on prolactin secretion *in vivo*.

The results presented in the present study suggest that progesterone has no effect on the PIF content of the rat hypothalamus. Ben-David et al. (1964) similarly reported that progesterone had no effect on hypothalamic inhibition of prolactin release by rat AP, when added to a pituitary-hypothalamus co-culture. However, in the present study progesterone did not increase pituitary prolactin content, as has been reported by Reece and Bivens (1942) and Meites and Turner (1948). The 4 mg daily dose of progesterone may have been insufficient fo influence prolactin secretion, and hence the present results cannot be considered conclusive.

There is some evidence that progesterone can influence gonadotropin release by acting at the hypothalamic level (Ralph and Fraps, 1960). Indirect evidence for a hypothalamic mechanism by which progesterone influences prolactin secretion has recently been reported (Barraclough and Cross, 1963).

Hypothyroidism and Pituitary Prolactin Release

Effect of Tapazole Injections on Pituitary Prolactin Content and Prolactin Release In Vitro

Experimental rats were injected subcutaneously with 5 mg of tapazole for 30 days. The tapazole was dissolved in saline and made up to give a concentration of 5 mg per 0.1 ml. The control animals were injected with an equal volume of saline. The data show that tapazole treatment did not significantly alter pituitary prolactin content (Table 11, Expts. 1, 2). No effect upon the weight of the AP was noted. When APs from the tapazole-treated rats and control rats were incubated, it was found that there were no significant differences in the amounts of prolactin released into the medium (Table 11, Expts. 3, 4). These results indicate that tapazole-treatment did not alter the capacity of the pituitary to release prolactin *in vitro*.

Table 11. Effect of tapazole injections on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
				AP PROLACTIN CONTENT			
1	-	1	7	1.52	1.16	- 24	NS
2	-	1	8	1.21	1.01	- 17	NS
				PROLACTIN RELEASED INTO MEDIUM			
3	3	3	9	1.84	1.76	- 4	NS
4	4	2	13	1.41	1.68	+ 19	NS

AP = Anterior pituitary
NS = Not significant

Effect of Hypothalamic Extract from Tapazole-treated Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from the tapazole-treated rats was added to each experimental flask and an equivalent amount of HE from control rats to each control flask. The data show that the amount of prolactin released by APs incubated with HE from tapazole-treated rats was not significantly different from the amount released by the APs incubated with HE from control rats (Table 12, Expts. 1, 2). These results suggest that HE from tapazole-treated rats and control rats have similar effects upon pituitary prolactin release *in vitro*.

In another series of experiments, medium containing HE from tapazole-treated rats was added to each experimental flask and medium without HE was added to each control flask. APs incubated with HE from tapazole-treated rats released an average of 54% less prolactin than the APs incubated without HE (Table 12, Expts. 3, 4). Similar results were obtained with HE from control cycling rats (Table 12, Expt. 5). These results suggest that tapazole-treatment did not alter the capacity of the hypothalamus to inhibit prolactin release *in vitro*.

Discussion

Several experimental observations indicate that thyroid hormones can influence prolactin secretion. Hypothyroidism, when induced by thyroidectomy or thiouracil

Table 12. Effect of hypothalamic extract from tapazole-injected rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
	HE FROM CONTROL CYCLING RATS vs HE FROM TAPAZOLE-TREATED RATS						
1	4	2	12	2.24	2.43	+ 8	NS
2	4	2	13	2.10	1.92	- 9	NS
		NO HE vs HE FROM TAPAZOLE-TREATED RATS					
3	3	3	10	1.84	1.21	- 63	.01
4	4	2	11	1.49	0.84	- 44	.01
		NO HE vs HE FROM CONTROL CYCLING RATS					
5	4	2	12	1.76	0.79	- 55	.01

AP = Anterior pituitary
HE = Hypothalamic extract
NS = Not Significant

treatment, elicits a marked reduction in the number and degree of granulation of the acidophils. These cytological changes can be reversed by thyroxine replacement therapy (see Purvis, 1961). Thyroidectomy reduced the pituitary prolactin content of the male rat (McQueen-Williams, 1935) and thiouracil treatment produced a similar effect in the female rat (Meites and Turner, 1947). Grosvenor (1961) reported that the pituitary prolactin content of rats, which were rendered hypothyroid by tapazole treatment, was reduced below that of normal control animals. Administration of thyroxine to these hypothyroid rats elevated the AP prolactin levels above the control values.

It can be inferred from these in vivo studies that thyroid hormones increase prolactin secretion, but more direct and definite evidence was supplied by Nicoll and Meites (1963). These workers demonstrated that thyroxine and triiodothyronine significantly increased prolactin release by rat pituitary cultured in vitro. This evidence indicated that thyroid hormones can influence prolactin secretion by an action at the pituitary level. Other studies also indicate that thyroid hormones influence TSH secretion by an action at the pituitary level (see D'Angelo, 1963). However, hypothalamic involvement cannot be excluded.

The results of the present study suggest that tapazole treatment, under the experimental conditions, had no effect on the PIF content of the hypothalamus. However, the finding that the particular dose of tapazole employed did not significantly reduce pituitary prolactin content, suggests that further work is necessary to establish whether hypothyroidism can alter hypothalamic PIF content.

Suckling and Pituitary Prolactin Release

Effect of Suckling on Pituitary Prolactin Content and Prolactin Release In Vitro

Experimental rats were lactating and kept with their litters constantly until killed on day 12-18 postpartum. The data show that the APs from the suckled rats contained considerably greater amounts of prolactin (110 and 80%) than APs from control cycling rats (Table 13, Expts. 1, 2). There was no significant difference in the weight of the APs from both groups. When prolactin release by the APs *in vitro* was compared, it was found that the APs from the suckled rats released considerably more prolactin (77 and 151%) into the medium than the APs from control cycling rats (Table 13, Expts. 3, 4). These results indicate that suckling enhances the capacity of the AP to release prolactin *in vitro*.

Effect of Hypothalamic Extract from Suckled Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from suckled rats was added to each experimental flask and an equivalent amount of HE from control rats to each control flask. The APs incubated with

Table 13. Effect of suckling on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
			AP PROLACTIN CONTENT				
1	-	1	8	1.81	3.80	+110	.01
2	-	1	7	1.31	2.36	+ 80	.01
			PROLACTIN RELEASED INTO MEDIUM				
3	6	3	15	1.24	2.19	+ 77	.01
4	4	2	13	0.88	2.21	+151	.01

AP = Anterior pituitary

HE from suckled rats released an average of 133% more prolactin into the medium than corresponding APs incubated with HE from control rats (Table 14, Expts. 1, 2, 3). These results suggest that HE from suckled rats has less capacity to inhibit prolactin release than HE from control rats.

In another series of experiments, medium containing HE from suckled rats was added to each experimental flask and medium without HE was added to each control flask. No significant difference was found in the amount of prolactin released by the APs incubated with HE from suckled rats as compared to APs incubated in medium containing no HE (Table 14, Expts. 4, 5, 6). HE from control cycling rats reduced prolactin release by 47% (Table 14, Expt. 7). These results indicate that suckling depleted the hypothalamus of its capacity to inhibit prolactin release *in vitro*.

Discussion

The first experimental indication that an exteroceptive stimulus influences prolactin secretion emerged from the observations of Selye (1934). He found that continued suckline of rats with ligated galactophores maintained the secretory activity and structural integrity of their mammary glands. This led to the suggestion that prolactin was released in response to suckling, and this has since been confirmed experimentally in several species. Suckling promotes mammary growth, initiates lactation and

Table 14. Effect of hypothalamic extract from suckled and from control cycling rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
HE FROM CONTROL CYCLING RATS vs HE FROM SUCKLED RATS							
1	6	3	22	1.13	2.66	+136	.001
2	3	3	13	0.82	2.24	+175	.001
3	4	2	14	1.71	3.25	+ 88	.01
NO HE vs HE FROM SUCKLED RATS							
4	4	2	11	1.67	1.54	- 6	NS
5	6	2	14	1.12	1.21	+ 9	NS
6	4	2	13	1.65	1.37	- 19	NS
NO HE vs HE FROM CONTROL CYCLING RATS							
7	4	2	15	3.00	1.64	- 47	.05

AP = Anterior pituitary
HE = Hypothalamic extract
NS = Not Significant

retards mammary involution in laboratory animals (see Meites, 1963). More direct evidence that suckling stimulates prolactin secretion was provided by studies on the effects of suckling on pituitary prolactin content. Grosvenor and Turner (1957) showed that a brief period of suckling results in a rapid discharge of prolactin from the pituitary. Regular suckling maintains pituitary prolactin content at higher than normal levels (Meites and Turner, 1948). The observation that suckling elicits degranulation of AP acidophils is further evidence that this stimulus elicits prolactin release (Desclin, 1947). Additional evidence that stimulation of sensory receptors in the mammary gland can influence prolactin secretion, as judged by mammary function, came from studies showing that involution of the mammary glands was retarded by repeated application of turpentine to the nipples of lactating rats from which litters were removed (Mixner and Turner, 1941), and that electrical stimulation of the nipples of estrogen-primed rats initiated mammary secretion (Maqsood and Meites, 1961).

Since the suckling stimulus stimulates the nerve endings in the nipples, it is reasonable to assume that its action is mediated through a neural circuit ending in the central nervous system. The importance of neural pathways in the suckling stimulus of rats was emphasized by the work of Eayrs and Baddely (1957).

It has been suggested that oxytocin, which is reflexly discharged by the suckling stimulus, may be responsible for inducing prolactin release from the pituitary (Benson and Folley, 1957). However, most of the recent evidence from *in vivo* and *in vitro* experiments do not support this concept (Meites et al., 1963; Nicoll and Meites, 1962b). The results of the present study suggest that the suckling stimulus promotes pituitary prolactin release by decreasing the production of PIF in the hypothalamus.

Reserpine and Pituitary Prolactin Release

Effect of Reserpine Injections on Pituitary Prolactin Content and Prolactin Release In Vitro

Experimental rats received daily subcutaneous injections of 50 μg of reserpine for 10 days. The reserpine was dissolved in a few drops of concentrated sulfuric acid and diluted with saline to reach a concentration of 50 μg per 0.1 ml. The control animals were injected with an equal volume of the solvent. The data show that reserpine did not significantly affect pituitary prolactin content when expressed on a 100 mg pituitary weight basis (Table 15, Expts. 1, 2). Pituitary weight was reduced by some 15% but this could be attributed to the loss of body weight exhibited by the reserpine-treated rats. When APs from reserpine-treated rats and control rats were incubated, it was found that there was no

Table 15. Effect of reserpine injections on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
			AP PROLACTIN CONTENT				
1	-	1	7	1.82	2.14	+ 18	NS
2	-	1	7	1.64	1.43	- 13	NS
			PROLACTIN RELEASED INTO MEDIUM				
3	4	2	13	1.92	2.24	+ 17	NS
4	4	2	11	1.53	1.64	+ 7	NS

AP = Anterior pituitary
NS = Not Significant

significant difference in the amount of prolactin released into the medium (Table 15, Expts. 3, 4). These results suggest that the dose of reserpine used did not alter pituitary prolactin content or the capacity of the pituitary to release prolactin *in vitro*.

Effect of Hypothalamic Extract from Reserpine-treated Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from the reserpine-treated rats was added to each experimental flask and an equivalent amount of HE from control rats was added to each control flask. APs incubated with HE from reserpine-treated rats released an average of 139% more prolactin into the medium than APs incubated with HE from control rats (Table 16, Expts. 1, 2, 3). These results indicate that HE from the reserpine-treated rats has less ability to inhibit prolactin release than HE from the control rats.

In another series of experiments, medium containing HE from reserpine-treated rats was added to each experimental flask and medium without HE was added to each control flask. No significant difference was found in the amount of prolactin released by the APs incubated with HE from reserpine-treated rats as compared to APs incubated in medium without HE (Table 16, Expts. 4, 5). APs incubated in medium containing HE from control cycling rats released 54% less prolactin than corresponding APs incubated in medium without HE (Table 16, Expt. 6). These results

Table 16. Effect of hypothalamic extract from reserpine-injected rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP Control	Prolactin IU/100 mg AP Experimental	% Difference	P C vs E
HE FROM CONTROL CYCLING RATS vs HE FROM RESERPINE-TREATED RATS							
1	4	2	13	1.35	2.88	+113	.01
2	3	3	10	1.40	3.12	+123	.01
3	3	3	11	1.29	3.63	+181	.01
NO HE vs HE FROM RESERPINE-TREATED RATS							
4	4	2	11	2.84	2.85	0	NS
5	4	2	12	1.90	2.30	+ 21	NS
NO HE vs HE FROM CONTROL CYCLING RATS							
6	4	2	13	2.60	1.20	- 54	.01

AP = Anterior pituitary
HE = Hypothalamic extract
NS = Not Significant

indicate that reserpine depleted the hypothalamus of its ability to inhibit prolactin release *in vitro*.

Effect of Adding Reserpine to Pituitary-hypothalamus Incubation on Prolactin Release

Medium containing 10 µg of reserpine per ml was added to each experimental flask. The reserpine was dissolved in a drop of sulfuric acid and diluted to the proper concentration and pH with medium 199. An equivalent amount of sulfuric acid was added to the control medium. APs incubated with hypothalamic pieces and reserpine released an average of 87% more prolactin into the medium than the corresponding APs incubated with hypothalamic pieces without reserpine (Table 17, Expts. 1, 2, 3). These results indicate that reserpine acted on the pituitary or hypothalamic tissue to cause an increase in prolactin release.

In order to determine whether reserpine acted directly on the AP, medium containing 10 µg reserpine was added to each experimental flask and medium containing no reserpine was added to each control flask. The results show that reserpine had no significant effect on prolactin release into the medium (Table 17, Expts. 4, 5, 6), indicating that reserpine did not act directly on the AP tissue. The effect of reserpine, thus, can probably be attributed to a direct effect upon the hypothalamic tissue.

Table 17. **Effect of incubating** reserpine with anterior pituitary and hypothalamus on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
				AP + HP vs AP + HP + R			
1	3	3	10	1.05	1.91	+ 83	.05
2	2	1	7	0.97	1.86	+ 92	.05
3	2	1	9	0.71	1.31	+ 85	.05
				AP vs AP + R			
4	3	3	11	1.20	0.78	- 35	NS
5	2	1	7	1.47	0.88	- 40	NS
6	3	1	7	3.22	2.39	- 26	NS

AP = Anterior pituitary
HP = Hypothalamic pieces
R = Reserpine
NS = Not Significant

Discussion

Reserpine has been shown to be effective in promoting prolactin release, as indicated by its ability to elicit mammary growth and lactation in a variety of species, and to induce pseudopregnancy in rats and retard mammary involution in pospartum rats after litter removal (see Meites, 1963). Studies on the effect of reserpine upon AP prolactin content have been inconclusive. Kanematsu et al. (1961) demonstrated that reserpine produced depletion of pituitary prolactin content 3 days after a single injection into rabbits, whereas Meites (1958) found an increase 7 days after injection. In the lactating rat, Moon and Turner (1959) reported that pituitary prolactin fell to 52% of the prenursing level following reserpine treatment. Gala and Reece (1963) reported that reserpine treatment for 10 days increased pituitary prolactin content of rats, but a decrease was observed when rats were sacrificed just 2 hours after a single injection. The apparent differences in pituitary prolactin response to reserpine treatment might be attributed to the injection dose, time sequence and species difference. Pasteels (1961a) examined the rat AP cyctochemically and noted that injections of reserpine resulted in acidophil cell stimulation, providing indirect evidence that reserpine acts to promote prolactin secretion.

Reserpine is presumed to have a depressing effect on the central nervous system, particularly the hypothalamus

(Bein, 1956), and it was suggested that its action upon prolactin secretion may depend on its ability to inhibit hypothalamic function (Gaunt et al. 1954). Reserpine has been observed to increase mammary secretion in hypophysectomized rats with single pituitary grafts under the kidney capsule (Desclin, 1960). Meites et al. (1963) reported similar results and suggested two possible mechanisms through which reserpine could have acted: (1) reserpine may have stimulated the pituitary directly to increase release of prolactin, (2) reserpine may have acted upon the hypothalamus to inhibit the release of a prolactin-inhibiting neurohumor which was otherwise acting on the pituitary graft through the systemic circulation.

The possibility that reserpine may stimulate the release of pituitary prolactin via a central nervous control mechanism was suggested by Sawyer (1957), following the observation that a minute amount of reserpine, too small to evoke the effect when administered systemically, could induce mammary gland activation when injected into the third cerebral ventricle. Kanematsu and Sawyer (1963b) showed that direct implantation of reserpine into the posterior median eminence-basal tuberal area of the hypothalamus initiated milk secretion and depleted the pituitary of prolactin in the estrogen-primed ovariectomized rabbit. Reserpine implantation into the AP had no effect. Placement of lesions in this area of the hypothalamus was similarly reported to initiate lactation (Haun and Sawyer, 1961; Kanematsu et al. 1963a). In animals with such a lesion, administration of reserpine exerted no further effects on pituitary prolactin release or mammary gland activation (Kanematsu et al. 1963b). These workers suggested that reserpine may act like a functional lesion in overcoming the hypothalamic mechanism which inhibits prolactin secretion. Evidence indicating that the focus of reserpine influence is the posterior basal tuberal region of the hypothalamus, was provided in these studies. Support of this concept was also provided by Ifft (1962), who reported that reserpine depressed nerve cell activity in this area of the hypothalamus.

The results presented in this study indicate that reserpine acts upon the hypothalamus to decrease the production of PIF. Reserpine depleted the hypothalamus of PIF when administered *in vivo*, and decreased hypothalamic inhibition of prolactin release when added *in vitro*. No direct effect upon AP prolactin release was noted. Danon et al. (1963) reported that a phenothiazine tranquilizer, perphenazine, acted similarly to block the inhibitory effect of hypothalamic tissue on rat pituitary prolactin release *in vitro*. Phenothiazine tranquilizers, like reserpine, can induce prolactin release and initiate mammary secretion (Barraclough and Sawyer, 1959; Talwalker et al., 1960).

Reserpine has been reported to deplete brain serotonin and norepinephrine levels (Page, 1958; Shore, 1962).

Both agents were found to elicit prolactin release *in vivo* and to initiate mammary secretion in estrogen-primed rats and rabbits (Meites, 1961). Conceivably serotonin, norepinephrine and related neuropharmacological agents can act at the hypothalamic level or directly on the pituitary to elicit prolactin release. However, Talwalker et al. (1963) observed that serotonin and norepinephrine did not induce prolactin release when added to rat AP *in vitro*. Preliminary investigations suggest that these agents may act on the hypothalamus to decrease production of PIF (Mittler and Meites, unpublished).

Cervical Stimulation and Pituitary Prolactin Release

Effect of Cervical Stimulation on Pituitary Prolactin Content and Prolactin Release In Vitro

Electrical stimulation was conveyed to the cervix of experimental rats by platinum electrodes which were insulated except for the tips. A current of 200 millivolts was supplied by an Electrodyne Stimulator set at a frequency of 20 cycles/second for 30 seconds. The rats were stimulated a second time 30 minutes after the first stimulation and killed 30 minutes after the second stimulation. The data show that cervical stimulation had no effect on AP prolactin content (Table 18, Expts. 1, 2). When APs from the cervically stimulated and control rats were incubated, it was found that the APs from the

Table 18. Effect of cervical stimulation on anterior pituitary prolactin content and on prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
				AP PROLACTIN CONTENT			
1	-	1	8	1.21	1.37	+ 13	NS
2	-	1	8	1.52	1.36	- 10	NS
				PROLACTIN RELEASED INTO MEDIUM			
3	4	2	14	1.17	1.94	+ 66	.01
4	4	2	12	1.03	1.74	+ 69	.05
5	4	2	12	1.19	3.10	+160	.01

AP = Anterior pituitary
NS = Not Significant

stimulated rats released considerably greater amounts of prolactin (66, 69 and 160%) per 100 mg pituitary tissue incubated than APs from control cycling rats (Table 18, Expts. 3, 4, 5). These results suggest that cervical stimulation increased the capacity of the pituitary to release prolactin *in vitro*. The increased release cannot be attributed to an increase in prolactin content, since pituitary prolactin content was found not to be significantly different. It is recognized, however, that pituitary prolactin content may not always be indicative of the amount of hormone released.

Effect of Hypothalamic Extracts from Cervically-stimulated Rats on Pituitary Prolactin Release In Vitro

Medium containing HE from cervically-stimulated rats was added to each experimental flask and an equivalent amount of HE from control rats was added to each control flask. The data show that APs incubated with HE from cervically-stimulated rats released an average of 157% more prolactin into the medium than APs incubated with HE from control rats (Table 19, Expts. 1, 2, 3). These results suggest that HE from stimulated rats has less capacity to inhibit prolactin release than HE from control rats.

In another series of experiments,medium containing HE from cervically-stimulated rats was added to each experimental flask and medium without HE was added to each control flask. APs incubation with HE from stimulated

Table 19. Effect of hypothalamic extract from cervically-stimulated rats on pituitary prolactin release *in vitro*.

Experiment No.	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
	HE FROM CONTROL CYCLING RATS vs HE FROM CERVICALLY STIMULATED RATS						
1	4	2	10	0.72	2.05	+184	.001
2	4	2	15	1.30	3.32	+155	.005
3	5	3	16	0.80	1.87	+133	.005
	NO HE vs HE FROM CERVICALLY STIMULATED RATS						
4	4	2	12	0.63	3.32	+427	.001
5	4	2	13	1.03	4.40	+327	.001
6	4	2	16	1.90	8.44	+344	.001
	NO HE vs HE FROM CONTROL CYCLING RATS						
7	4	2	14	1.43	0.56	- 61	.01

AP = Anterior pituitary
HE = Hypothalamic extract

rats released an average of 388% more prolactin than APs incubated without HE (Table 19, Expts. 4, 5, 6). HE from control cycling rats reduced prolactin release by 61% (Table 19, Expt. 7). These results suggest that HE from cervically-stimulated rats may stimulate prolactin release by rat pituitary *in vitro*.

Discussion

Electrical stimulation of the uterine cervix can induce pseudopregnancy (Meites, 1959) and initiate lactation in rats (Meites et al., 1959). It was suggested (Meites et al., 1959) that these effects may be mediated through the central nervous system, resulting in prolactin release from the pituitary. The findings that section of the pelvic nerves (Kollar, 1952) and spinal anesthesia (Meyer et al., 1929) bring about complete blockage of the pseudopregnancy response to cervical stimulation adds support for the participation of the central nervous system.

The results of the present study demonstrate that cervical stimulation does act at the hypothalamic level. HE from the cervix stimulated rats was shown to stimulate prolactin release by rat AP *in vitro*. This suggests that the hypothalamus from these rats stimulated prolactin release by the rat AP. This requires confirmation.

Recently, Kragt and Meites (1965) demonstrated that hypothalamus of parent pigeons contains prolactin stimulating activity and presumably a prolactin stimulating factor (PSF). Their results also suggest that the PIF of the rat hypothalamus and the PSF of the pigeon hypothalamus are not the same, since prolactin release by the AP from either of these two species could not be altered by HE from the other species.

Grosvenor and Turner (1957) reported that in lactating rats isolated from their litters for several hours, acute depletion of pituitary prolactin occurred within 30 minutes after suckling. The present study shows that the AP had a greater capacity to release prolactin *in vitro* 30 minutes after cervical stimulation. The results of these experiments can be explained in part on the basis of a withdrawal of the PIF factor. However, this does not entirely explain the apparent large increase in prolactin release following cervical stimulation.

Control of pituitary prolactin by a dual mechanism would seem to allow for a more efficient regulation of its secretion than would a single regulating mechanism. Proof, however, that a stimulating factor is contained in the hypothalamus of normal cycling mammals will require isolation and identification of the factor and establishment of its specificity. It should be noted, however, that no other stimulus except cervical stimulation of the uterine cervix has been reported to result in hypothalamic stimulation of AP prolactin release. Further work is necessary to resolve this problem.

Effect of Corticotropin Releasing Factor (CRF) on Pituitary Prolactin Release In Vitro

Several purified CRF preparations were supplied by Dr. M. Saffran (McGill University, Montreal, Canada). The rat CRF preparations were of stalk-median eminence origin and were partially purified by acid extraction. Each CRF preparation was added to medium 199 and made up so that each ml contained an equivalent of 1 rat hypothalamus. The medium containing CRF was incubated together with 6 AP halves in an experimental flask, and an equal number of AP halves from the same 6 rats were incubated in medium 199 without CRF in a corresponding control flask. The data (Table 20) show that the addition of CRF to the incubation medium did not affect prolactin release by the AP. These results suggest that CRF has no effect on pituitary prolactin release *in vitro*.

Discussion

Several workers have demonstrated that hypothalamic extracts can induce release of adrenocorticotropic hormone (ACTH) from the AP (see Ganong, 1963). This factor, designated as corticotropin-releasing factor (CRF) has been purified and its amino acid sequence has been reported (see Guillemin and Schally, 1963).

Numerous drugs and stressful stimuli have been shown to initiate lactation in the estrogen-primed rat (see Meites, 1963). These drugs and stimuli are believed

Table 20. Effect of corticotropin releasing factor (CRF) on anterior pituitary prolactin release *in vitro*.

Preparation No.	Dose CRF Hypothalami/cc	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
					Control	Experimental		
1	1	4	4	16	1.32	1.39	+ 5	NS
2	1	2	2	10	1.01	1.21	+ 20	NS

AP = Anterior pituitary
NS = Not Significant

to induce release of prolactin and probably ACTH from the AP, since the administration of prolactin alone is ineffective in initiating lactation in intact rats and requires the addition of ACTH or glucocorticoids (Reece, 1939). Meites et al. (1960) noted that injection of hypothalamic tissue could initiate mammary secretion in estrogen-primed rats. Presumably this tissue induced discharge of prolactin and probably also ACTH from the pituitary. A crude CRF preparation was also shown to elicit secretion in the estrogen-primed rat (Meites et al., 1963), suggesting that this factor induced the release of both prolactin and ACTH. The exact mechanism whereby this occurred was unknown. It is possible that the primary action of the hypothalamic tissue and CRF was on ACTH release, resulting in adrenal cortical stimulation, and this in turn elicited release of prolactin from the pituitary. In the present study, crude CRF preparations did not have any effect on prolactin release by the rat AP *in vitro*, suggesting that CRF does not contain prolactin-stimulating activity.

Effect of Luteinizing Hormone-Releasing Factor (LRF) on Pituitary Prolactin Release In Vitro

Does LRF Contain PIF Activity?

Purified LRF preparations were supplied by Dr. A. V. Schally (Veterans Administration Hospital, New Orleans, La.). Purification of LRF from acid extract of bovine and ovine median eminence tissue was performed by gel filtration on Sephadex G-25. Each LRF preparation was added to medium 199 and incubated together with 6 AP halves in an experimental flask. An equal number of AP halves from the same 6 rats were incubated without LRF in a corresponding control flask. The dosages of the LRF preparations are given in Table 21.

Addition of the LRF preparations to the incubation medium did not significantly affect prolactin release by the isolated rat pituitary (Table 21). The LRF preparations were tested for LH releasing activity and were found to be active, using both in vivo and in vitro procedures (Schally et al., 1964). In some cases the doses tested on pituitary prolactin release were 10 times larger than doses found active in the LRF tests. These results suggest that LRF does not contain PIF activity, and that they are separate factors.

Discussion

Several workers have demonstrated that HE can induce release of luteinizing hormone (LH) from the anterior pituitary (see McCann et al., 1964). This factor, designated as luteinizing hormone releasing factor (LRF), has been partially purified, and has been shown to be active in vitro as well as in vivo (Schally and Bowers, 1964; Guillemin, 1964).

There is considerable evidence for a reciprocal relationship between the secretion of FSH and LH on the

Table 21. **Effect of luteinizing hormone-releasing factor (LRF) on anterior pituitary prolactin release *in vitro*.**

Preparation No.	Dose LRF μg	No. of Flask Pairs	No. of Assays	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
					Control	Experimental		
1-Ovine	2300	2	1	6	1.93	1.76	- 9	NS
2-Ovine	12000	2	1	7	1.06	1.68	+ 68	NS
	6000	2	1	7	1.93	3.42	+ 78	NS
3-Bovine	4800	2	1	7	1.12	2.87	+158	NS
4-Bovine	1500	2	1	8	1.31	1.55	+ 18	NS

AP = Anterior pituitary
NS = Not Significant

one hand, and prolactin on the other hand. Thus, pituitary stalk section (Nikitovitch-Winer, 1957), transplantation of the pituitary (Everett, 1956), placement of appropriate hypothalamic lesions (Haun and Sawyer, 1960) or implantation of estrogen into the hypothalamus (Ramirez et al., 1963) promote prolactin release but inhibit gonadotropin release. Gonadotropin release has also been shown to be depressed during lactation and psuedopregnancy (Greenwald, 1962; Rothchild, 1960a). It has been suggested (Everett, 1956) that the same neurohumoral agent which stimulates gonadotropin secretion may inhibit the secretion of prolactin.

Haun and Sawyer (1960) placed lesions in several regions of the hypothalamus of ovariectomized rabbits whose mammary glands had been developed by estrogen treatment. Lactation occurred only in animals with lesions in the basal tuberal region, suggesting that this area may inhibit release of prolactin. Taleisnik and McCann (1961) reported similar results in the rat. Since this same area apparently controls the release of LH, they suggested, in agreement with the view of Everett (1956) that a single neurohumoral factor may stimulate LH release and inhibit prolactin release.

The results of the present study indicate that LRF preparations do not inhibit prolactin release by the isolated rat pituitary. This suggests that LRF and PIF are not the same. Prolactin release appears to be controlled independently of the gonadotropins. Everett (1964) has recently provided additional physiological evidence that LH and prolactin release can occur independently of each other.

Effect of Intracarotid Injection of Hypothalamic Extract on Pituitary Prolactin Content

Experimental rats were anesthetized with ether and injected into the left common carotid artery with 1 ml of HE from control cycling rats. The HE was prepared as described under materials and methods. Thirty minutes after injection the AP was removed, weighed and placed in a freezer at -20° C until ready for assay. The dose levels are given in Table 22. Control rats were injected with an equal volume of medium 199. APs from rats injected with HE were injected over one side of the crop sac and APs from control rats were injected over the other side in the same birds. This provided a direct comparison between the two samples.

Prolactin content of APs from rats injected with HE was not found to be significantly different from the content of control rats (Table 22). These results indicated that HE injected intracarotidly had no effect on pituitary prolactin content.

Discussion

The demonstration that HE can inhibit prolactin release by isolated AP indicated that the hypothalamus

Table 22. Effect of intracarotid injection of hypothalamic extract on anterior pituitary prolactin content.

Experiment No.	Dose Hypothalami/Rat	No. of Rats	No. of Pigeons	Prolactin IU/100 mg AP		% Difference	P C vs E
				Control	Experimental		
1	2	3	10	1.42	1.31	- 8	NS
2	4	3	8	1.21	1.46	+ 21	NS
3	4	4	12	1.63	1.74	+ 7	NS

AP = Anterior pituitary
NS = Not Significant

contains a prolactin-inhibiting factor (Talwalker et al., 1963). Such _in vitro_ evidence is particularly worthwhile in that it demonstrates a direct effect of the agent under well controlled conditions. But many workers believe that _in vitro_ evidence does not supply proof of a particular effect unless it can also be duplicated _in vivo_.

In the present study, intracarotid injections of HE did not alter pituitary prolactin content. Prolactin release may have been inhibited, but this could not be demonstrated by assaying AP prolactin content. The intracarotid procedure has been used by Pecile et al. (1964) and Meites and Fiel (1965) to provide _in vivo_ evidence for a somatotropin releasing factor. Wolthuis (1963) has developed a sensitive blood assay for prolactin using the number of corpus luteal cell nuclei as an end point. Possibly, such a blood assay might be used to demonstrate an _in vivo_ inhibiting effect by HE on prolactin release. Recently, Grosvenor et al. (1964) reported that an injection of HE, given shortly before mother rats were allowed to suckle their young, prevented the depletion of prolactin from the AP which occurs within 30 minutes after suckling. These results indicate that the hypothalamus contains a factor which inhibits the release of prolactin _in vivo_ as well as _in vitro_.

GENERAL DISCUSSION

It is apparent from these studies that certain agents and stimuli, which have been shown to alter prolactin secretion *in vivo*, can induce changes in the prolactin-inhibiting activity of the hypothalamus. Alterations in content of hypothalamic prolactin-inhibiting factor (PIF) were shown to be in agreement with parallel changes in prolactin secretion under most but not all conditions. These observations provide evidence for the physiological role of this particular neurohumoral factor.

In these studies PIF content has been used as an indicator of hypothalamic activity, but it should be understood that PIF content *per se* merely reflects the differential between the rate of synthesis and release of the factor. If there is no change in factor content, one cannot state with complete certainty that there is no change in factor synthesis or release. Nor is a decrease in factor content necessarily indicative of reduced factor synthesis--it may actually reflect increased release. The assay of hypothalamic concentration of a particular factor is believed to be of greatest physiological significance when it can be co-related with pituitary hormone content and release into the circulation. A more convincing indicator of factor release would be a measure of the concentration of the factor in the portal vessel blood or general circulation. CRF activity has been assayed in the blood (Brodish, 1960; Eik-Ness and Brizzee, 1958) but PIF activity has not yet been detected in blood. In the present studies, prolactin release *in vitro* was shown in most cases to correlate well with AP content, with the exception of APs from cervically-stimulated rats. Pituitary prolactin content of these rats was not shown to be significantly different from control cycling rats; yet they released considerably more prolactin *in vitro*. Thus, the capacity to release prolactin *in vitro*, does not always correlate with gland content. Van Rees (1961) using a similar incubation procedure to the one employed here observed that APs from male rats released relatively less FSH than APs from female rats, even though they contained more FSH. Estradiol administration reduced pituitary FSH content, yet the APs released more FSH *in vitro*. These observations suggest, that in some cases, the capacity of the AP to release hormones *in vitro* may be indicative of AP hormone release *in vivo*.

Several recent studies have provided other proof that the production of neurohumoral factors can be altered. Maszkowska (1961) studied the effects of hypothalamic tissue on pituitary LH release *in vitro*. Hypothalami from adult female rats were more potent than those from male donors. Castration rendered the female hypothalamus less effective, while the same operation greatly enhanced the capacity of the male hypothalamus to effect LH release. A reversal of the effects of male and female hypothalamic explants was noted after the administration of testosterone and prolonged illumination. These data require confirmation. Kobayashi et al. (1963) reported an increase in total gonadotropin-releasing activity of rat hypothalamus after ovariectomy, and Vernikos-Dannellis (1964) observed an increase in corticotropin-releasing activity of rat median-eminence extract after stress. Meites and Fiel (1965) have recently noted that starvation reduced the hypothalamic content of somatotropin-releasing factor (SRF) and pituitary STH content in rats. Sinha and Meites (1965) found that thyriodectomy increased the thyrotropin-releasing factor (TRF) levels in the hypothalamus and pituitary TSH content in the rat. These observations support the concept that hypothalamic factors regulate the secretion of AP hormones.

Estradiol, reserpine and the suckling stimulus were shown in the present studies to suppress hypothalamic inhibition of prolactin secretion by depleting the rat hypothalamus of PIF. Depletion of PIF is attributed to reduced PIF synthesis and release, rather than to increased release, since an increase in PIF release would cause an inhibition of pituitary prolactin release. On the contrary, these agents induce increased prolactin secretion. The change in PIF content thus correlates with prolactin release *in vivo*.

PIF content of HE from male, ovariectomized and tapazole treated rats was not significantly different from PIF content of HE from control cycling rats. The lack of effect on PIF content suggest that these conditions do not affect the activity of the hypothalamus, and suggest that the *in vivo* effects of these conditions on prolactin secretion may be via other mechanisms. These results cannot be considered conclusive, however, since ovariectomy and tapazole in particular failed to alter pituitary prolactin content, as had been reported previously (Reece and Turner, 1937; Grosvenor, 1961). Evidence indicates that estrogen and thyroxine can act directly on the AP to increase prolactin secretion (Nicoll and Meites, 1962a, 1963), and in addition estrogen has also been shown to act on the hypothalamus to depress its PIF content (Ratner and Meites, 1964). No effect by the 4 mg dose of progesterone upon hypothalamic PIF content was evident in these studies, but it also failed to increase pituitary prolactin content, as reported in previous studies (Meites and Turner, 1948). Nicoll and Meites (1964) reported that progesterone failed to alter prolactin secretion when added to rat AP *in vitro*. It would be of interest to determine whether intra-hypothalamic or intra-pituitary implants of progesterone could stimulate prolactin secretion.

Cervical stimulation changed the hypothalamus from one which inhibits prolactin release, to one which apparently stimulated prolactin release. There is no convincing evidence as yet that mammalian hypothalamus contains a factor which stimulates prolactin release.

It has been suggested that LRF may act upon the AP to inhibit prolactin secretion (Everett, 1956). Under many conditions there is a reciprocal release of prolactin and LH. This suggested that LRF and PIF may be the same neurohumoral agent. In the present studies, the failure of LRF to alter prolactin release in vitro, indicates that this factor differs from PIF. CRF preparations were also shown to be free of PIF activity, indicating that these are also separate factors. It is of interest, however, that many stresses elicit both ACTH and prolactin release, and some of these may also induce LH release (see Meites et al., 1963). This suggests that similar stimuli may induce release of all three hypothalamic factors.

Recently, two other methods have been employed to localize and evaluate functions in the hypothalamus in relation to prolactin secretion. One procedure uses the nucleolar size of particular hypothalamic nuclei to evaluate functional nerve cell activity. Ifft (1962) reported that the nucleoli of neurons in the arcuate nucleus of rats enlarge during light-induced constant estrus and become smaller during treatment with reserpine or chloropromazine, which induced diestrus in these animals. The decrease in nucleoli size indicated depressed activity of the arcuate nucleus. Direct implants of reserpine into this area of the hypothalamus initiated milk secretion and reduced pituitary content of prolactin in the estrogen-primed rabbit (Kanematsu and Sawyer, 1963b). Lisk and Newlon (1963) demonstrated that estradiol implanted in the arcuate nuclei of the rat reduced the size of the nucleoli of the neurons. These changes were accompanied by ovarian and uterine atrophy. Kanematsu and Sawyer (1963a) found that estrogen implants into this hypothalamic region promoted synthesis of prolactin in the pituitary of the rabbit.

Destruction of the posterior-basal tuberal area of the rabbit hypothalamus induced ovarian atrophy (Sawyer, 1959) and lactation (Haun and Sawyer, 1960), presumably through release of pituitary prolactin (Haun and Sawyer, 1961). In rats, similar lesions, induced gonadal atrophy and released prolactin as evidenced by a luteotropic action on the ovaries and activation of the mammary glands (McCann and Friedman, 1960). The reports on the functional state of the nuclei, are thus in agreement with lesion and implantation studies, and suggest that the cells of the arcuate nucleus function as neural links in the mechanism that controls pituitary gonadotropic and prolactin release.

The recording of localized changes in electrical activity of the hypothalamus and other CNS regions provides another means of studying the functional activity of neural centers. Such work provides examples of definite influences of afferent stimuli upon neurophysiological thresholds that can contribute to the identification of neural pathways involved in neuroendocrine mechanisms. Barraclough and Cross (1963) attempted to study the response of hypothalamic neurones to genital stimulation in the female rat, in an attempt to gain information concerning the neural control of prolactin release. Relating hypothalamic activity to various stages of the cycle, they found that estrogen depressed lateral hypothalamic activity. Injection of progesterone induced a selective depression of the response of the lateral hypothalamic neurones to cervical probing. The response to progesterone, suggested that the lateral hypothalamus might be involved in a feedback control by progesterone upon prolactin release. Kawakami and Sawyer (1959) working with rabbits, observed that sex steroids influence the rhinencephalic-hypothalamic system, which includes the basal tuberal region. Estrogen lowered the threshold for the EEG reaction to electrical stimulation, while progesterone had a biphasic effect. Progesterone first lowered the thresholds and subsequently elevated them to high levels. The early phase is related to estrus and pituitary gonadotropic secretion, whereas the elevated threshold correlates with anestrus or pseudopregnancy, gonadotropic inhibition and prolactin release. In its broader implications, these findings suggest the possibility that a change in brain thresholds may well be a basic factor regulating the duration of the luteal phase of the female cycle and pseudopregnancy.

An influence of olfactory sensibility on prolactin secretion in mice has been elucidated by Parkes and Bruce (1961), who have shown that pregnancy is blocked in a large proportion of newly mated female mice exposed to the smell of males of a different strain. Since the effect can be overcome by administration of prolactin, it seems that the primary action of the strange odor is to suppress prolactin secretion. The removal of the olfactory lobes left the females virtually immune to the influence of alien males. This study provides evidence that neural centers above the hypothalamus can play an important role in prolactin release. It is possible that afferent nervous impulses from various parts of the brain play a role in the regulation of prolactin release. Nauta (1961, 1963) pointed out that the hypothalamus represents a "nodal point" in a series of nerve networks which connect the midbrain and limbic system. The pathways to and from both of these locations make it proper to consider the hypothalamus and limbic system as part of a midbrain-limbic circuit with functions that include, in addition to complex regulation of behavior and homeostatic mechanisms, the regulation of endocrine secretion. Studies have provided evidence that the midbrain and limbic system function in the neuroendocrine regulation of ACTH and FSH-LH release (see Bovard, 1961; Harris, 1958). The hypothalamus and its neurohumoral factors may be considered the final link in the neuroendocrine control of the pituitary but it is probable that its activity is inseparably related to the patterns of neural activity in the limbic-midbrain circuit as a whole. What functional role this circuit plays in the regulation of prolactin release, and by which pathway or mechanism external and internal stimuli influence this system remains to be investigated.

There is some question as to whether the neurohomornes adrenaline, noradrenaline, acetylcholine and serotonin have any physiological role in prolactin release. These substances normally are present in relatively high concentrations in the CNS and hypothalamus, and all of these agents have been shown to elicit mammary secretion in estrogen-primed rats (Meites et al., 1963). The administration of these agents to hypophysectomized rats with a pituitary transplant, resulted in an increase in prolactin release by the transplanted pituitary (Meites, et al., 1963). This suggested that the agents acted either directly on the transplanted pituitary to increase prolactin release or acted on the hypothalamus to depress the production of PIF, which was still able to exert a weak inhibition on prolactin release by the transplanted AP. It remains to be demonstrated whether hypothalamic PIF can exert an influence on the transplanted AP, since it would be greatly diluted in the blood and would be subject to possible inactivation. Preliminary investigations indicate that norepinephrine and acetylcholine can suppress the production of PIF (Mittler and Meites, unpublished). No direct effect on pituitary prolaction release was noted when these agents were added to rat AP *in vitro* (Talwalker et al., 1963).

It is apparent that the hypothalamus serves as a final pathway by which many internal and external stimuli can influence prolactin secretion. The specific neurosecretory system involved in prolactin secretion appears to be located in the basal tuberal region of the hypothalamus. Presumably PIF is produced in cells located in this area, possibly in the arcuate nucleus, and transported to nerve endings, from which it is secreted into the primary plexus of the portal system in the median eminence. The available evidence indicates that the neural mechanism involves the inhibition of prolactin secretion, but the possibility that some other mechanism exists cannot be excluded. Experimental approaches have been centered almost exclusively upon the hypothalamus. The increase in prolactin secretion resulting from the removal of hypothalamic influence has been interpreted as an inhibitory mechanism. However, the increase in prolactin secretion could represent a net effect, in which excitatory and inhibitory stimuli from supra- and intra-hypothalamic pathways have been nullified. The properly integrated activity of the neuroendocrine mechanism controlling prolactin secretion may require a balanced production and intervention of a number of chemical substances in the hypothalamus and related areas.

Our knowledge of CNS regulation of prolactin secretion is far from complete. The precise neural pathways and agents by which stimuli converge on the hypothalamus to alter prolactin secretion have yet to be identified. The hypothalamic factor (PIF) which has been related to prolactin secretion in mammals, is representative of only very crude hypothalamic extracts. The precise origin, number and nature of CNS factors which may be involved in regulation of prolactin secretion is unknown. Final proof that an inhibitory (or stimulatory) factor is contained in the hypothalamus will require isolation, purification and identification of the substances and studies to establish their specificity.

REFERENCES

Ahren, K. The secretory capacity of the autotransplanted hypophysis as indicated by the effects of steroid hormones on the mammary glands. I. Effects of ovarian hormones. Acta Endocrinol. 38:449-468, 1961.

Alloiteau, J. J., and A. Vignal. Pseudogestations après traitement lutèotrophe de courte durèe chez la Ratte. Compt. Rend. 247:2465-2467, 1958.

Baker, B. L., and N. B. Everett. The effect of diethylstilbesterol on the anterior hypophysis of thyroidectomized rats. Endocrinology. 41:144-57, 1947.

Barraclough, C. A., and B. A. Cross. Unit activity in the hypothalamus of the cyclic female rat: Effect of genital stimuli and progesterone. J. Endocrinol. 26:339-359, 1963.

Barraclough, C. A., and C. H. Sawyer. Induction of pseudopregnancy in the rat by reserpine and chloropromazine. Endocrinology. 65:563-571, 1959.

Batson, H. C. An Introduction to Statistics in the Medical Sciences. Burgess Publishing, Minneapolis, p. 16, 1961.

Bein, N. J. The pharacology of Rauwolfia. Pharmacol. Rev. 8:435-483, 1956.

Ben-David, M., S. Dikstein, and F. G. Sulman. Effect of different steroids on prolactin secretion in pituitary-hypothalamus organ co-culture. Proc. Soc. Exptl. Biol. Med. 117:511-513, 1964.

Benson, G. K., and S. J. Folley. The effect of oxytocin on mammary gland involution in the rat. J. Endocrinol. 16:189-201, 1957.

Bovard, E. W. A concept of hypothalamic functioning. Perspect. Biol. and Med. 5:52-60, 1961.

Brodish, A. The role of a hypothalamic neurohumor in the secretion of ACTH. Acta Endocrinol. 35 (Suppl. 51), 1960.

D'Angelo, S. A. Central nervous regulation of the secretion and release of thyroid stimulating hormone. In A. V. Nalbandov (ed.), Advances in Neuroendocrinology. Univ. of Illinois Press, Urbana, Chapt. 6, 1963.

Danon, A., S. Dikstein, and F. G. Sulman. Stimulation of prolactin secretion by perphenazine in pituitary-hypothalamus organ culture. Proc. Soc. Exptl. Biol. Med. 114:366-368, 1963.

Desclin, L. Concerning the mechanism of diestrum during lactation in the albino rat. Endocrinology. 40:14-29, 1947.

Desclin, L. A propos du méchanism d'action des oestrogènes sur le lobe antérieure de l'hypophyse chez le Rat. Ann. Endocrinol. 11:656-659, 1950.

Desclin, L. Influence of reserpine, oxytocin and adrenaline on the structure, secretory activity and involution of mammary gland in virgin and postpartum rats. Anat. Record. 136:182, 1960.

Eayrs, J. T., and R. M. Baddeley. Neurol pathways in lactation. J. Anat. 90:161-171, 1956.

Eik-Nes, K. B., and K. R. Brizzee. Some aspects of corticotrophin secretion in the trained dog. I. The presence of a corticotrophin releasing factor in the blood of dogs shortly after hypophysectomy. Acta Endocrinol. 29:219-23, 1958.

Everett, J. W. Functional corpora lutea maintained for months by autografts of rat hypophysis. Endocrinology. 58:786-796, 1956.

Everett, J. W. Pseudopregnancy in the rat from brief treatment with progesterone. Nature. 198:695-696, 1963.

Everett, J. W. Central neural control of reproductive functions of the adenohypophysis. Physiol. Rev. 44:373-431, 1964.

Folley, S. J. The Physiology and Biochemistry of Lactation. Charles C. Thomas, Springfield, Illinois, 1956.

Gala, R. R., and R. P. Reece. Effect of a reserpine analogue, methyl-18-epi-O-methyl-reserpate hydrochloride, on lactogen release from rat anterior pituitary. Endocrinology. 72:649-657, 1963.

Gala, R. R., and R. P. Reece. Influence of estrogen on anterior pituitary lactogen production. Proc. Soc. Exptl. Biol. Med. 115:1030-1035, 1964a.

Gala, R. R., and R. P. Reece. Influence of hypothalamic fragments and extracts on lactogen production in vitro. Proc. Soc. Exptl. Biol. Med. 117:833-835, 1964b.

Ganong, W. F. The central nervous system and the synthesis and release of adrenocorticotropic hormone. In A. V. Nalbandov (ed.), Advances in Neuroendocrinology. Univ. of Illinois Press, Urbana, Chapt. 5, 1963.

Gaunt, R., A. A. Renzi, N. Antonchack, G. J. Miller, and M. Gilman. Endocrine aspects of the pharmacology of reserpine. Ann. N.Y. Acad. Sci. 59:22-43, 1954.

Green, J. D., and G. W. Harris. The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocrinol. 5:136-146, 1947.

Greenwold, G. S. Luteinizing hormone content of the pituitary of the lactating mouse. J. Gen. Comp. Endocrinol. 2:453-457, 1962.

Grosvenor, C. E. Effect of experimentally induced hypo- and hyperthyroid states upon pituitary lactogenic hormone concentration in rats. Endocrinology. 69:1092-1994, 1961.

Grosvenor, C. E., and C. W. Turner. Release and restoration of pituitary lactogen in response to nursing stimuli in lactating rats. Proc. Soc. Exptl. Biol. Med. 96:723-25, 1957.

Grosvenor, C. E., S. M. McCann, and R. Nallar. Inhibition of suckling-induced release of prolactin in rats injected with acid extract of bovine hypothalamus. Program of the 46th Meeting of the Endocrine Society. p. 96, 1964.

Guillemin, R. Hypothalamic polypeptides releasing pituitary hormones. Metabolism. 13:1206-1211, 1964.

Guillemin, R., and A. V. Schally. Recent advances in the chemistry of neuroendocrine mediators originating in the central nervous system. In A. V. Nalbandov (ed.), Advances in Neuroendocrinology. Univ. of Illinois Press, Urbana, Chapt. 10, 1963.

Harris, G. W. Neural Control of the Pituitary Gland. Edward Arnold Ltd., London, 1955.

Harris, G. W. The reticular formation, stress, and endocrine activity. In: Reticular Formation of the Brain. Henry Ford Hosp. Symp. Little, Brown and Co., Boston, p. 207, 1958.

Haun, C. K., and C. H. Sawyer. Initiation of lactation in rabbits following placement of hypothalamic lesions. Endocrinology. 67:270-272, 1960.

Haun, C. K., and C. H. Sawyer. The role of the hypothalamus in initiation of milk secretion. Acta Endocrinol. 38:99-106, 1961.

Hymer, W. C., W. H. McShan, and R. G. Christiansen. Electron microscopic studies of anterior pituitary glands from lactating and estrogen-treated rats. Endocrinology. 69:81-90. 1961.

Ifft, J. D. Evidence of gonadotropic activity of the hypothalamic arcuate nucleus in the female rat. Anat. Record. 142:1-8, 1962.

Kanematsu, S., and C. H. Sawyer. Effects of intrahypothalamic and intrahypophysial estrogen implants on pituitary prolactin and lactation in the rabbit. Endocrinology. 72:243-252, 1963a.

Kanematsu, S., and C. H. Sawyer. Effects of intrahypothalamic implants of reserpine on lactation and pituitary prolactin content in the rabbit. Proc. Soc. Exptl. Biol. Med. 113:967-969, 1963b.

Kanematsu, S., J. Hilliard, and C. H. Sawyer. Effects of reserpine and hypothalamic lesions on pituitary prolactin content in the rabbit. Program of the 43rd Meeting of the Endocrine Society, p. 3, 1961.

Kanematsu, S., J. Hilliard, and C. H. Sawyer. Effect of hypothalamic lesions on pituitary prolactin content in the rabbit. Endocrinology. 73:345-348, 1963a.

Kanematsu, S., J. Hilliard, and C. H. Sawyer. Effect of reserpine on pituitary prolactin content and its hypothalamic site of action in the rabbit. Acta Endocrinol. 44:467-474, 1963b.

Kawakami, M., and C. H. Sawyer. Neuroendocrine correlates of changes in brain activity thresholds by sex steroids and pituitary hormones. Endocrinology. 65:652-668, 1959.

Kim, U., J. Furth, and K. Yannopoulos. Observations on hormonal control of mammary cancer. I. Estrogen and mammotropes. J. Natl. Cancer Inst. 31:233-259, 1963.

Kobayashi, T., T. Kobayashi, T. Kigawa, M. Mizuno, and Y. Amenomori. Influence of rat hypothalamic extract on gonadotropic activity of cultivated anterior pituitary cells. Endocrinol. Japonica. 10:16-24, 1963.

Kollar, E. J. Reproduction in the female rat after pelvic nerve neurectomy. Anat. Record. 115:641-658, 1953.

Kragt, C. L., and J. Meites. Stimulation of pigeon pituitary prolactin release by pigeon hypothalamic extract in vitro. Endocrinology. (In Press), 1965.

Lisk, R. D., and M. Newlon. Estradiol: Evidence for its direct effect on hypothalamic neurons. Science. 139:223-224, 1963.

Lyons, W. R. The preparation and assay of mamotropin. Cold Spring Harbor Symposium on Quantitative Biology. V:198, 1937.

Maqsood, M., and J. Meites. Induction of mammary secretion in rats by electrical stimulation. Proc. Soc. Exptl. Biol. Med. 106:104-106, 1961.

Marshall, F. H. A. Sexual periodicity and the causes which determine it. The Croonian Lecture: Philos. Trans. B. 226:423-456, 1936.

Marshall, F. H. A. Exteroceptive factors in sexual periodicity. Biol. Rev. 17:68-90, 1942.

McCann, S. M., and H. M. Friedman. The effect of hypothalamic lesions on the secretion of luteotrophin. Endocrinology. 67:597-608, 1960.

McCann, S. M., V. D. Ramirez, and M. Igarashi. Hypothalamic FSH and LH releasing factors. Metabolism. 13:1177-1189, 1964.

McQueen-Williams, M. Decreased mammotropin in pituitaries of thyrodectomized male rats. Proc. Soc. Exptl. Biol. Med. 33:406-409, 1935.

Meites, J. Effect of reserpine on prolactin content of rabbit pituitary. Proc. Soc. Exptl. Biol. Med. 97:742-744, 1958.

Meites, J. Mammary growth and lactation. In H. H. Cole and P. T. Cupps (eds.), Reproduction in Domestic Animals. Academic Press, New York, Vol. I, Chapt. 16, 1959.

Meites, J. Farm animals: Hormonal induction of lactation and galactopoiesis. In S. K. Kon and A. T. Cowie (eds.), Milk: The Mammary Gland and its Secretion. Academic Press, New York, Vol. I, p. 321, 1961.

Meites, J. Pharmacological control of prolactin secretion and lactation. In R. Giullemin (ed.), Proceedings of the First International Pharmacological Meeting. Pergamon Press, Oxford, Vol. I, p. 151, 1963.

Meites, J., and N. J. Fiel. Effect of starvation on hypothalamic content of "somatotropin releasing factor" and pituitary growth hormone content. Endocrinology. (In Press), 1965.

Meites, J., and C. W. Turner. Effect of thiouracil and estrogen on lactogenic hormone and weight of the pituitaries of rats. Proc. Soc. Exptl. Biol. Med. 64:488-492, 1947.

Meites, J., and C. W. Turner. Studies concerning the induction and maintenance of lactation. Missouri Agri. Exp. Sta. Res. Bulls., Nos. 415 and 416, 1948.

Meites, J., C. S. Nicoll, and P. K. Talwalker. Induction and maintenance of lactation in rats by electrical stimulation of uterine cervix. Proc. Soc. Exptl. Biol. Med. 102:127-131, 1959.

Meites, J., C. S. Nicoll, and P. K. Talwalker. The central nervous system and the secretion and release of prolactin. In A. V. Nalbandov (ed.), Advances in Neuroendocrinology. Univ. of Illinois Press, Urbana, Chapt. 8, 1963.

Meites, J., P. K. Talwalker, and C. S. Nicoll. Initiation of lactation in rats with hypothalamic or cerebral tissue. Proc. Soc. Exptl. Biol. Med. 103:298-300, 1960.

Meyer, R. K., C. Biddulph, and J. C. Finerty. Pituitary-gonad interaction in immature female parabiotic rats. Endocrinology. 39:23-31, 1946.

Meyer, R. K., S. L. Leonard, and F. L. Hisaw. Effect of anesthesia on artificial production of pseudopregnancy in the rat. Proc. Soc. Exptl. Biol. Med. 27:340-342, 1929.

Michael, R. P. Oestrogen-sensitive systems in mammalian brains. Proc. 22nd. Intern. Congr. Physiol. Sci., Leiden, 1(2):650-652, 1962.

Mixner, J. P., and C. W. Turner. Influence of local applications of turpentine on mammary gland growth and involution. Proc. Soc. Exptl. Biol. Med. 46:437-440, 1941.

Mizuno, H., P. K. Talwalker, and J. Meites. Influence of hormones on tumor growth and plasma prolactin levels in rats bearing a pituitary "mammotropic" tumor. Cancer Research. 24:1433-1436, 1964.

Moon, R. C., and C. W. Turner. Effect of reserpine on oxytocin and lactogen discharge in lactating rats. Proc. Soc. Exptl. Biol. Med. 101:332-335, 1959.

Moszkowska, A. Contribution à la recherche des relations du complexe hypothalamo-hypophysaire dans la fonction gonadotrope: méthode in vivo et in vitro. Compt. Rend. Soc. Biol. 153:1945-48, 1959.

Nauta, W. J. H. Limbic system and hypothalamus: Anatomical aspects. Physiol. Rev. 40 (Suppl. 4):102, 1961.

Nauta, W. J. H. Central nervous organization and the endocrine motor system. In A. V. Nalbandov (ed.), Advances in Neuroendocrinology. Univ. of Illinois Press, Urbana, Chapt. 2, 1963.

Nicoll, C. S., and J. Meites. Estrogen stimulation of prolactin production by rat adenohypophysis in vitro. Endocrinology. 70:272-277, 1962a.

Nicoll, C. S., and J. Meites. Failure of neurohypophysial hormones to influence prolactin secretion in vitro. Endocrinology. 70:927-929, 1962b.

Nicoll, C. S., and J. Meites. Prolactin secretion in vitro. Comparative aspects. Nature. 195:606-607, 1962c.

Nicoll, C. S., and J. Meites. Prolactin secretion in vitro: Effects of thyroid hormones and insulin. Endocrinology. 72:544-551, 1963.

Nicoll, C. S., and J. Meites. Prolactin secretion in vitro: Effects of gonadal and adrenal cortical steroids. Proc. Soc. Exptl. Biol. Med. 117:579-583, 1964.

Nicoll, C. S., P. K. Talwalker, and J. Meites. Initiation of lactation in rats by non-specific stresses. Am. J. Physiol. 198:1103-1106, 1960.

Nikitovitch-Winer, M. B. The influence of the hypothalamus on luteotropin secretion in the rat. In: Memoirs of the Society for Endocrinology. No. 9, Cambridge Univ. Press, London, Part I, p. 70, 1960.

Page, I. H. Serotonin (5-hydroxytryptamine); the last four years. Physiol. Rev. 38:277-335, 1958.

Parkes, A. S., and H. M. Bruce. Olfactory stimuli in mammalian reproduction. Science. 134:1049-1054, 1961.

Pasteels, J. L. Comparison entre l'action des androgèns et de la réserpine sur l'hypophyse et la glande mammaire chez le Rat. Ann. Endocrinol. 22:257-268, 1961a.

Pasteels, J. L. Première résultats de culture combinée in vitro d'hypophysis et d'hypothalamus dans le but d'en apprecier la sécrétion de prolactine. Compt. Rend. 253:3074-3075, 1961b.

Pasteels, J. L. Administration d'extraits hypothalamique à l'hypophyse de Rat in vitro, dans le but d'en contrôler la sécrétion de prolactine. Compt. Rend. 254:2664-2666, 1962.

Pecile, A., E. Muller, G. Falconi, and L. Martini. Growth hormone releasing activity of hypothalamic extracts at different ages. Program of the 46th Meeting of the Endocrine Society. p. 132, 1964.

Purves, H. D. Morphology of the hypophysis related to its function. In W. C. Young (ed.), Sex and Internal Secretions. The Williams and Wilkens Co., Baltimore, Vol. I, Chapt. 3, 1961.

Ramirez, V. D., R. M. Abrams, and S. M. McCann. Effect of estrogen implants in the hypothalamo-hypophysial region on the secretion of LH in the rat. Fed. Proc. (Abstract) 22:2063, 1963.

Ratner, A., and J. Meites. Depletion of prolactin-inhibiting activity of rat hypothalamus by estradiol or suckling stimulus. Endocrinology. 75:377-382, 1964.

Reece, R. P. Initiation of lactation in the albino rat with lactogen and adrenal cortical hormone. Proc. Soc. Exptl. Biol. Med. 40:25-27, 1939.

Reece, R. P., and J. A. Bivino. Progesterone effect on pituitary lactogen content and on mammary glands of ovariectomized rats. Proc. Soc. Exptl. Biol. Med. 49:582-584, 1942.

Reece, R. P., and C. W. Turner. The lactogenic and thyrotropic hormone content of the anterior lobe of the pituitary gland. Missouri Univ. Agr. Expt. Sta. Research Bull. No. 266, 1937.

Rothchild, I. The corpus luteum-pituitary relationship: The association between the cause of luteotrophin secretion and the cause of follicular quiescence during lactation; the basis for a tentative theory of the corpus luteum-pituitary relationship in the rat. Endocrinology. 67:9-41, 1960a.

Rothchild, I. The corpus luteum-pituitary relationship: The lack of an inhibitory effect of progesterone on the secretion of pituitary luteotrophin. Endocrinology. 67:54-61, 1960b.

Rothchild, I., and R. Schubert. The corpus luteum-pituitary relationship: The induction of pseudopregnancy in the rat by progesterone. Endocrinology. 72:969-972, 1963.

Sawyer, C. H. Induction of lactation in the rabbit with reserpine. Anat. Record. 127:362-363, 1957.

Sawyer, C. H. Effects of brain lesions on estrous behavior and reflexogenous ovulation in the rabbit. J. Exptl. Zool. 142:227-246, 1959.

Sawyer, C. H., C. K. Haun, J. Hilliard, H. M. Radford, and S. Kanematsu. Further evidence for the identity of hypothalamic areas controlling ovulation and lactation in the rabbit. Endocrinology. 73:338-344, 1963.

Schally, A. V., and C. Y. Bowers. Corticotropin-releasing factor and other hypothalamic peptides. Metabolism. 13:1190-1205, 1964.

Schally, A. V., J. Meites, C. Y. Bowers, and A. Ratner. Identity of prolactin inhibiting factor (PIF) and luteinizing hormone-releasing factor (LRF). Proc. Soc. Exptl. Biol. Med. 117:252-254, 1964.

Selye, H. On the nervous control of lactation. Am. J. Physiol. 107:535-39, 1934.

Shore, P. A. Release of serotonin and catecholamines by drugs. Pharmacol. Rev. 14:531-550, 1962.

Sinha, D., and J. Meites. Effects of thyroidectomy and thyroxine on hypothalamic content of "thyrotropin releasing factor" (TRF). Fed. Proc. (In Press), 1965.

Taleisnik, S., and S. M. McCann. Effects of hypothalamic lesions on the secretion and storage of hypophysial luteinizing hormone. Endocrinology. 68:263-272, 1961.

Talwalker, P. K. Central nervous system inhibition of prolactin secretion. Ph.D. Thesis. Michigan State Univ., E. Lansing, Mich., 1964.

Talwalker, P. K., J. Meites, C. S. Nicoll, and T. F. Hopkins. Effect of chloropromazine on mammary glands of rats. Am. J. Physiol. 199:1073-1076, 1960.

Talwalker, P. K., A. Ratner, and J. Meites. *In vitro* inhibition of pituitary prolactin synthesis and release by hypothalamic extract. Am. J. Physiol. 205:213-218, 1963.

Van Rees, G. P. Influence of steroid sex hormones on the FSH release by rat hypophyses *in vitro*. Acta Endocrinol. 36:385-497, 1961.

Vernikos-Daniellis, J. Estimation of corticotropin-releasing activity of rat hypothalamic and neurohypophysis. Endocrinology. 75:514-520, 1964.

Wolthuis, O. L. The effects of sex steroids on the prolactin content of hypophyses and serum in rats. Acta Endocrinol. 43:137-146, 1963.

Zeilmaker, G. H. Luteotropic hormone secretion in the male rat. Acta Endocrinol. (Suppl. 67):70, 1962.

Zeilmaker, G. H. Experimental studies on the regulation of corpus luteum function in castrated male rats bearing a transplanted ovary. Acta Endocrinol. 43:246-254, 1963.

APPENDIX

Curriculum Vitae and List of Published Papers

CURRICULUM VITAE

Name: Ratner, Albert

Date of Birth: September 10, 1937

Place of Birth: New York, U.S.A.

Marital Status: Single

Permanent Address: 2 Bradley Street
Planview, L.I., New York

Education Qualifications:

Institution	Major field of Study	Degree	Date
Brooklyn College Brooklyn, N.Y.	Biology-Chemistry	B.S.	1959
Michigan State Univ. East Lansing, Mich.	Physiology	M.S.	1962
Michigan State Univ. East Lansing, Mich.	Physiology Biochemistry-minor Endocrinology as special field Languages - French and German	Ph.D.	1965

Positions Held:

Position	Place of Employment	Date
Teaching Assistant	Department of Physiology Michigan State Univ.	1959-1960
Research Associate	Department of Physiology Michigan State Univ.	1961-1962 1963-1964

Honors:

Elected as a full member of honorary national scientific society, Sigma Xi, U.S.A.

Awarded NIH postdoctoral fellowship.

Talks Presented at Scientific Meetings:

Meeting	Topic	Date
45th Ann. Meeting Fed. Am. Soc. Exptl. Biol. Atlantic City, N.J.	Effects of hormone injections upon milk production in underfed rats.	April, 1961
46th Ann. Meeting Fed. Am. Soc. Exptl. Biol. Atlantic City, N.J.	Prolactin production by rat anterior pituitary during short-term incubation.	April, 1962
47th Ann. Meeting Fed. Am. Soc. Exptl. Biol. Chicago, Ill.	Removal of prolactin inhibiting activity of rat hypothalamus by estrogen, reserpine, or suckling.	April, 1963

RESEARCH PUBLICATIONS

1. Hopkins, T. F., J. Meites and A. Ratner. Inactivation of prolactin by blood serum and plasmin. *Proc. Soc. Exp. Biol. & Med*. *106*:140-141, 1961.
2. Ratner, A. and J. Meites. Effects of hormone injections upon milk production in underfed rats. *Fed. Proc.* *20*: 1961 (abstract).
3. Ratner, A., P. K. Talwalker and J. Meites. Prolactin production by rat anterior pituitary during short-term incubation. *Fed. Proc*. *21*:1962(abstract).
4. Talwalker, P. K., A. Ratner and J. Meites. *In vivo* and *in vitro* prolactin production by "mammotropic" pituitary tumors. *Fed. Proc*. *21*: 1962(abstract).
5. Ratner, A., P. K. Talwalker, and J. Meites. Effect of estrogen administration *in vivo* on prolactin release by rat pituitary *in vitro*. *Proc. Soc. Exp. Biol. & Med*. *112*:12-15, 1963.
6. Ratner, A., and J. Meites. Effects of hormone administration on milk production of underfed rats. *Am. J. Physiol*. *204*:268-270, 1963.
7. Talwalker, P. K., A. Ratner and J. Meites. *In vitro* inhibition of pituitary prolactin synthesis and release by hypothalamic extract. *Am. J. Physiol*. *205*:213-218, 1963.

8. Ratner, A., and J. Meites. Removal of prolactin inhibiting activity of rat hypothalamus by estrogen, reserpine, or suckling. Fed. Proc. 23:1963 (abstract).

9. Ratner, A., and J. Meites. Depletion of prolactin inhibiting activity of rat hypothalmus by estradiol or suckling stimulus. Endocrinology. 75:377-382, 1964.

10. Schally, A. V., J. Meites, C. Y. Bowers and A. Ratner. Identity of prolactin inhibiting factor (PIF) and luteinizing hormone-releasing factor (LRF). Proc. Soc. Exp. Biol. & Med. 117:252-254, 1964.

11. Talwalker, P. K., A. Ratner, and J. Meites. In vivo and in vitro prolactin secretion by transplanted rat "mammotropic" pituitary tumors. Cancer Research 24:1723-1726, 1964.

12. Ratner, A., P. K. Talwalker and J. Meites. Effect of reserpine on prolactin-inhibiting activity of rat hypothalamus. Endocrinology (In Press).