# EFFECTS OF HYDROCORTISONE ACETATE, OXYTOCIN AND PROLACTIN ON LACTATIONAL PERFORMANCE OF RATS AFTER PARTURITION

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Purnachandra Keshavrao Talwalker

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BY

PURNACHANDRA KESHAVRAO TALWALKER

AN ABSTRACT

Submitted to the College of Science and Arts,
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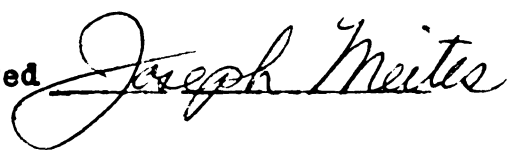
Approved 

ABSTRACT

1. In experiment I, the effects of administration of hydrocortisone acetate, prolactin and oxytocin, singularly and in combination, on lactational performance of rats were studied. Ninety mature female rats of the Carworth strain were bred and on the 4th day postpartum the litters were reduced to six young each. The rats were divided into nine groups, each group containing ten rats. All injections were given subcutaneously, in 0.1 ml saline, once daily, except oxytocin which was given twice daily. The injections were given from the 4th day to 17th day postpartum. On the 18th day the dams were killed and the pituitary, adrenals and ovaries were removed, cleaned and weighed. Each group received the following treatment:

    a. Controls, saline 0.85%.
    b. Hydrocortisone acetate 0.25 mg.
    c. Hydrocortisone acetate 0.5 mg.
    d. Hydrocortisone acetate 1.0 mg.
    e. Oxytocin 1 I.U.
    f. Prolactin 1 mg.
    g. Hydrocortisone acetate 0.25 mg and prolactin 1 mg. and oxytocin 1 I.U.

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h. Hydrocortisone acetate 0.5 mg. and prolactin 1 mg. and oxytocin 1 I.U.

i. Hydrocortisone acetate 1 mg. and prolactin 1 mg. and oxytocin 1 I.U.

2. When injected individually into lactating rats, hydrocortisone acetate, oxytocin and prolactin, only hydrocortisone acetate at the 0.5 mg. dose level increased milk secretion significantly, whereas oxytocin and prolactin had no effect. When all hormones were injected in combination, increases in lactation were no greater than with hydrocortisone acetate alone. (Hydrocortisone acetate was effective in increasing milk secretion probably because during lactation the adrenal cortex secretes less than optimal amounts of glucocorticoids and hence small amounts of additional glucocorticoid have beneficial effects in increasing milk secretion. Oxytocin and prolactin were not effective in increasing milk secretion. During lactation these two hormones are secreted in optimum amounts and hence additional amounts have no effect.)

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3. Dams receiving saline, oxytocin and prolactin alone each gained in average body weight by approximately 20-25 gms., while those receiving 0.25 or 0.5 mg. hydrocortisone acetate gained in average body weight by only 7-10 gms. Dams injected with 1.0 mg. hydrocortisone acetate lost about 8 gms. in average body weight during the injection period.

4. No significant difference was observed in the average weight of the pituitaries between the different groups. However, the average weights of the adrenals and ovaries were significantly lower than controls in the groups which received 0.5 and 1.0 mg. hydrocortisone acetate.

5. In experiment II, the effect of prolactin on lactational performance of oophorectomized rats was studied. Twenty mature female rats of the Carworth strain were bred. On the 2nd day postpartum both the ovaries were removed while the rats were under light ether anesthesia. They were divided into two groups of ten rats each. Litters were reduced to six young each on the 6th day postpartum. Group I

ABSTRACT

received 0.85% saline and group II received prolactin (1 mg. daily) during the 4-17 day-period of lactation. On the 18th day, the dams were killed and the adrenals, ovaries and pituitaries were removed, cleaned and weight. Dams and litters were weighed daily.

6. No significant difference was observed in average litter weight gain during the 4-18 day-period between the oophorectomized and intact groups. This shows that sufficient prolactin is secreted after parturition to luteinize the ovaries (luteotropic action) and to maintain optimal lactation. This also shows that the functional ovaries of the rat after parturition do not inhibit milk production.

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# INTRODUCTION

It is well known that completion of mammary growth alone does not initiate lactation; thus, factors other than those necessary for mammary growth must be present to initiate and later, to maintain lactation. Although this subject is still not free from controversy, there is sufficient evidence to show that several endocrine factors are essential for the maintenance of established lactation. Folley and Young (1940) and Bergman and Turner (1940) independently suggested the term "galactopoiesis" to designate the stimulation of established lactation, and thus they drew a distinction between the initiation of lactation (lactogenesis) and its maintenance, once established.

It is very well known that lactation in most mammals rapidly reaches a peak and subsequently declines over a long period of time. Various studies have been carried out in order to determine the factors responsible for the decline in lactation. These studies include:

1) Administration of hormones during the decline to stimulate milk production in farm and laboratory animals.

2) Removal of an endocrine gland or glands and replacement therapy to maintain normal lactation.

3) Maintenance of mammary structure and functional integrity by hormonal administration after removal of milking stimulus.

4) Biochemical studies.

5) Nutritional studies.

The removal of the hypophysis in any stage of lactation in laboratory animals has been shown to cause a rapid and abrupt cessation of lactation (Gomez and Turner, 1936a; Hill et al., 1935). However, it was shown that lactation could be maintained in hypophysectomized animals by administration of crude hypophyseal extracts (Gomez and Turner, 1936b; Houssay, 1935). These earlier studies showed the importance of the role of the pituitary in maintaining lactation. However, it is not still precisely known how many hormones of the pituitary are involved in normal maintenance of lactation. Variations in kind and amount of hormonal requirements have been found to vary in different species.

The importance of the adrenal cortical hormones for the maintenance of lactation is very well established. Adrenalectomy during lactation results in cessation of lactation, but replacement with adrenal cortical hormones can maintain lactation partially or completely. However, the precise role of the various adrenal cortical hormones in maintaining lactation is not known.

It is very well established that the maintenance of

the lactating mammary gland in a state of functional integrity is dependent upon the continued application of suckling or milking stimulus (Selye, 1939; Bruce, 1958; Nicoll and Meites, 1959). There is evidence that the suckling stimulus during lactation causes the release of oxytocin (Petersen et al., 1942; Cross and Harris, 1950, 1951), prolactin (Reece and Turner, 1937; Meites and Turner, 1948) and ACTH (Tabachnick and Trentin, 1951; Gregoire, 1946) from the pituitary. It has been reported that injections of prolactin (Williams, 1945; Johnson, 1957) or oxytocin (Johnson, 1957; Benson and Folley, 1956) to lactating mother rats after removal of their litters inhibit involutionary changes in the mammary glands. It has been shown that cortisone administration can significantly retard mammary involution in rats after litter removal, and can maintain milk secretion at a higher level during the declining phase (Johnson and Meites, 1958). Meites and Nicoll (1959) have shown that injections of hydrocortisone acetate, oxytocin and prolactin, in combination, can retard mammary involution as long as 70 - 75 days after litter removal on the 4th day postpartum.

These studies suggested that secretion of oxytocin, prolactin and ACTH or corticoids may be limiting factors

in the lactational performance of rats after parturition. Therefore it was of interest to know whether these three hormones administered singularly or in combination could increase normal milk production in the rat.

In the rat, prolactin has been shown to have luteotropic activity. Lyons (1958b) suggested that prolactin injected into rats bearing corpora lutea of lactation, might be utilized by the corpora lutea to promote the secretion of progesterone, thus stimulating mammary growth and antagonizing lactation. Therefore it was of interest to determine the effects of ovariectomy and injections of prolactin into ovariectomized rats on milk yields.

# I

# REVIEW OF LITERATURE

Reviewing the literature is a very informative and interesting experience. One gains a feeling of gratitude towards those pioneers in the field of the physiology of milk secretion who were so instrumental in laying the ground work for a better understanding of such a complex process as milk secretion on the basis of keen observation, curiosity and painstaking effort. From this background comes much of the knowledge we presently have of this subject.

The literature on lactation is voluminous. This well indicates the importance of this subject, not only from a commercial point of view but also from the point of view of acquiring basic understanding of the process of milk secretion. In recent years great advances have been made, and much is known about the general and some specific aspects. However, many of the fundamental processes still remain to be elucidated.

## 1. Endocrine Control of Postpartum Lactation in Hypophysectomized Animals.

The earliest reports on the maintenance of lactation after hypophysectomy are those of Gomez (1939, 1940) who found that rats hypophysectomized during lactation could

rear their litters, if given anterior pituitary extract, adrenal cortical extract, glucose and posterior pituitary extract. He used posterior pituitary extract at a time when the role of posterior pituitary in lactation was not fully understood.

Recently, experiments have been carried out in hypophysectomized rats to determine which hormones of the pituitary are necessary for maintaining lactation adequate to support average-sized litters. It is now commonly accepted that hypophysectomized test animals should be used in lactational studies in order to eliminate possible disturbing factors due to the action of endogenous pituitary hormones.

Nelson et al. (1943) showed that lactation could not be induced in the hypophysectomized guinea pig with lactogenic hormone and deoxycorticosterone. They also reported that they could induce and partially maintain lactation in hypophysectomized guinea pigswith lactogenic hormone and 17-hydroxy-11-dehydrocorticosterone.

Cowie (1957) showed that slight maintenance of milk secretion, in rats hypophysectomized on the 4th day of lactation, could be obtained by administration of crude anterior-pituitary extracts or prolactin alone (25 I.U. twice daily). Slightly higher milk yield was obtained with

a larger dose of prolactin (50 I.U. twice daily). Neither GH nor ACTH when administered alone had any replacement value, although a slight replacement was obtained when they were administered together. In no case was complete maintenance of lactation achieved, although yields of almost half the normal were obtained with prolactin and ACTH, or prolactin and GH.

Bintarningsih et al. (1957) were able to obtain considerable maintenance of milk secretion in the hypophysectomized rat with prolactin and cortisol acetate or prednisolone acetate. Lyons et al. (1958) hypophysectomized rats on the 12th day of pregnancy. In these animals the mammary gland developed in response to placental and ovarian hormones. Normally such animals after parturition secrete a small amount of milk for a day before regressing. They found that they could maintain lactation by injecting prolactin and prednisolone acetate, and assumed that the endogenous secretion of oxytocin continued because the milk ejection reflex was found to be regenerated within ten days after hypophysectomy. They showed that GH or thyroxine administrations had no effect in enhancing lactation; the milk yield, however, of the rats injected with prolactin and either ACTH or cortisol was only about 50% of normal. The hypophysectomized mother rats showed gains in body

weight when GH and thyroxine were given. But those rats which received prolactin plus cortisone lost considerable body weight. Thus in hypophysectomized rats prolactin, ACTH and possibly GH appear to be components of a pituitary complex concerned in the maintenance of milk secretion.

## 2. Neural Control of Anterior and Posterior Pituitary Secretions during Lactation.

Much of the milk present in the mammary gland just before suckling or milking is in the alveoli and ducts from which it can be removed by the squeezing action of a contractile effector tissue, the myoepithelium, which forms a network surrounding each alveolus. This is a reflex response to the stimulation of teat or other stimuli, often psychic, to which the animal has been conditioned. Thus the active but unconscious participation of the nursing mother is essential if the milk is to be made available to the young. Once it was believed that this milk-ejection reflex consisted of a purely neural arc (Harris, 1958).

Gains (1915) reported that pups suckling an anesthetized bitch usually obtained no milk. Thus anesthesia inhibited the milk-ejection reflex. However this inhibition was overcome by the injection of posterior pituitary extract. Turner and Slaughter (1930) suggested that milk-ejection reflex might act through the posterior pituitary gland. Ely and Petersen (1941) showed that milk-ejection could occur in response to the normal milking stimulus in one half of the bovine udder in which two nerves, believed to carry all the efferent fibers to the gland, had been cut.

Petersen and Ludwick (1942) showed that blood collected from cows after stimulation produced ejection of milk when perfused through the isolated cow udder. These studies indicated that this reflex consists of a neurohormonal arc. This involves the release of a hormone, probably oxytocin from the posterior pituitary which causes contraction of the myoepithelium surrounding the alveoli and thus squeezing out the milk (Harris, 1958).

There is now considerable evidence that the suckling stimulus during lactation also causes release of prolactin (Reece and Turner, 1937; Meites and Turner, 1948) and ACTH (Tobachnick and Trentin, 1951; Gregoire, 1946) from the anterior pituitary, which are essential for the maintenance of normal lactation. Thus the suckling stimulus appears to play a central role in the two main phases of lactation -- milk secretion and milk ejection. Harris, (1955) suggested that there is considerable evidence in favor of the view that anterior pituitary secretions are stimulated by the liberation from the hypothalamus of some humoral substance (s) into the primary plexus of the hypophysial portal system. It is believed that this substance is then carried in the portal circulation to the anterior lobe. Various suggestions have been made regarding the nature of the humoral substance (s).

Benson and Folley (1956, 1957) suggested that oxytocin might be a humoral link for the release of prolactin or the whole galactopoietic complex from the anterior pituitary. The basis for such speculation was mainly the ability of oxytocin to retard mammary gland involution in the rat and galactopoietic effect observed with oxytocin in ruminents by many other workers (Adams and Allen, 1952; Sprain et al., 1954; Donker et al., 1954) and in the rat by Johnson (1958). However, oxytocin injections have not been shown to alter pituitary prolactin content in rats (Meites and Turner, 1948; Johnson and Meites, 1957), guinea pigs or rabbits (Meites and Turner, 1948). Grosvenor and Turner (1958) reported that injection of oxytocin in anesthetized lactating rats failed to alter pituitary prolactin content from the control prenursing level. Donovan and Vander Werff ten Bosch (1957) reported that milk secretion could be maintained in rabbits in which the hypophyseal portal system was destroyed and which received only oxytocin. These studies are in contrast to the views held by Benson and Folley that oxytocin is the humoral link in anterior pituitary secretions.

Meites (1959) and Meites, Nicoll and Talwalker (in press) have recently shown that acetylcholine, epinephrine and serotinin can initiate milk secretion in virgin female

rats primed with estrogen. These three substances also retarded mammary involution after litter removal on the 4th day postpartum. This suggests that probably one or more of these substances may be involved in the humoral link between the hypothalamus and anterior pituitary, resulting in the release of prolactin and perhaps other hormones favorable to lactation.

### 3. Endocrine Control of Postpartum Lactation in Adrenalectomized Animals.

Carr (1931) reported that removal of adrenals resulted in complete cessation of lactation in rats. These results were confirmed by Swingle and Pfiffner (1932), Brownell et al. (1933), Gaunt (1933), and Britton and Kilne (1936). Meites et al. (1942) showed that adrenalectomy did not prevent initiation of milk secretion at parturition, but lactation was only of short duration, in rats.

In these earlier studies both partial and complete cessation of milk secretion after adrenalectomy had been reported. On the other hand it has been shown by many workers that lactation can be maintained in adrenalectomized rats by administrating adrenal cortical extracts (Nelson and Gaunt, 1937; Schultz, 1937; Gomez and Turner, 1937). The characterization and synthesis of different adrenal cortical hormones stimulated further studies in order to ascertain the role of the adrenals in lactation.

Gaunt (1941) reported that deoxycorticosterone was incapable of maintaining lactation in adrenalectomized rats. However, Nelson and Gaunt (1937) observed that deoxycorticosterone had a slightly beneficial effect upon established lactation in the rats. They concluded that

alterations in the electrolyte and water balance had an adverse effect upon lactation.

Gomez and Turner (1937), while studying the effect of adrenotropic principle on lactation, suggested that a carbohydrate metabolism disturbance appears to be more important than water and electrolyte balance in maintaining lactation. Gaunt et al. (1942) found that in adrenalectomized-lactating rats complete restoration of lactation could be obtained by the administration of 17-hydroxy-11-dehydro corticosterone (cortisone). They concluded that while restoration of normal electrolyte metabolism was helpful for maintenance of normal lactation, the limiting factor for maximum lactation appeared to be glucocorticoids which are concerned with carbohydrate metabolism.

Folley and Cowie (1944) reported that they could obtain better maintenance of lactation in adrenalectomized rats with deoxycorticosterone compared with 11-oxygenated steroids. However they could not obtain complete maintenance with deoxycorticosterone alone (Cowie and Folley, 1947). Cowie (1952) reported complete maintenance of lactation in adrenalectomized rats by administering cortisone and deoxycorticosterone together.

Cowie and Tindal (1957) showed that in lactating goats there was a rapid inhibition of milk secretion after

adrenalectomy. They could obtain partial to complete maintenance of lactation by implanting tablets of cortisone or cortisone acetate and deoxycorticosterone acetate. Deoxycorticosterone acetate appeared to be the more critical component of the combination.

## 4. Galactopoietic Studies in Laboratory and Farm Animals.

Following the demonstration of lactogenic properties of anterior-pituitary extracts by Stricker and Grüter (1928) in ovariectomized, pseudopregnant rabbits, galactopoietic studies of anterior pituitary extracts were carried out in laboratory and farm animals.

The earliest report is that of Azimov and Krauze (1937) who showed that injections of ox-anterior-pituitary into cows produced a marked temporary increase in milk production. They found that the treatment was most effective during the first 4 months of lactation during which time the yield was increased slightly above the natural peak value.

Asdell et al. (1936) reported that in goats the anterior pituitary extracts were effective during the declining phase but not at the peak of lactation. Similar results have been obtained in cows. Folley and Young (1938, 1939, 1940) showed that they could obtain higher milk yeild during the declining phase by administering anterior pituitary extracts on milk secretion in cows was only temporary even when injections were continued over a longer period of time. They also reported that they

could not delay the onset of decline of lactation by administering crude saline extracts. Folley and Young (1945) obtained a sigmoid dose response curve for single injections of crude ox-anterior-pituitary extracts in cows.

Riddle et al. (1933) were the first to extract the lactogenic hormone following its discovery by Stricker and Gruter (1928). This hormonal principle caused enlargement and secretion of the pigeon crop gland. After this discovery various prolactin preparations were prepared and studies were made to determine its galactopoietic effect.

Folley and Young (1938, 1939, 1940) showed that single injections of partially purified prolactin preparations in cows were ineffective in causing increases in milk production during the declining phase of lactation. This was in contrast with the marked galactopoietic effect shown with single injection of anterior pituitary extracts. However they could obtain higher milk yield with repeated injections of high doses of prolactin preparations, but in some cows this initial stimulating effect soon disappeared though the treatment was continued. They also found that their prolactin preparation showed no correlation between prolactin content and galactopoietic effect.

It became apparent that prolactin was not the only pituitary hormone involved in the maintenance of lactation, and the galactopoietic activity of crude anterior pituitary extract was not due to only a single hormone. Folley and Young (1941) suggested the concept of a pituitary galactopoietic hormone-complex. Recent advances in identification, purification and knowledge of chemical and biochemical properties of anterior pituitary hormones, has made it possible to study their galactopoietic activity, singularly and in combination, in order to elucidate the pituitary galactopoietic hormone-complex.

Roy (1947) studied the relative galactopoietic activities of anterior pituitary extracts, prolactin, thyroxine, ACTH, whole anterior pituitary extracts plus thyroxine and ACTH plus prolactin in cows. He found that all hormonal preparations were active in stimulating milk production in cows during the declining phase of lactation. However, the responses to the combined treatment were found to be greater than any single treatment. Cotes et al. (1949b) showed that single injections of 40 mg. purified prolactin had no effect on the milk yield of lactating cows. However, these cows were responsive to unfractioned ox pituitary extracts.

Balmain and Folley (1952) studying the galactopoietic

effects of various anterior pituitary extracts, found that diabetogenic anterior pituitary fractions showed marked galactopoietic activity in the cow. Cotes et al. (1949a) showed that purified growth hormone (GH) from ox anterior pituitary had diabetogenic effects in the intact cat. Cotes et al. (1949b) reported that single injection of purified GH (30 mg.) resulted in marked temporary increase in milk yield during the declining phase of lactation in cows. This galactopoietic effect of GH in cows has been confirmed by other workers (Donker and Petersen, 1951, 1952) in single injection tests, and also by Chung et al. (1953), Wrenn and Sykes (1953) and Brumby and Hancock (1955) who observed substantial increases in milk yield in response to series of daily injections. Jordan and Shaffhausen (1954) have reported marked increases in milk production with GH in lactating ewes.

Shaw (1955) injected cows in various stages of lactation with 50 to 100 mg GH daily for periods varying from a few days to seven weeks. He could elicit and maintain a 25-50 per cent increase in milk production during the injection period. The fat percentage in milk was found to be increased by 120 during a 14 day injection period. It was also found that administration of GH prior to or following parturition resulted in lasting effects in

milk production after GH was discontinued. Shaw suggested that the action of GH on lactation may be that of increasing the availability of milk precursors in the blood, increasing the efficiency of milk secretion or producing an increase in growth of mammary tissue.

Meites and Reineke (1955) studied the effect of GH on lactation in goats. They obtained about a 12-15 per cent increase in milk production by injecting 50 mg. of GH daily for 5 days. These goats were receiving Protamone, a thyroid-active material, before GH treatment was started. Meites and Reineke (1955) showed that the marked decrease in milk production following removal of Protamone could be corrected partially by GH. They interpreted these results as suggesting that the galactopoietic actions of thyroid-active substances may be mediated through an increased secretion of GH by the pituitary. However, additional studies by these workers (unpublished) indicated that the effects of GH and Protamone were exerted independently.

Hutton (1957) studied the effect of GH on the yield and composition of cow's milk. The results obtained showed a highly significant linear relationship between the log weight of a single injection of GH and increase in the milk yield obtained in cows during the decline of lactation. When he decreased the doses of GH, he obtained a significant

increase in fat yield relative to the yield of non-fatty solids. Administration of GH also appeared to increase the efficiency of conversion of food to milk. Hutton suggested that the galactopoietic effect of GH appeared to be due to its ability to increase the functional efficiency of the alveolar cells.

In contrast to the positive findings with GH in cows reported by many workers, there is a report by Flux (1955) who found decreasesin milk yield after single injections of purified growth hormone in the cow (40 mg.) and goat (20 and 30 mg.). The results in goats are also in contrast to that reported by Meites (1955) who obtained a 12-15% increase with GH. The virtually negative results obtained by Hutton may be due to lower doses of GH used (20 and 30 mg.). Meites in his experiment used 50 mg. daily for five days.

Meites (1957) reported that injections of one mg. GH in rats for 12 days starting on the 5th day postpartum had no galactopoietic effect. The mothers receiving GH injections benefited by a significant increase in body weight. Grosvenor and Turner (1959a) claimed about a 40% increase in milk production in rats by daily injection of one mg. of GH during the 7-13th days postpartum. However their method of measuring milk yield is open to criticism.

They considered the amount of milk obtained (stomach content) by a litter of 6 during 30 minutes nursing on the 14th day postpartum as an index of lactation, and yet found no increase in growth by these litters. They also reported that the milk yield was greater when oxytoxin was injected in combination with GH.

It thus appears that GH may be a limiting factor in the decline of lactation in the cow (Cotes et al., 1949b; Folley, 1955; Shaw, 1955; Donker and Petersen, 1951-1952; Chung et al., 1953; Wrenn and Sykes, 1953; and Brumby and Hancock, 1955), sheep (Jordan, 1959), and goat (Meites, 1955). However it does not appear to be a limiting factor in the decline of lactation in the rat.

Roy (1947) reported that injections of ACTH or ACTH and prolactin caused significant increases in milk production in cows. However Cotes et al. (1949b) found that single injections of approximately 100 or 200 I.U. of ACTH in cows caused temporary decreases in milk production rather than an increase. Similar results were reported by Flux et al. (1954). Shaw (1955) reported that administrations of purified ACTH (100-300 I.U.), hydrocortisone alcohol, cortisone acetate or hydrocortisone acetate (5 gms.) caused pronounced decreases in milk production in cows. He also found that the decline in milk production due to ACTH administration could be corrected by simultaneous administration of GH in cows.

Meites (1955) reported that administration of cortisone, 100 mg./day for 5 days did not cause any reduction in milk production in goats. Johnson and Meites (1958) showed that daily injection of 0.5 mg. cortisone acetate in rats resulted in a significant rise in milk production, as judged by growth rate of litters during an 18 day postpartum period. Apparently pure corticosteroids vary in their effectiveness in replacing the adrenals in lactation. ACTH might favor or antagonize lactation, depending upon the quantity and quality of steroidal output by the adrenals.

Although thyroid-active substances were not used in the present study, they are briefly considered here since they have been shown to increase milk production in ruminants. The earlier experiments by Graham (1934a) showed that either thyroxine injection (1934b) or feeding of dried thyroid gland (1934a) increased the milk and fat yeild of dairy cows. These results were confirmed with goats and cows by many other workers (Jack and Bechdel, 1935; Folley and White, 1936; Herman et al., 1938). On the other hand complete thyroidectomy or thiourea feeding have been shown to reduce milk secretion in cows (Blaxter et al., 1949). Experiments with iodinated casein, first reported by Reineke (1942); Reineke and Turner (1942) and confirmed by others indicated that properly prepared

iodinated casein has galactopoietic effects in ruminants. However great variability in milk production has been obtained by feeding iodinated casein or injecting thyroxine. These variations are due to a number of factors such as the dose employed, physiological status of the cow, length of treatment, nutritional status, etc. (Blaxter, et al., 1949).

Chen et al. (1955) reported that the thyroid was unnecessary for mammary development and milk secretion in hypophysectomized, oophorectomized, adrenalectomized and thyroidectomized rats. Lyons et al. (1958) and Cowie (1957) found that thyroxine had no effect in enhancing milk secretion alone or in combination with prolactin and ACTH or cortisone in hypophysectomized rats.

Meites (unpublished) observed that thyroxine had no effect in retarding mammary involution and maintaining milk secretion after litter removal on the 4th day postpartum in rats. On the other hand Desclin (1949) and Grosvenor and Turner (1959b) reported increases in milk secretion in rats following daily administrations of l-thyroxine. The amount of milk obtained by a litter of 6 young during 30 minutes suckling on the 14th day postpartum was used as the index of response by Grosvenor and Turner. This method of accessing the milk yield is open to criticism, since the young did not show any increase in growth rate despite an apparent increase in milk intake.

# II

## MATERIALS AND METHODS

It is difficult to accurately measure lactational responses in small laboratory animals when compared to larger animals such as the cow and goat, in which, by use of milking machines, the milk can be almost quantitatively withdrawn. Since it is difficult to measure milk output, it is necessary to employ survival of litters and litter growth curves as measures. In these experiments litter growth rates have been employed. This is not an absolute but only relative index of lactational response, since the daily weight loss due to excreta and insensible perspiration are not measured.

Cowie (1946) used a logistic equation and plotted a mean growth curve for litters of lactating dams from birth to 16 days of age and obtained a sigmoid curve. The points from the 6th to 11th days of lactation, in this curve fall on a straight line. He found that during this 5 day period the average increment in litter weight was approximately constant as well as at a maximum for each rat. The weight gain of litters during this period, Cowie termed the "litter growth index" and he used it as a quantitative measure for lactational responses in rats. This is not believed to be a true reflection of milk yield but is

estimated by Cowie to be roughly 50-60 per cent of the true output.

Johnson (1957) showed that cortisone administration during the initial phase of lactation (0-5th day) did not have any significant effect on litter growth. However significant increases over that of the controls were obtained when cortisone was administered during later periods of lactations, 6th-10th days and 11th-18th days. Therefore in this experiment it was decided to carry out hormonal treatments during the 4th-17th days of lactation.

In Experiment I, 90 mature female albino rats of the Carworth strain were bred. Once pregnancy was established, the dams were placed in individual cages. These cages were designed to make the dam's ration inaccessible to the young throughout the experimental period. This insured that the young received food only from their mothers. The food and water was available to dams ad libitum. The animals were kept in an air-conditioned room with a temperature of 74±1° F throughout the experiment. Constant artificial lighting was provided in the room from 7:30 A.M. until 9:30 P.M. The rats weighed between 220-275 gms. at the beginning of the experiment.

From the day of parturition to the 18th day of lactation the rats and litters were weighed daily at approximately the

same hour of each day. On the 4th day after parturition the litters were reduced to 6 young each. This gives a better chance for survival of the young and standardizes the number per group.

The litters were randomly divided into 9 groups of uniform weight. All injections were administered subcutaneously, daily, the 4th day postpartum to the 17th day postpartum. On the 18th day the dams were killed. The ovaries and adrenals were removed, dissected free from connective tissue and their weights were recorded. Similarly the pituitary was removed and weighed on a Roller-Smith balance.

The following injection schedule was carried out once daily except for oxytocin which was injected twice daily:

(1) Controls, saline (0.85%).

(2) Hydrocortisone acetate, 0.25 mg.

(3) Hydrocortisone acetate, 0.5 mg.

(4) Hydrocortisone acetate, 1.0 mg.

(5) Prolactin (20 I.U.), 1 mg.

(6) Oxytocin (20 I.U./mg.), 1 I.U.

(7) Hydrocortisone acetate, 0.25 mg.; prolactin, 1 mg. and oxytocin, 1 I.U.

(8) Hydrocortisone acetate, 0.5 mg.; prolactin, 1 mg. and oxytocin, 1 I.U.

(9) Hydrocortisone acetate, 1 mg.; prolactin, 1 mg. and oxytocin, 1 I.U.

All injections were given in a 0.1 cc. solution.

In Experiment II, 20 mature female albino rats of the Carworth strain were bred. On the 2nd day after parturition both the ovaries were removed under light ether anesthesia. The rats were divided into two groups. Group I received saline daily while Group II received 1 mg. prolactin (20 I.U./ mg.) daily during the 4th-17th days postpartum. Dams and litters of six young each were weighed daily. On the 18th day postpartum the dams were killed and the adrenals, ovaries and pituitary were removed and weighed on a Roller-Smith balance.

# III

# EXPERIMENTAL RESULTS

## 1. Effects of Hydrocortisone Acetate, Oxytocin and Prolactin on Lactational Performance in Rats

The results of this experiment are summarized in Tables 1-4. Dams receiving saline, oxytocin or prolactin gained in average body weight by about 20-25 gms. during the 18 days period. Dams receiving 0.25 or 0.5 mg. hydrocortisone acetate gained in average body weight by only 7-10 gms. However the dams which received 1.0 mg. hydrocortisone acetate lost in average body weight by about 8 gms. during the 18 days period.

No significant differenceswere found in the average weights of pituitaries in the groups. However the average weights of the adrenals and ovaries are significantly lower than the controls in the groups which received 0.5 and 1.0 mg. hydrocortisone respectively.

There was no significant difference in average litter growth rate, total and daily, during the 6-10 day period (Cowie's litter growth index) between the controls and the groups receiving oxytocin, prolactin, or hydrocortisone acetate at a dose of 0.25 mg. These groups also did not show any significant difference in their average litter growth rate during the 11-18th days or 4-18th days from the controls.

The group which received 0.5 mg. hydrocortisone acetate daily, showed significant increases over the controls in average litter growth rate, total and daily, during the first 6-10 days period (Cowie's litter growth index), 11-18 days period and 4-18 days period. This group gained approximately 18 per cent more in average body weight during 4-18 day period than the controls. The group which received 1 mg. hydrocortisone acetate showed a significant decrease in average litter weight gain during the 4-18 days period. However there was no significant difference during the first 6-10 days between the groups receiving 0.5 and 1.0 mg. of hydrocortisone acetate, respectively.

There were no significantly greater increases in average litter growth rate when the rats were given 1 I.U. oxytocin twice daily and 1 mg. prolactin in addition to 0.25, 0.5 or 1.0 mg. hydrocortisone acetate. Thus oxytocin and prolactin do not appear to have any synergistic effects with hydrocortisone acetate in promoting average litter growth rate. The magnitude of the response with 0.5 mg. hydrocortisone acetate was the same when given alone or in combination with 1 mg. prolactin and 1 I.U. oxytocin.

Figure 1 shows the average daily weight gains of the

young of the control group and the groups receiving 0.25, 0.5 and 1.0 mg. hydrocortisone acetate, respectively. Figure 2 shows the average daily weight gains of the young of the control group and the groups receiving 0.25, 0.5 and 1.0 mg. hydrocortisone acetate, respectively, in addition to 1 mg. prolactin and 1 I.U. oxytocin twice daily.

2. Effects of Prolactin on Lactational Performance in Oophorectomized Rats

The results of this experiment are summarized in Tables 5 and 6. Dams in both groups gained an average of 20 gms. in body weight during the 18-day postpartum period. No significant difference in the average weight of the pituitary were observed between groups. Also no significant differences in average litter growth rate, total and daily, during the first 6-10 days (Cowie's litter growth index), 11-18th days and 4-18th days postpartum were observed between the two groups.

TABLE 2

Effects of Hydrocortisone Acetate, Prolactin and Oxytocin in Combination on Body, Adrenal, Ovarian and Pituitary Weights of Lactating Rats

| Group No. and Treatment | Av. Litter Size At Birth | Av. Dam Wt. at Parturition gms. | Av. Dam Wt. at 18 Days Postpartum gms. | Av. Adrenal Wt. at 18 Days Postpartum mg./100 gms. BW. | Av. Pituitary Wt. at 18 Days Postpartum mg./100 gms. BW. | Av. Wt. of Ovaries at 18 Days Postpartum mg./100 gms. BW. |
|---|---|---|---|---|---|---|
| 1) Controls Saline daily (10)* | 11.8 | 251.9 ±9.2** | 276.6 ±9.0 | 27.06 ±1.24 | 4.63 ±0.32 | 29.10 ±1.41 |
| 2) Hydrocortisone 0.25 mg. daily, Prolactin 1 mg. daily Oxytoxin 1 I.U. twice daily (10) | 10.6 | 263.2 ±9.7 | 283.2 ±10.2 | 27.13 ±1.51 | 4.93 ±0.39 | 29.72 ±1.44 |
| 3) Hydrocortisone 0.5 mg. daily Prolactin 1 mg. daily Oxytocin 1 I.U. twice daily (10) | 10.7 | 262.2 ±8.9 | 256.2 ±10.8 | 23.5 ±1.1 | 5.24 ±0.47 | 25.65 ±1.39 |
| 4) Hydrocortisone 1.0 mg. daily Prolactin 1 mg. daily Oxytocin 1 I.U. twice daily (10) | 12.3 | 257.8 ±10.1 | 248.8 ±12.4 | 19.18 ±1.22 | 4.32 ±0.42 | 24.44 ±1.28 |

* Number of rats per group
** Standard error of the mean

TABLE 3

Effects of Hydrocortisone Acetate, Prolactin and Oxytocin on Body Weights of Litters

| Group No. and Treatment | Average Litter Growth Rate 6–10th days Total gms. | 6–10th days Daily+ gms. | 11–18th days Total gms. | 11–18th days Daily gms. | 4–18th days++ Total gms. | 4–18th days++ Daily gms. |
|---|---|---|---|---|---|---|
| 1) Controls Saline daily (10)* | 44.8 ±4.12** | 8.96 ±0.51 | 79.7 ±5.91 | 9.96 ±0.60 | 157.1 ±8.36 | 10.47 ±0.67 |
| 2) Hydrocortisone 0.25 mg. daily (10) | 50.0 ±4.57 | 10.0 ±0.54 | 89.4 ±6.66 | 11.17 ±0.74 | 165.7 ±8.77 | 11.71 ±0.81 |
| 3) Hydrocortisone 0.5 mg. daily (10) | 53.5 ±3.91 | 10.71 ±0.48 | 93.0 ±5.96 | 11.62 ±0.68 | 184.9 ±9.67 | 12.32 ±0.61 |
| 4) Hydrocortisone 1 mg. daily (10) | 49.7 ±3.12 | 9.94 ±0.61 | 86.5 ±6.24 | 10.81 ±0.59 | 164.4 ±10.1 | 10.94 ±0.78 |
| 5) Oxytocin 1 I.U. twice daily(10) | 45.3 ±4.12 | 9.08 ±0.49 | 77.8 ±7.01 | 9.72 ±0.70 | 153.6 ±8.62 | 10.24 ±0.71 |
| 6) Prolactin 1 mg. daily (10) | 44.1 ±3.93 | 8.82 ±0.57 | 76.9 ±5.81 | 9.61 ±0.82 | 152.1 ±9.45 | 10.14 ±0.81 |

* Number of litters per group, 6 pups per litter

** Standard error of the mean

\+ Litter growth index

++ Total period of treatment

TABLE 4

Effect of Hydrocortisone Acetate, Prolactin and Oxytocin in Combination on Body Weights of Litters

| Group No. and Treatment | Average Litter Growth Rate 6-10th days Total gms. | 6-10th days Daily+ gms. | 11-18th days Total gms. | 11-18th days Daily gms. | 4-18th days++ Total gms. | 4-18th days++ Daily gms. |
|---|---|---|---|---|---|---|
| 1) Controls Saline daily (10)* | 44.8 ±4.12** | 8.96 ±0.51 | 79.7 ±5.91 | 9.96 ±0.60 | 157.1 ±8.36 | 10.47 ±0.67 |
| 2) Hydrocortisone 0.25 mg. daily Prolactin 1 mg. daily Oxytocin 1 I.U. twice daily (10) | 47.9 ±3.91 | 9.58 ±0.48 | 88.1 ±6.82 | 11.01 ±0.79 | 170.4 ±9.45 | 11.36 ±0.72 |
| 3) Hydrocortisone 0.5 mg. daily Prolactin 1 mg. daily Oxytocin 1 I.U. twice daily (10) | 57.4 ±3.67 | 11.48 ±0.45 | 93.2 ±6.79 | 11.65 ±0.72 | 189.0 ±8.88 | 12.60 ±0.74 |
| 4) Hydrocortisone 1 mg. daily Prolactin 1 mg. daily Oxytocin 1 I.U. twice daily (10) | 55.8 ±4.23 | 10.16 ±0.56 | 83.5 ±5.82 | 10.43 ±0.84 | 165.9 ±9.96 | 11.06 ±0.86 |

* Number of litters per group, 6 pups per litter
** Standard error of the mean
\+ Litter growth index
++ Total period of treatment

TABLE 5

Effect of Prolactin on Body Weights of
Litters of Oophorectomized Lactating Rats

| | Group No. and Treatment | Average Litter Growth Rate: 6–10th days Total gms. | 6–10th days Daily+ gms. | 11–18th days Total gms. | 11–18th days Daily gms. | 4–18th days++ Total gms. | 4–18th days++ Daily gms. |
|---|---|---|---|---|---|---|---|
| +++ | 1) Controls | 45.4 | 9.08 | 81.6 | 10.20 | 158.9 | 10.60 |
| | Saline daily (10)* | ±3.89** | ±0.54 | ±5.42 | ±0.68 | ±10.12 | ±0.74 |
| +++ | 2) Prolactin 1 mg. | 47.3 | 9.46 | 84.2 | 10.52 | 163.4 | 10.86 |
| | daily (10) | ±4.11 | ±0.57 | ±5.89 | ±0.76 | ±9.87 | ±0.69 |
| | 3) Controls (Intact) | 44.8 | 8.96 | 79.7 | 9.96 | 157.1 | 10.47 |
| | Saline daily (10) | ±4.12 | ±0.51 | ±5.91 | ±0.60 | ±8.36 | ±0.67 |

* Number of litters per group, 6 young per litter

** Standard error of the mean

\+ Litter growth index

++ Total period of treatment

+++ Oophorectomized rats

TABLE 6

Effect of Prolactin on Body, Adrenal, and Pituitary Weights of Oophorectomized Lactating Rats

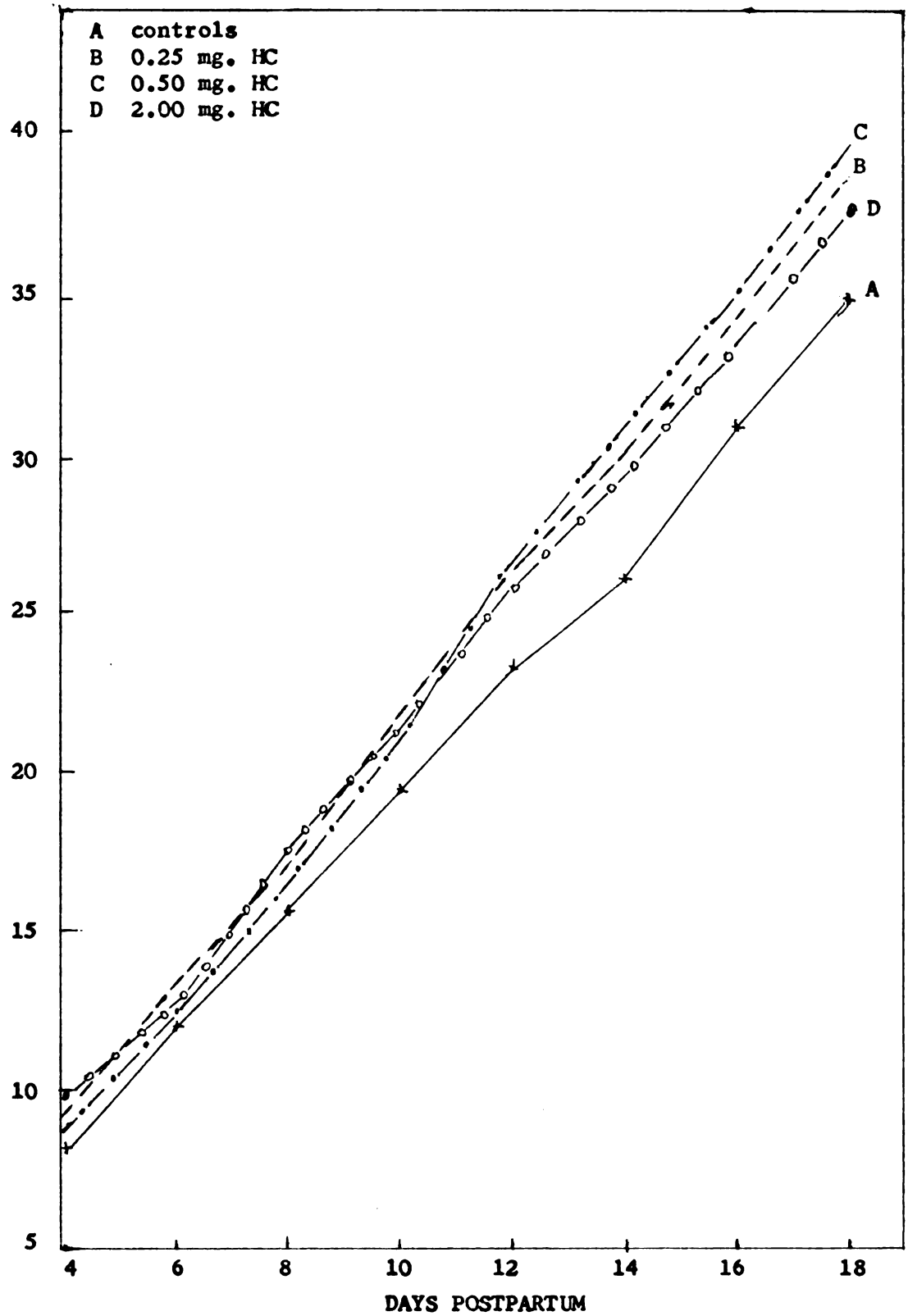
| | Av. Litter Size | Av. Dam Wt. at Parturition gms. | Av. Dam Wt. at 18 Days Postpartum gms. | Av. Adrenal Wt. at 18 Days Postpartum gms. | Av. Pituitary Wt. at 18 Days Postpartum mg./100 gms. BW. |
|---|---|---|---|---|---|
| + 1) Controls saline daily (10)* | 10.8 | 258.6 ±8.4** | 279.2 ±8.8 | 28.12 ±1.17 | 5.16 ±0.42 |
| + 2) Prolactin 1 mg. daily (10) | 11.0 | 252.4 ±8.9 | 273.8 ±9.4 | 27.45 ±1.41 | 4.67 ±0.33 |
| 3) Controls (Intact) saline daily (10) | 11.8 | 251.9 ±9.2 | 276.6 ±9.0 | 27.06 ±1.24 | 4.63 ±0.32 |

* Number of rats per group

** Standard error of the mean

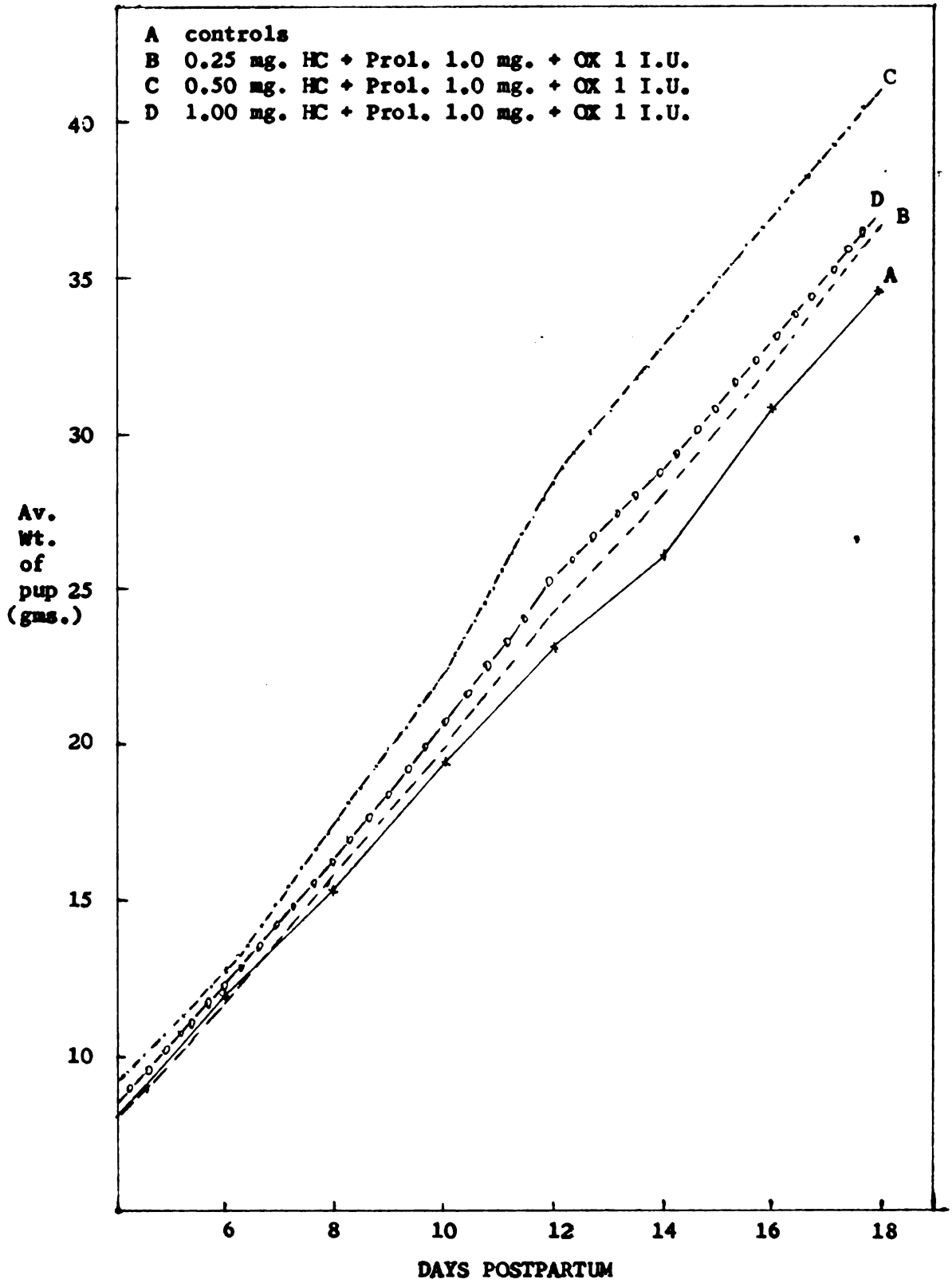
\+ Oophorectomized rats

FIGURE 1




Effect of Hydrocortisone acetate on Litter Weight Gains in the Rat

FIGURE 2




Effect of Hydorcortisone acetate, Prolactin and Oxytocin on Litter Weight Gains in the Rat

# IV

# DISCUSSION

It has been very well established that the adrenals are essential for the maintenance of lactation in rats (Nelson and Gaunt, 1937; Cowie and Folley, 1947) and in goats (Cowie and Tindal, 1957). Lyons (1958) observed that in hypophysectomized rats prolactin and ACTH or a glucocorticoid are the minimum hormones necessary to maintain lactation. It has also been reported that the suckling stimulus causes release of ACTH from the pituitary, which would subsequently induce release of adrenal cortical steroids (Tabachnick and Trentin, 1951; Gregoire, 1946). From these studies it appears that ACTH or a corticoid could be a limiting factor in the decline of lactation. However, the galactopoietic studies with ACTH or corticoids in ruminants have been reported to have either inhibitory effect (Cotes et al., 1949b; Flux et al., 1954; Shaw, 1955) or no effect (Meites, 1955), the exception being the report by Roy (1947) who obtained stimulation of milk secretion in the cow.

The results of the present study indicate that hydrocortisone acetate can increase the milk secretion during the 5-10 day period (Cowie's litter growth index) and during the remaining period of lactation in rats. These findings confirm the results obtained by

Johnson and Meites (1958) with cortisone acetate. The daily dose of 0.5 mg. hydrocortisone acetate appears to be optimal. With a lower dose (0.25 mg. daily) of hydrocortisone acetate there was slight stimulation but this was not significant. However at a higher dose level (1 mg. daily) the rat mothers lost considerable body weight and there was a slight decrease in milk secretion as judged by litter growth rate. Johnson (1958) showed that when cortisone was administered in combination with GH and prolactin to lactating rats, they did not lose body weight, but the lactational response was of the same magnitude as with cortisone alone. Also Lyons (1958) reported that the hypophysectomized lactating rats given prolactin and prednisolone acetate lost considerably more in body weight than rats also given GH. However, the litter growth rates in both cases were essentially similar.

These results present one additional support to the earlier findings that the adrenal cortical hormones are essential for the maintenance of milk secretion in rats. Hydrocortisone acetate was effective in increasing milk secretion during lactation, probably because the adrenal cortex of the rat secretes less than optimal amounts of glucocorticoids. It is probable that ruminants, in contrast, already secrete sufficient amounts of adrenal

cortical hormones for lactation, and hence additional amounts are harmful, producing catabolic rather than anabolic effects. It is known that physiological doses of adrenal glucocorticoids are essential for normal metabolism of carbohydrate, protein and fat, whereas large amounts are detrimental to these processes.

It has been reported that injections of prolactin to lactating mother rats after removal of their litters inhibit involutionary changes in the mammary gland (Williams, 1945; Johnson, 1957). It has also been reported that in hypophysectomized rats prolactin together with an adrenal corticoid is essential for the maintenance of milk secretion (Cowie, 1957; Bintarningsih et al., 1957; Lyons et al., 1958). The results of the present study indicate that daily injections of 1 mg. of prolactin (20 I.U./mg.) had no effect on the lactational performance in rats. These results corroborate the negative findings in ruminants, but are in disagreement with the positive findings reported by Johnson (1958) in the rat. The principal difference between this experiment and that of Johnson (1958) is that a different prolactin preparation was employed and the diets were not the same.

In the rat prolactin has been shown to have luteotropic

activity. Moore and Nolbandov (1955) have also reported that prolactin is luteotropic in sheep. Lyons (1958b) suggested that prolactin, injected in rats bearing corpora lutea during lactation, might be utilized by the corpora lutea. This would promote secretion of progesterone, and thus stimulate mammary growth and antagonize lactation. However, prolactin, at the dose level used, did not show any stimulating effect on milk secretion in either oophorectomized or intact lactating rats. Therefore it appears that during lactation the suckling stimulus releases prolactin in sufficient amounts to luteinize ovaries and maintain lactation. The presence of luteinized ovaries in the lactating rat were shown not to interfere in any way with milk production even when the rats were injected with prolactin, which presumably stimulated greater luteal secretion by the ovaries.

The suckling stimulus during lactation has been shown to cause the release of oxytocin from the neurohypophysis (Petersen and Ludwick, 1942; Cross and Harris, 1950, 1951). Oxytocin acts on the myoepithelial cells surrounding the alveoli, causing contraction, and thus squeezes out the milk from the lumen of the mammary alveoli. This milk ejection effect of oxytocin has been very well established. There are various reports that regular injections of oxytocin

have galactopoietic effects in lactating cows (Adams and Allen, 1952; Sprain et al., 1954; Donker et al., 1954). Johnson (1958) reported that oxytocin had galactopoietic effects in rats during lactation. It has been also shown that oxytocin inhibits mammary involution after litter removal on the 4th day of parturition in rats (Benson and Folley, 1957; Meites and Nicoll, 1959). In the present study injections of oxytocin 1 I.U. twice daily in lactating rats appear to have no galactopoietic effect. This is in contrast to the findings of Johnson (1958). The same doses of oxytocin were used in both experiments, and the same strain of rats were employed. However, the diets were not the same in the two experiments and this might have accounted for the difference in results.

# V

## SUMMARY AND CONCLUSIONS

1. The effects of administering 0.25, 0.5 or 1.0 mg. of hydrocortisone acetate, 1 mg. (20 I.U./mg.) prolactin or 1 I.U. oxytocin, singularly or all 3 in combination were studied on the lactational performance of postpartum rats. In a second experiment the effects of prolactin on lactational performance of postpartum rats ovariectomized on the 2nd postpartum day were observed. The growth rate of litters of 6 young each during the 4th-18th days postpartum was used as an index of milk production.

2. When hydrocortisone acetate, oxytocin or prolactin were injected individually in lactating rats, only hydrocortisone acetate at the 0.5 mg. dose level increased milk secretion significantly. When all three hormones were injected in combination, the increases in lactation were no greater than with hydrocortisone acetate alone. Hydrocortisone acetate was effective in increasing milk secretion, perhaps because the adrenal cortex of the rat secretes less than optimal amounts of hormones during lactation. Oxytocin and prolactin were not effective in increasing

milk secretion. These two hormones are probably secreted in sufficient amounts during lactation in the rat, and hence additional amounts of oxytocin and prolactin might have no effect.

3. Prolactin at the dose level used did not induce a significant increase in milk secretion over that of saline injected controls in ovariectomized lactating rats. Also no significant difference in milk secretion was observed between the ovariectomized and intact controls. These results suggest that the suckling stimulus during lactation causes release of sufficient prolactin from anterior pituitary in sufficient amounts to luteinize the ovaries and maintain milk secretion. These results also show that the functional corpus-luteum during lactation has no inhibitory effect on milk secretion in the rat.

# BIBLIOGRAPHY


Adams, H. P. and Allen, N. N. The effect of removal of residual milk by the use of oxytocin upon the yield and fat content of subsequent milkings, J. Dairy Sci. 35:112, 1952.

Asdell, S. A., Brooks, H. J., Salisbury, G. W and Seidenstein, H. R. Experiments in the physiology of mammary development and lactation. Mem. Cornell Univ. Agri. Exptl. Sta. No. 198, 1936.

Azimov, G. J., and Krouze, N. K. The lactogenic preparations from the anterior pituitary and the increase of milk yield in cows. J. Dairy Sci. 20:289, 1937.

Balmain, J. H., Folley, S. J. and Glascock, R. F. Inhibition of fatty acid synthesis in rat mammary gland slices by cortisone in vitro and its antagonism by insulin. Nature 169:447, 1952.

Benson, G. K. and Folley, S. J. Oxytocin as a stimulator for the release of prolactin from anterior pituitary. Nature 177:700, 1956.

Benson, G. K. and Folley, S. J. Retardation of mammary involution in the rat by oxytocin. J. Endocrinology 14:XI, 1956.

Benson, G. K. and Folley, S. J. Effect of oxytocin on mammary gland involution in the rat. J. Endocrinology 16:189, 1957.

Bergman, A. J. and Turner, C. W. The Specificity of lactogenic hormone in the initiation of lactation. J. Dairy Sci. 23:1229, 1940.

Bintarningsih, Lyons, W. R, Johnson, R. E. and Li, C. H. Hormonal requirement for lactation in hypophysectomized rats. Anat. Rec. 127:266, 1957.

Blaxter, K. L., Reineke, E. P., Cramton, E. W., Petersen, W. E., The Role of thyroidal materials and of synthetic goitrogens in animal production and an appraisal of their practical use. J. Anim. Sci. 8:318, 1949.

Britton, S. W. and Kline, R. F. Relation of the adrenal cortex to reproduction and lactation. Am J. Physiol. 115:266, 1936.

Brownell, K. A., Lockwood, J.E. and Hartman, F. A. A lactation hormone of the adrenal cortex. Proc. Soc. Exptl. Biol. 30:783, 1933.

Bruce, H. M. Suckling stimulus and lactation. Proc. Royal Soc. 149:421, 1958.

Carr, J. L. Effect of ovaries in various stages of activity and of pregnancy upon adrenalectomized rats. Proc. Soc. Exptl. Biol. 29:128, 1931.

Chen, T. T., Johnson, R. E., Lyons, W. R., Li, C. H. and Cole, R. D. Hormonally induced mammary growth and lactation in the absence of the thyroid. Endocrinology 57:153, 1955.

Chung, A. C., Shaw, J. C. and Gill, W. M. Effect of somatotropin upon milk production and various blood substances of lactating cows. J. Dairy Sci. 36:589, 1953.

Cotes, P. M., Reid, E. and Young, F. G. Diabetogenic action of pure anterior pituitary growth hormone. Nature 164:209, 1949a.

Cotes, P. M., Crichton, J. A., Folley, S. J. and Young, F. G. Galactopoietic activity of purified anterior pituitary growth hormone. Nature 164:992, 1949b.

Cowie, A. T. Influence on the replacement value of some adrenal-cortex steroids of dietary sodium and synergism of steroids in lactating adrenalectomized rats. Endocrinology 51:217, 1952.

Cowie, A. T. Maintenance of lactation in rat after hypophysectomy. J. Endocrinology 16:135, 1957.

Cowie, A. T. and Folley, S. J. The measurement of lactational performance in the rat in studies of the endocrine control of lactation. J. Endocrinology 5:9, 1947.

Cowie, A. T. and Tindal, J. S. Maintenance of lactation in adrenalectomized rats with aldosterone and 9,a-halo derivatives of hydrocortisone. Endocrinology 56:612, 1955.

Cowie, A. T. and Tindal, J. S. Adrenalectomy in goat: Replacement therapy and the maintenance of lactation. J. Endocrinology 16:403, 1957.

Cross, B. A. and Harris, G. W. The role of the neurohypophysis in the milk ejection reflex. J. Endocrinology 8:148, 1952.

Cross, B. A. and Harris, G. W. Milk ejection following electrical stimulation of the pituitary stalks in rabbits. Nature 166:999, 1950.

Cross, B. A. and Harris, G. W. The neurohypophysis and "let-down" of milk. J. Physio. 113:35P, 1951.

Desclin, L. Effet de l'administration de thyroxine a des rats femelles en lactation sur la croissance des jeunes. C.R. Soc. Biol. Paris 143:1156, 1949.

Donker, J. D. and Petersen, W. E. The milk secretion response of the bovine to injected growth hormone. J. Anim. Sci. 10:1074, 1951.

Donker, J. D. and Petersen, W. E. Hormones in lactation: Administration of hormones in declining phases of lactation. J. Dairy Sci. 35:503, 1952.

Donker, J. D., Koshi, J. H. and Petersen, W. E. The influence of oxytocin-induced udder evacuation on milk and butterfat production in a complete lactation. J. Dairy Sci. 37:299, 1954.

Donovan, B. T. and Van der Werff ten Bosch, J. J. J. Dairy Sci. 37:299, 1954.

Ely, F. and Petersen, W. E. Factors involved in the ejection of milk. J. Dairy Sci. 24:211, 1941.

Flux, D. S. (ed.), Hypophyseal Growth Hormone, Nature and Actions, pp. 476-479, Blackston Division, McGraw-Hill Book Co., Inc., New York, 1955.

Flux, D. S., Folley, S. J. and Rowland, S. J. The effect of adrenocorticotropic hormone on the yield and composition of the milkof the cow. J. Endocrinology 10:133, 1954.

Folley, S. J. (ed.) Hypophyseal Growth Hormone, Nature and Actions, pp. 493-494, Blackston Division, McGraw-Hill Book Co., Inc., New York, 1955.

Folley, S. J. and Cowie, A. T. Adrenalectomy and replacement therapy in lactating rats. Yale J. Biol. Med. 17:67, 1944.

Folley, S. J. and Young, F. G. The effect of anterior pituitary extracts on established lactation in the cow. Proc. Royal Soc. London, B. 126:45, 1938.

Folley, S. J. and Young, F. G. The effect of continued treatment with anterior pituitary extracts on milk volume and milk fat production in the lactating cow. Biochem. J. 33:192, 1939.

Folley, S. J. and Young, F. G. Further experiments on the continued treatment of lactating cows with anterior pituitary extracts. J. Endocrinology 2:226, 1940.

Folley, S. J. and Young, F. G. Effects of estrogens on lactation. Lancet 240:380, 1941.

Folley, S. J. and Young, F. G. The galactopoietic action of pituitary extracts in lactating cows 1. dose-response relations and total yields during declining lactation. J. Endocrinology 4:194, 1945.

Folley, S. J. and White, P. The effect of thyroxine on milk secretion and on the phosphatase of the blood and milk of the lactating cow. Proc. Royal Soc. London, B. 120:346, 1936.

Gaines, W. L. A contribution to the physiology of lactation. Am J. Physiol. 38:285, 1915.

Gaunt, R. Adrenalectomy in the rat. Am. J. Physiol. 103:494, 1933.

Gaunt, R. Inability of deoxycorticosterone to maintain lactation. Proc. Soc. Expl. Biol. Med. 47:28, 1941.

Gaunt, R., Eversoll, W. J. and Kendall, E. C. Influence of some steroid hormones on lactation in adrenalectomized rats. Endocrinology 31:84, 1942.

Gomez, E. T. The relation of posterior hypophysis in the maintenance of lactation in hypophysectomixed rats. J. Dairy Sci. 22:4, 1939.

Gomez, E. T. Effects of post-hypophyseal extract on lactation in hypophysectomized post gravid rats. J. Dairy Sci. 23:537, 1940.

Gomez, E. T. and Turner, C. W. Effect of hypophysectomy on development and function of the mammary gland. J. Dairy Sci. 19:450, 1936a.

Gomez, E. T. and Turner, C. W. Initiation and maintenance of lactation in hypophysectomized guinea pigs. Proc. Soc. Exptl. Biol. Med. 35:365, 1936b.

Gomez, E. T. and Turner, C. W. The adrenotropic principle of the pituitary in relation to lactation. Proc. Soc. Exptl. Biol. Med. 36:78, 1937.

Graham, W. R., Jr. The effect of thyroidectomy and thyroid feeding on milk secretion and milk fat production of cows. Jour. Nutr. 7:907, 1939a.

Graham, W. R., Jr. The action of thyroxine on milk and fat production of cows. Biochem. J. 28:1368, 1934b.

Gregoire, C. Factors involved in maintaining involution of the thymus during suckling. J. Endocrinology 5:68, 1946.

Grosvenor, C. E. and Turner, C. W. Effects of oxytocin and blocking agents upon pituitary lactogen discharge in lactating rats. Proc. Soc. Exptl. Biol. Med. 97:463, 1958.

Grosvenor, C. E. and Turner, C. W. Effect of growth hormone and oxytocin upon milk yield in the lactating rat. Proc. Soc. Exptl. Biol. Med. 100:158, 1959a.

Grosvenor, C. E. and Turner, C. W. Thyroid hormone and lactation in the rat. Proc. Soc. Exper. Biol. Med. 100:162, 1959b.

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

Harris, G. W. The central nervous system, neurohypophysis and milk ejection. Proc. Royal Soc., B. 149:336, 1958.

Herman, H. A., Graham, W. R. Jr. and Turner, C. W. The effect of thyroid and thyroxine on milk secretion in dairy cattle. Mo. Agr. Exp. Sta. Res. Bul. 275.

Hill, R. T., Turner, C. W., Uren, A. W. and Gomez, E. T. Hypophysectomy of the goat. Mo. Agr. Exp. Sta. Res. Bul. 230, 1935.

Houssay, B. A. Action de L'hypophysectomie sur la grossesse et la secretion lactee chez la chienne. compt. rend. soc. biol. 120:496, 1936.

Hutton, J. B. The effect of growth hormone on the yield and composition of cow's milk. J. Endocrinology 16:115, 1957.

Jack, E. L. and Bechdel, S. I. A study of the influence of thyroxine on milk secretion. J. Dairy Sci. 18:195, 1935.

Johnson, R. M. and Meites, J. Effects of cortisone, hydrocortisone, and ACTH on mammary growth and pituitary prolactin contents of rats. Proc. Soc. Exptl. Biol. Med. 89:455, 1955.

Johnson, R. M. and Meites, J. Effects of suckling, pitocin and cervical stimulation on release of prolactin in the parturient rat. J. Animal Sci. 16:1045, 1957a.

Johnson, R. M. Ph.D. thesis, Michigan State University, 1957b.

Johnson, R. M. and Meites, J. Effect of cortisone acetate on milk production and mammary involution in parturient rats. Endocrinology 63:290, 1958.

Jordan, R. M. and Shaffhausen, D. D. Effect of somatotropin on milk yield of ewes. J. Anim. Sci. 13:706, 1954.

Lyons, W. R. Hormonal synergism in mammary growth. Proc. Royal Soc. London, B. 149:313, 1958.

Lyons, W. R., Johnson, R. E., Li, C. H. Recent Progress in Hormone Research, Chapter II, pp. 228-245, Academic Press Inc., New York, 1958.

Meites, J. (ed.) Hypophyseal Growth Hormone, Nature and Actions, pp. 493-95, Blackston Division, McGraw-Hill Book Co., Inc., New York, 1955.

Meites, J. Effect of growth hormone on lactation and body growth of parturient rats. Proc. Soc. Exptl. Biol. Med. 96:730, 1957.

Meites, J. Induction and maintenance of mammary growth and lactation in rats with acetylcholine or epinephrine. Proc. Soc. Exptl. Biol. Med. 100:750, 1959.

Meites, J. and Nicoll, C. S. Hormonal prolongation of lactation for 75 days after litter removal in parturient rats. Fed. Proc. 18:103, 1959.

Meites, J., Nicoll, C. S. and Talwalker, P. K. (in press) 1959, Proc. Soc. Exptl. Biol. Med., Effect of serotonin and reserpine on mammary growth and lactation.

Meites, J., Trentin, J. J. and Turner, C. W. Effect of adrenalextomy on the lactogenic hormone and initiation of lactation. Endocrinology 31:607, 1942.

Meites, J. and Turner, C. W. Studies concerning the induction and maintenance of lactation I. The mechanism controlling the initiation of lactation at parturition. II. The normal maintenance and experimental inhibition of milk secretion in goats with diethylstilbesterol. Mo. Agr. Exptl. Sta. Res. Buls. 415 and 416, 1948.

Moore, W. W. and Nalbandov, A. V. Maintenance of corpora leutea in sheep with lactogenic hormone. J. Endocrinology 13:18, 1955.

Nelson, W. O. and Gaunt, R. The adrenal pituitary relationship. Cold Spring Harbour, Symposia Quant. Biol. 5:398, 1937.

Nelson, W. O. and Gaunt, R. and Schweizer, M. Effects of adrenal cortical compounds on lactation. Endocrinology 33:325, 1943.

Nicoll, C. S. and Meites, J. Prolongation of lactation in the rat by litter replacement. Proc. Soc. Exptl. Biol. Med. (in press)

Petersen, W. E. and Ludwick, T. M. The humoral nature of the factor causing the let down of milk. Fed. Proc. 1:66, 1942.

Reece, R. P. and Turner, C. W. The lactogenic and thyrotropic hormone content of the anterior lobe of the pituitary gland. Mo. Agr. Exptl. Sta. Res. Bul. 226, 1937.

Riddle, O., Bates, R. W. and Dykshorn, S. W. Preparation, identification and assay of prolactin - a hormone of the anterior pituitary. Am. J. Physiol. 105:191, 1933.

Roy, A. The galactopoietic activity of hormones in cows in declining lactation. J. Endocrinology 5:XXXV, 1947.

Sawyer, W. M. Inactivation of oxytocin by homogenates of uteri and other tissues from normal pregnant rats. Proc. Soc. Exptl. Biol. (N.Y.) 87:463, 1954.

Schultze, A. B., Experimental initiation of milk secretion in the albino rat. J. Dairy Sci. 16:129, 1937.

Selye, H. On the nervous control of lactation. Am. J. Physiol. 107:394, 1954a.

Selye, H. The effect of cortisole upon mammary glands. Acta Endocronologica 17:394, 1954b.

Shaw, J. C. (ed.) Hypophyseal Growth Hormone, Nature and Actions, pp. 486-492, Blackston Division, McGraw-Hill Book Co., Inc., New York, 1955.

Shaw, J. C., Chung, A. C., and Bunding, I. The effect of pituitary growth hormone and adrenocorticotropic hormone on established lactation. Endocrinology 57:162, 1955.

Sprain, D. G., Smith, V. R., Tyler, W. J. and Fosgate, O.T. The effect on milk and fat production of injections of oxytocin at alternate 14-day periods during lactation. J. Dairy Sci. 37:195, 1954.

Stricker, P. and Grueter, F. Action du lobe anterieur de l'hypophyse sur la montee laiteuse. C.R. Soc. Biol. Paris 99:1978, 1928.

Tabachnick, I. A. and Trentin, J. J. Effect of suckling on adrenal ascorbic acid level of the mice. Fed. Proc. 10:339, 1951.

Turner, C. W. and Slaughter, I. S. The physiological effect of pituitary extract (posterior lobe) on the lactating mammary gland. J. Dairy Sci. 13:8, 1932.

Williams, W. L. The effect of lactogenic hormone on post parturient unsuckled mammary glands of the mouse. Anat. Rec. 93:171, 1945.

Wrenn, T. R. and Sykes, J.F. The response of hormonally induced bovine mammary tissue to anterior pituitary hormones. J. Dairy Sci. 36:1313, 1953.

SEP 26 1960

AUG 30 1961

JAN 6 1961

SEP 28 1961

Jan 20, 1961

JAN 13 1962

FEB 13 1964

Feb 3

MAR 30 1961

MAY 6- 1961

MAY 25 1961

JUN 27 1961

JUL 13 1961