STUDIES OF THE ANTAGONISM BETWEEN PROLACTIN AND THE OVARIAN HORMONES ON THE INITIATION OF LACTATION

By

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AN ABSTRACT

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ABSTRACT

1. In Experiment I prolactin was incubated *in vitro* for 1 hour at 38° C. with the following tissues: liver, kidney, brain and muscle of rats; mammary gland from lactating and non-lactating guinea pigs and rabbits; pigeon crop glands; corpora lutea and the remaining ovarian tissue from pseudopregnant rabbits. After incubation the prolactin was assayed in pigeons and directly compared with an equivalent amount of prolactin which had been incubated in a tissue-free solution.

On a fresh tissue basis, mammary gland, pigeon crop gland and the two ovarian tissues showed the greatest capacity to inactivate prolactin. Lactating mammary slices appeared to be more effective in this respect than nonlactating mammary slices. Liver and kidney inactivated only about half as much prolactin as the preceding tissues, while muscle and brain had no effect on the prolactin. Liver and kidney homogenates were more effective in removing prolactin than slices of these tissues. When any of the tissues were boiled for 10 minutes prior to incubation with prolactin, they lost their ability to inactivate the hormone.

2. In Experiment II the inguinal mammary glands were removed from 17 rats on the 12-15th days of gestation and from 19 rats on the 4th day postpartum. The glands were

homogenized and 100-mg. samples were incubated with 0.2 mg. of Squibb prolactin (20-25 I.U./mg.) at 38 ± 0.1° C. for 1 hour. For controls, the same amount of prolactin was similarly incubated without mammary tissue or with boiled mammary tissue. After incubation, the tissues were removed and assayed in White Carneau pigeons for prolactin activity.

The mammary homogenate from the lactating rats inactivated 65.6 per cent of the prolactin compared with 19.5 per cent for the tissue from the pregnant rats. When corrected for milk content, by lactose determinations, the former tissue showed 8 times as much capacity to remove prolactin as the latter. These data suggest that the mammary glands of pregnant rats cannot utilize prolactin to any marked extent, and that this contributes to the absence of copious lactation during gestation.

3. In Experiment III a total of 63 albino rabbits of both sexes, intact and castrate, were injected with 960 I.U. of estrone and 1 I.U. of progesterone daily for 25 days in order to induce optimal mammary development. From the 26th to 35th days, all except three rabbits were injected daily with 2 mg. of prolactin (20-25 I.U./ mg.), with or without one or both of the steroid hormones. At the end of the 35th day, the rabbits were killed and the mammary glands were exposed and rated for intensity of lactation. The extent of mammary growth induced by these treatments was determined by injecting radioactive phosphorus

(P^{32}) 4 hours prior to sacrifice of the rabbits, and then making radioautographs of the mammary glands.

The rabbits given prolactin and both steroids during the last 10 days of the 35 day experimental period had practically no milk in their mammary glands (average rating of less than 1); those injected with prolactin alone had mammary glands filled with milk (average rating of 3 to 4); those given prolactin and progesterone showed no inhibition in lactational response (average rating of 3 to 4); and the rabbits injected with prolactin and estrone showed only a slight decrease in milk secretion (average rating of 2 to 3). The radioautographs indicated the presence of intensive mammary growth in the rabbits treated with both steroids throughout the entire 35 day period but not in the rabbits given both steroids only for the first 25 days. It is concluded that in the doses employed, estrone and progesterone together can effectively inhibit the milk-secreting action of prolactin on the mammary gland of the rabbit, whereas progesterone alone is ineffective and estrone alone is only slightly effective in this respect.

4. In Experiment IV 36 young rabbits were ovariectomized, and after 10 to 14 days were injected daily with 0.96 mg. of estrone and 1 mg. of progesterone for 25 days to induce optimal mammary development. From the 26th to 35th days, 3 groups of 6 rabbits each were continued on the same doses of steroid hormones together with 2, 4 and 8 mg. of prolactin daily, respectively; 3 other groups of 6 each

were injected with 2 mg. of prolactin daily together with 0, 1/4 or 1/2 of the 2 steroid hormones used previously. On the 36th day the rabbits were killed and their mammary glands were exposed and rated visually for lactational response.

In the first 3 groups lactation was almost completely inhibited when only 2 mg. of prolactin was injected daily in the presence of the 2 steroid hormones, while 4 or 8 mg. daily elicited good lactational responses. In the second 3 groups, the greatest amount of milk was secreted when 2 mg. of prolactin was given daily without the steroid hormones, while the same dose of prolactin in the presence of either 1/4 or 1/2 of the steroid hormones produced a smaller flow of milk.

It is concluded that the antagonism between mammary growth and lactation is relative, depending on the balance between the levels of prolactin and the 2 ovarian hormones in the body. When the mammary growth stimulus exerted by the 2 ovarian hormones is greater than the lactational stimulus of prolactin, milk flow will be inhibited, and vice versa. The application of these findings to explain the absence of lactation during a non-lactational pregnancy or the simultaneous coexistence of lactation and pregnancy is discussed.

5. In Experiment V, extracts of mammary tissue taken from parturient rats and rabbits were prepared and injected subcutaneously into mature and lactating rats. Thirty mature rats were divided into three groups and were injected daily

for 10 days as follows: group 1 received a normal saline solution; group 2 received 1.5 ml. rat mammary extract daily; group 3 received 1.0 ml. rabbit mammary extract daily. At the end of the experimental period the rats were killed and their pituitaries were assayed for prolactin content. The rat extract increased pituitary prolactin level by 41.8 per cent, while the rabbit extract decreased it by 27.7 per cent.

Sixteen additional rats on the 4th day of lactation were isolated for 4 hours from their litters and divided into two groups of 9 and 6, respectively. Group 1 received 1 ml. of normal saline, while group 2 received 1.5 ml. rat mammary extract in a single injection. At the end of 24 hours they were killed and their pituitaries were assayed for prolactin content. The rat mammary extract elicited a 16.9 per cent increase in prolactin content.

On the basis of these limited data it appears that mammary extracts from parturient rats and rabbits do not influence the pituitary prolactin content when injected into mature and lactating female rats. Further work is necessary before a definite conclusion can be drawn regarding the effects of mammary tissue on pituitary prolactin secretion.

6. In Experiment VI, the <u>in vitro</u> effects of prolactin on the QO_2 of mammary slices from lactating rats was determined. Eighteen rats on the 4th, 5th, 8th, 9th, 10th, and 12th day of lactation were decapitated and their inguinal mammary glands were removed. QO_2 was determined on slices in

Warburg flasks containing acetate-glucose-phosphate buffer solution in the absence or presence of prolactin. Prolactin was found to increase oxygen consumption, particularly in the latter stages of lactation. This suggests that the mammary glands from parturient rats are susceptible to prolactin stimulation.

7. Lactation is thought to be held in abeyance during pregnancy by two mechanisms: (1) the growth stimulating levels of estrogen and progesterone on the mammary gland, render it refractory to prolactin stimulation; (2) the relatively low level of prolactin secretion is insufficient to overcome the inhibitory effects of the 2 ovarian hormones on the mammary gland. At parturition, the high levels of the two ovarian hormones are greatly reduced, permitting the increased amounts of prolactin from the pituitary to initiate lactation. Lactation can continue during a subsequent pregnancy because (a) prolactin secretion is maintained at a high level by the milking act and (b) estrogen and progesterone are not secreted in sufficient quantities, particularly during the first half of pregnancy, to overcome the action of prolactin on the mammary gland. Other hormones may also influence the initiation and maintenance of lactation, but they are believed to play a secondary role to the factors mentioned above.

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Dedicated

to my beautiful wife Katherine

and son Tommy

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INTRODUCTION

The onset of lactation following parturition was formerly thought to be a natural consequence of the completion of mammary development. However, during the last fifty years new concepts involving endocrine control of the mammary gland have arisen. Thus it has been shown that the initiation of lactation is primarily under the control of the endocrine system.

Numerous theories attempting to explain the absence or relatively minor secretory activity in the mammary gland during pregnancy and the initiation of copious lactation at parturition have been postulated. These theories fall into two categories: (a) inhibitory theories, according to which the lack of lactation during pregnancy is attributed to the presence of inhibitory agents while the initiation of lactation is believed due to the removal of these agents; (b) stimulatory theories, primarily that of Meites and Turner (1942a, b, c), according to which the absence of milk secretion during gestation was attributed to low lactogenic hormone production. Estrogen, a stimulating agent, was believed to be prevented from increasing prolactin secretion during pregnancy by the overriding influence of progesterone. At the completion of pregnancy, estrogen was believed to gain the upper hand and stimulate prolactin secretion by the pituitary, resulting in the initiation of lactation.

None of the theories postulated gave adequate consideration to the possibility that mammary growth may be antagonistic to the initiation of lactation or adequately explained the ability of some animals to maintain lactation and pregnancy simultaneously.

This thesis is an attempt to throw additional light upon this classical problem by determining: (1) the degree of inactivation, if any, of the lactogenic hormone by the mammary gland and other body tissues in vitro; (2) whether a differential could actually be demonstrated between the ability of mammary tissue from pregnant and parturient rats to inactivate prolactin; (3) whether the ovarian hormones (estrogen and progesterone), either individually or together, could render the mammary gland refractory to prolactin stimulation; (4) the effects of altering the balance between prolactin and ovarian hormones on the initiation of lactation in rabbits; (5) whether mammary tissue from parturient animals could alter the pituitary prolactin content of rats; (6) the effects of prolactin upon respiratory activity of mammary tissue of rats during lactation. It is hoped that the results of these experiments have helped clarify the endocrine control of lactation during pregnancy and at parturition, although additional work will be required before this problem can be completely resolved.

REVIEW OF LITERATURE

It is evident that in the mammary gland a transition from a growth phase to a secretory phase occurs during the second half of pregnancy, culminating in the initiation of copious milk secretion at parturition. Under normal conditions the growth phase is essentially completed by the middle or two-thirds of pregnancy (Turner, 1939), and lactation is possible at this time. Thus, if pregnancy is terminated after mid-pregnancy, it is usually followed by lactation. However, normally full lactation does not set in until after the completion of gestation. There are a number of theories that attempt to account for this lack of abundant lactation during pregnancy. The classical ideas held during the early days of physiology was that the initiation of lactation was a passive phenomenon following parturition, due to the removal of inhibitory factors that maintained the gravid reproductive system. The idea that lactation was a passive process fell into disrepute with the discovery by Stricker and Grüter (1929) that anterior pituitary extracts contained a lactogenic factor capable of initiating lactation in non-lactating animals. Some of the most important theories dealing with the initiation of lactation prior to and following the discovery of prolactin will be reviewed here.

NERVOUS INFLUENCES

Following parturition most animals nurse their young. Therefore, the possibility that the nursing stimulus may be responsible for the postpartum milk flow must be considered. If the suckling stimulus is the primary factor involved in eliciting copious milk secretion, then one would expect to be able to initiate lactation in animals during pregnancy by stimulating the nipples. Masson (1948) attempted this in rats with no success. However, Selye and McKeown (1934a, b) found in non-pregnant rats and mice that it was possible to cause the mammary gland to proliferate and actually lactate through stimulating the nipples by actively suckling litters.

Reece and Turner (1937) in the rat and Meites and Turner (1942b) in the rabbit set up unique experiments in which pregnant animals were placed in cages with floors of wire mesh containing large openings. With the completion of pregnancy the newborn dropped through the openings so that the mothers were not suckled. Lactation occurred and pituitary prolactin was increased as in normal animals. One must conclude, therefore, in view of these observations, that suckling is not necessary for the initiation of lactation at parturition. Suckling is necessary, however, for the maintenance of postpartum lactation (Selye, Collip and Thomson, 1934a; Selye and McKeown, 1934a, b; Turner and Reineke, 1936; Meites and Turner, 1948a, b).

FETUS INHIBITION THEORY

The fetus undergoes a tremendous spurt in growth and development, particularly during the last third of pregnancy. This caused many to speculate that lactation is not initiated during the latter half of pregnancy because the fetus takes nutrients from the blood that would otherwise serve as precursors of milk.

Another version stated that the developing fetus elicited an inhibitory influence upon the milk secreting alveolar cells of the mammary gland (Hildebrandt, 1904). This inhibition supposedly prevented the breakdown of the alveolar cells during lactation. It is known now that the alveolar cells *per se* do not constitute the milk, but only manufacture it. This theory appeared to receive additional support in the experiments of Freud and Wijsenbeck (1938), who transferred the fetuses from the uterus into the abdominal cavity of rats and noted the continued absence of lactation: when the fetuses were removed, lactation ensued. This type of experiment subjects the animal to extremely abnormal conditions and one might suspect disturbances to any normal process such as lactation.

The termination of pregnancy by caesarian section in its later stages in the rat results in the onset of lactation within thirty-six hours; however, if the pituitary is removed when the uterus is empty, no milk secretion occurs (Collip *et al*, 1933). Nelson (1934) partially confirmed this finding in an experiment in which he removed the ovaries and embryos

from female guinea pigs during the 35th to 45th days of pregnancy, leaving the placenta intact. He found a continued absence of lactation. However, lactation occurred after the expulsion of the placenta, indicating that the placenta may be the primary inhibitory factor involved.

Therefore, in view of these observations and the well-known fact that lactation may persist in the presence of developing fetuses in subsequent pregnancies one is led to conclude that the fetus plays no role in the inhibition of lactation.

THE ROLE OF THE PLACENTA

Halban (1905) observed that when the placenta was retained following delivery in parturient women, lactation did not occur during the period of retention. He believed that the placenta was responsible for mammary growth during pregnancy, preventing milk secretion until it was expelled. Frankl (1923) implanted placental tissue into rats near term and observed that no secretion occurred following delivery as long as the graft remained active. Smith and Smith (1933) observed that rabbits with retained living placental remnants did not lactate, and thought the inhibitory substance was estrogen secreted by this retained tissue.

There is other evidence, however, which does not favor the placental inhibition theory. Placentas are frequently retained in cows and do not inhibit the onset of lactation (Petersen, 1942). Copious lactation may also be maintained

during subsequent pregnancies in some species, such as cattle. Successful transplantation of fetal placental tissues of pregnant rats into their eyes had no effect upon lactation following parturition (Litt, 1933). Comparable conclusions were drawn by Selye et al. (1934a) in experiments with mice. They implanted whole mouse placentas daily for 8 days into 6 lactating mice and found no deleterious effects upon lactation.

ROLE OF UTERINE DISTENSION

The tremendous growth of the fetus during the last third of pregnancy submits the uterus to an abnormal distension. It was postulated that the absence of lactation during this time may be due in some way to mechanical distension of the uterus (Selye, 1934; Selye et al., 1934a). They removed the young by caesarian section during pregnancy or on the day of parturition and distended the uteri with paraffin. This resulted in an apparent inhibition of lactation. Inability of this theory to meet the test of repeatability by Bradbury (1941) and Greene (1941) resulted in its lack of acceptance. The latter showed that the apparent failure to lactate was due to the effects of the operation, since following recovery in their animals they observed lactation even when the uteri were filled with paraffin. Again the observation that pregnancy and lactation can occur simultaneously in several species may be taken as evidence against this theory.

THE ROLE OF CORPORA LUTEA OR PROGESTERONE

It is well known that ovariectomy or removal of the corpora lutea in most animals usually terminates pregnancy. If this occurs in late pregnancy and is accompanied by abortion, lactation is initiated (Masson, 1948). Therefore, one is led to suspect that the ovary or one of its component parts may be a possible inhibiting agent. Hammond (1917) postulated that the corpora lutea of pregnancy inhibited lactation. He based this on the observation that removal of the corpora lutea from rabbits on the 12th day of pregnancy resulted in lactation. Drummond-Robinson and Asdell (1926) found that removal of the corpora lutea in goats after mid-pregnancy also resulted in initiation of lactation. They attributed a mammary growth stimulus to the corpora lutea. It was believed that the corpus luteum degenerated, thus removing the growth stimulus, the alveolar cells would begin to secrete and lactation would set in. The decline of milk yield with the advance of a subsequent pregnancy was also explained by the progressive increase of this mammary growth substance during pregnancy.

With the isolation of progesterone as the active factor from the corpus luteum, and the demonstration of its increase during pregnancy (Bachman et al., 1940; Lyon, 1946), the concept arose that progesterone may prevent the onset of lactation. Walker and Matthews (1949) found that daily treatment of rats with progesterone begun prepartally (2.5

mg.) or postpartally (5 mg.) could neither prevent nor inhibit lactation. The criterion of lactation was the growth rate of nursing young. Other workers (Anselmino et al., 1936a, b; Folley and Kon, 1938; Folley, 1942; Barsantini and Masson, 1947) found no suppression of established lactation in rats given either moderate or massive doses of progesterone. Abarbanel (1941) also reported that large doses of progesterone were ineffective in suppressing the initiation or maintenance of lactation in the postpartum human. Only Reece and Turner (1937) found some degree of depression of established lactation. They gave crude progestin to ovariectomized lactating rats and found that milk secretion was decreased in about one-half of the animals. Thus most workers are in general agreement that progesterone per se does not inhibit the initiation or the maintenance of lactation.)

ROLE OF ESTROGEN

Beerstecher (1941, 1942) observed an increased level of estrogen in the urine of rats and rabbits as pregnancy progressed. Turner et al. (1930) and Levin (1945) observed a maximum concentration of estrogen in the urine and feces of cows at term. Zondek (1931) and Goldberger and Frank (1942) found that this hormone increased in the urine and blood of women during pregnancy. This indicates that in several species there is a rise in estrogen during pregnancy. \The progressive increase in the estrogen titer

throughout pregnancy and its decrease at parturition suggested that this hormone may play a role in the inhibition of lactation during pregnancy.

Barsantini and Masson (1947) found that estradiol at daily doses of 10 mcg. and 1 mg. caused a marked inhibition of lactation in normal rats. Walker and Matthews (1949) found that prepartal injection of estrone did not prevent the initiation of lactation either in ovariectomized (25 mcg.) or in intact (25 mcg. and 100 mcg.) rats, but did produce a delayed depression in the intact animals. Postpartal treatment with 200 mcg. of estrone begun on the second day of lactation did not inhibit established lactation in ovariectomized rats while moderate daily doses (5 mcg. and 10 mcg.) of estrone inhibited lactation in intact rats. This inhibition did not appear until the 10th to 12th day following the initiation of treatment. Large doses of estrone (1 mg.) inhibited lactation in both intact and ovariectomized animals, but this was accompanied by marked loss in weight of the mother.

Other workers (Reece and Turner, 1937; Folley and Kon, 1938; and Folley, 1941) have established that estrogen will inhibit lactation in the rat and cow, but the amount of this hormone required was much larger than would be present in a normal pregnancy. In addition, it was necessary to begin treatment with estrogen early in order to establish this inhibition.

Nelson (1936) developed a lactation hypothesis attributing an inhibitory role to estrogen. His theory not only involves a mammary growth-promoting role for estrogen, but in addition, an inhibitory effect on lactogenic secretion by the pituitary. He postulated that during pregnancy the ovarian hormones, primarily estrogen, induced the proliferative changes that occur in the mammary gland, but that estrogen inhibited the actual secretion of milk. This inhibitory influence was thought to act in two ways (a) by suppressing the secretion or release of prolactin by the pituitary, (b) by directly inhibiting the mammary gland. At parturition, when the ovarian hormones sharply declined, the inhibitory agent was removed and the gland was susceptible to stimulation by lactogenic hormone. The basis for this theory of Nelson (1936) rests on a number of observations: (1) Lactation was inhibited in normal parturient female guinea pigs by injections of estrone. If prolactin was given simultaneously with estrone, lactation occurred. However, estrone dosage could be increased to the point where large amounts of prolactin failed to stimulate lactation. (2) The injection of purified lactogenic hormone into pregnant animals failed to induce lactation. (3) Crude prolactin preparations did induce lactation but only after pregnancy was terminated by abortion. (4) Extirpation of the ovaries was not invariably followed by abortion. Lactation occurred only after fetal expulsion, whether they were aborted or were expelled normally at term. (5) Lactation did

not occur when the ovaries and embryos were removed and the placents was left intact, but did occur following expulsion of the placenta.

The inhibitory role attributed to estrogen by Nelson (1936) can be criticized when one considers the following: (1) all of the experiments were conducted in guinea pigs, a species which reacts to estrogen differently from most other species. Thus, estrogen induces complete mammary growth in this species whereas estrogen and progesterone are both required in other species for complete lobule-alveolar growth. (2) Although estrogen increases during pregnancy, it does not exert many of its characteristic actions: (a) the animals are not in estrus, (b) uterine motility is absent or at a minimum, (c) there is no or little increase in pituitary weight in several species, (d) estrogen usually induces a decrease in body weight in rats but the opposite occurs during pregnancy. The absence of these estrogenic effects is believed to be due to the predominance of progesterone during pregnancy. (3) Artificial induction and enhancement of lactation with estrogen is possible in goats and cows. (4) Meites and Turner (1948a) have shown that estrogen increases rather than decreases pituitary prolactin content in the rat, guinea pig, and rabbit and increases prolactin in the blood of rabbits. (5) The inhibition of established lactation which is observed with large doses of estrogen may be due in part to the toxic effects that it exerts on body metabolism. Meites

and Turner (1948b) noted a progressive loss in appetite and a reduction of food and water intake in goats, while Walker and Matthews (1949) reported a drastic decrease in body weight in lactating rats given estrogens.

ROLE OF ESTROGEN AND PROGESTERONE

Masson (1948) observed that when estradiol was given in combination with progesterone to spayed rats the hormones inhibited established lactation when the ratio of estrogen to progesterone was 1 to 1000. The milk secreting activity of the mammary gland was estimated from the growth curve and survival of the young. Walker and Matthews (1949) found that simultaneous injections of estrone and progesterone begun prepartally or postpartally in the rat did not prevent the initiation of lactation, but inhibited established lactation after 10-12 days of treatment. A marked proliferation of the glands indicated that growth of the mammary parenchyma may play a role in the inhibition of milk secretion.

MAMMARY GROWTH VERSUS SECRETION

Since mammary growth is completed by the middle or two-thirds of pregnancy in most animals, one would presume that growth *per se* is not responsible for the inhibition of lactation after mid pregnancy. However, Loeb and Hesselberg (1917) postulated that mammary growth and milk secretion are incompatable and therefore lactation is absent during pregnancy. Using as a criterion the number of mitotic figures in the

mammary gland, they found in the guinea pig and rat that as secretion progressively increased, mitosis in the mammary cells became more rare. This relationship was confirmed by Reece et al. (1940) who observed that when established lactation was inhibited by estrogen, the number of mitotic figures in the mammary gland of rats increased. Nelson et al. (1943) postulated that since estrogen, testosterone and desoxycortisone all stimulate mammary growth and inhibit lactation, all substances which stimulate mammary growth have an inhibitory effect on lactation. Hammond (1927) suggested that the decrease in milk production about the fifth month of a subsequent pregnancy in the cow was due to active growth of the udder.

Folley and Kon (1938) reported that testosterone promoted mammary growth and inhibited lactation and postulated, as did Nelson et al. (1943), that the ability of certain hormones to cause mammary growth is accompanied by an ability to inhibit lactation. However, Folley (1942) later found that massive doses of progesterone and desoxycorticosterone produced no inhibition of established lactation in rats, which was in disagreement with their hypothesis.

The ability to elicit growth and simultaneously initiate lactation in the udder of goats by Folley (1941) and Mixner, Meites and Turner (1944) and in the udder of a cow by Walker and Stanley (1941) constitute additional evidence against this theory. Cells are constantly being replenished in the various organs of the body without disturbance to the normal

function of the organ. As a new cell is formed and developed others carry out the duties of the organ. The ability of the mammary duct system to secrete milk (Turner, 1939) indicates that lobule-alveolar growth is not essential for a limited degree of lactation to occur. The ability of prolactin to stimulate alveolar cell developments in the mammary gland (Lyons, 1942) indicates further that mammary growth and lactation can go on simultaneously. This has actually been observed during normal lactation in rats by Jeffers (1935) and by Kuramitsu and Loeb (1921) who found a direct correlation between amitotic proliferation and intensity of milk secretion.

MEITES AND TURNER THEORY OF LACTATION

The majority of the theories explaining the onset of lactation at parturition were based on the removal of prepartum inhibitory agents. Meites and Turner (1942a, b, c) postulated that stimulatory as well as inhibitory factors are involved in the initiation of lactation. Their theory is based on the following: (1) Pituitary prolactin content remains low during pregnancy, begins to increase prior to parturition and increases sharply following parturition. It is maintained at a high level during lactation in most animals. (2) Only estrogens, of all the hormones tested, can significantly increase the pituitary prolactin content. Even in physiological doses, estrogens can increase the pituitary prolactin content to lactational levels in the

rat, guinea pigs, and rabbits, and this is accompanied by an increased blood prolactin level in the rabbit. During pregnancy, when estrogen and progesterone are secreted in increasing quantities, progesterone is thought to override estrogen, preventing it from stimulating prolactin secretion. However, at parturition, the levels of these hormones declines rapidly and estrogen is believed to become predominant, as indicated by the appearance of estrogenic effects at parturition or shortly thereafter, i.e., estrus 1-2 days postpartum in the rabbit, rat, and mouse. Estrogen is believed to activate the pituitary to secrete prolactin in sufficient amounts to initiate lactation.

The primary criticisms of this theory as given by Folley and Malpress (1948) are: (1) An increased pituitary prolactin content does not necessarily indicate an increased secretion of prolactin. However, Meites and Turner (1948a) have shown a simultaneous increase in blood prolactin with increased pituitary prolactin in the rabbit. (2) The pigeon assay involves a tissue of mesodermal origin while the mammary gland is of ectodermal origin. Therefore, the identity of prolactin with the pigeon crop proliferating factors is questioned. (3) Based on experiments with hypophysectomized animals in which they were unable to initiate lactation with purified prolactin alone, Folley and Malpress (1948) claimed that prolactin is not the only hormone involved in the initiation of lactation. However, Meites (1954) has pointed out that in the normal intact animal, with developed mammary

glands, only an increase in prolactin is essential for the initiation of lactation. Only prolactin has been shown to be capable of directly stimulating the mammary alveolar cells to secrete milk (Lyons, 1942; Meites and Turner, 1947), which also proves its identity with the pigeon crop stimulation factor. The other hormones are believed to have secondary albeit important supporting roles in the initiation and maintenance of lactation.

It can be seen that no theory has yet been evolved which can completely explain the absence of lactation during an uncomplicated pregnancy, the initiation of lactation at parturition, or the existence of a simultaneous pregnancy and lactation in the same animal. In the Discussion at the end of this thesis, an attempt will be made to coordinate the various lactation theories with the experimental results presented herein.

EXPERIMENTAL

EXPERIMENT I. INACTIVATION OF PROLACTIN BY DIFFERENT BODY TISSUES *in vitro*

Purpose:

Little is known of the fate of lactogenic hormone in the body. It has been shown that a number of anterior pituitary hormones are concentrated or inactivated by their respective target organs (Seidlin, 1940). Since injected prolactin rapidly disappears from the bloodstream and only a small fraction can be accounted for in the urine (Meites, 1940), it may be withdrawn or inactivated by its target organ or other tissues in the body. This experiment was planned to determine the degree of inactivation, if any, of the lactogenic hormone by mammary gland and other body tissues *in vitro*.

Method:

The following tissues were tested for their ability to inactivate prolactin: liver, kidney, brain and muscle from female rats; mammary glands from lactating and non-lactating guinea pigs and rabbits; pigeon crop glands; corpora lutea and the remaining ovarian tissue from pseudo-pregnant rabbits. After killing the animals, the tissues were quickly removed and placed in ice-cold Ringer-phosphate solution. The corpora

lutea were carefully shelled out from the luteinized ovaries. It is improbable, however, that the ovaries were entirely freed of luteal tissue. Tissue slices were cut with a Stadie-Riggs microtome or homogenized with a glass tube and plunger. For tissue controls, several of the tissues were boiled for 10 minutes in order to destroy enzymatic activity.

Approximately 500 mg. duplicate samples of tissue were placed in standard Warburg flasks with 1.9 ml. of Ringer-phosphate solution and 0.1 ml. of a solution containing 0.2 mg. of purified prolactin (Squibb: 1 mg. = 22.5 I.U.). For prolactin controls, only prolactin and Ringer-phosphate solutions were incubated together at least six times during the course of the experiments. All flasks were placed in a water bath at a constant temperature of $38 \pm 0.1^{\circ}$ C. for a period of 1 hour, except in one experiment liver slices were incubated for 2 hours. After incubation, the solutions were removed and the flasks were rinsed with 2 ml. of distilled water. The fluids were combined and diluted so that 1.0 ml. contained 0.005 mg. of the original prolactin.

The pigeon method of Reece and Turner (1937) was used to assay the prolactin. White Carneau pigeons were injected intradermally over both crop sacs with 0.1 ml. volumes daily for 4 days. A prolactin control solution was always injected over one crop sac and compared with an equal amount of tissue-incubated prolactin injected over the other crop sac. On the 5th day the pigeons were killed and the crop glands

were removed and rated visually for degree of proliferation. Hall (1944) reported that a 20 per cent difference in potency between two lactogenic preparations could be detected by this method of direct comparison in the same pigeon.

Results:

The data on the rat tissues are presented in Table 1. It can be seen that when liver slices and prolactin were incubated for 2 hours, 67 per cent of the prolactin was inactivated, while when incubated for 1 hour, only 43 per cent was inactivated. Liver homogenate inactivated 70 per cent of the prolactin in 1 hour, indicating that the homogenate was more effective in this respect than the liver slices. Kidney slices removed 46 per cent and kidney homogenate 76 per cent of the prolactin activity, again demonstrating a greater inactivation by the homogenate. When liver or kidney slices were boiled, they lost their ability to destroy lactogenic hormone. Neither brain nor muscle slices showed any significant capacity to inactivate prolactin.

Table 2 shows the results of incubating prolactin with mammary, crop gland and ovarian tissues. Lactating mammary slices or homogenate from guinea pigs removed all of the lactogenic activity, while boiled mammary slices were completely ineffective. Mammary slices from lactating rabbits showed somewhat more ability to inactivate prolactin than mammary slices from non-lactating, pseudopregnant rabbits.

TABLE 1. EFFECTS OF VARIOUS RAT TISSUES ON INACTIVATION OF PROLACTIN *in vitro*

Tissue	Wt. of tissue, mg.	Treatment	Assay results		
			No. pigeons/ assay	Av. rating/ pigeon	Inactivation, %
Liver slices[1]	500	Fresh	5	0.55	67
Control[1]			5	1.65	
Liver slices	489	Fresh	5	1.05	43
Control			5	1.85	
Liver homog.	481	Fresh	5	0.55	70
Control			5	1.85	
Kidney slices	498	Fresh	5	1.00	46
Control			5	1.85	
Kidney homog.	480	Fresh	3	0.41	76
Control			3	1.75	
Liver slices	498	Boiled	5	1.90	5
Control			5	2.00	
Kidney slices	490	Boiled	5	1.20[2]	4
Control			5	1.25[2]	
Brain slices	513	Fresh	4	1.44	15
Control			4	1.69	
Muscle slices	499	Fresh	4	0.81[2]	0
Control			4	0.75[2]	

[1]Incubation time was 2 hours.
[2]Immature pigeons.

TABLE 2. EFFECTS OF MAMMARY, OVARIAN AND PIGEON CROP TISSUES ON INACTIVATION OF PROLACTIN *in vitro*

Tissue	Wt. of tissue, mg.	Treatment	Assay results		
			No. pigeons/ assay	Av. rating/ pigeon	Inactivation, %
Lact. mam. slices (g. pig)	454[1]	Fresh	5	0.15	92
Control			5	1.85	
Lact. mam. homog. (g. pig)	485[1]	Fresh	5	0.00	100
Control			5	2.30	
Lact. mam. slices (g. pig)	452[1]	Boiled	5	1.95	3
Control			5	2.01	
Lact. mam. slices (rabbit)	508[1]	Fresh	4	0.25[2]	84
Control			4	1.50[2]	
Nonlact. mam. slices (rabbit)	489	Fresh	4	0.44[2]	68
Control			4	1.38[2]	
Corpora lutea, homog. (rabbit)	320	Fresh	4	0.00	100
Control			4	1.75	
Other ovarian tissue, homog. (rabbit)	319	Fresh	4	0.00	100
Control			4	1.63	
Crop slices (pigeon)	460	Fresh	5	0.15[2]	86
Control			5	1.05[2]	
Crop homog. (pigeon)	425	Fresh	5	0.00[2]	100
Control			5	1.25[2]	
Crop slices (pigeon)	407	Boiled	5	1.40[2]	0
Control			5	1.40[2]	

[1]Since considerable milk was present in these tissues, their true weights were much less than indicated here.
[2]Immature pigeons.

Since the lactating mammary slices contained considerable quantities of milk, there was actually more inactivation per unit of tissue than indicated in the data. Homogenates of corpora lutea or of the reamining ovarian tissue from pseudopregnant rabbits completely destroyed the prolactin. Pigeon crop slices and homogenates inactivated 86 and 100 per cent, respectively, of the prolactin, while boiled crop slices showed no capacity to inactivate the hormone.

Conclusion:

In general these data indicate that, when computed on a fresh tissue basis, mammary, crop gland and ovarian tissues were outstanding in their ability to inactivate prolactin. Homogenates of these tissues appeared to possess at least twice the ability of liver or kidney slices to inactivate lactogenic hormone. Brain or muscle slices showed little or no capacity to remove prolactin, indicating that these tissues probably do not normally metabolize prolactin in the body.

The importance of tissue enzymes in the inactivation of prolactin is indicated by two observations. First, liver and kidney homogenates were more active than slices of these tissues. It seems reasonable to explain this on the basis that more of the enzymes were in direct contact with prolactin in the homogenates than in the tissue slices. Second, when the tissues were boiled, thus inactivating the enzymes, the tissues were no longer able to alter prolactin activity.

EXPERIMENT II. DIFFERENTIAL INACTIVATION OF PROLACTIN BY MAMMARY TISSUES FROM PREGNANT AND PARTURIENT RATS

Purpose:

In experiment I there was some indication that mammary tissue from lactating guinea pigs and rabbits had more ability to inactivate prolactin than similar tissues from pregnant guinea pigs and rabbits. There is other evidence that mammary tissue from lactating animals is more reactive to prolactin than tissue from pregnant animals. Thus Folley (1952) has shown that prolactin can increase respiratory activity only in lactating mammary tissue of rats and not in mammary tissue of pregnant rats. Evidence will also be presented in Experiment III demonstrating that the ovarian hormones, which are secreted in large quantities during pregnancy, are antagonistic to the action of prolactin on the mammary glands. It was the purpose of this experiment to determine whether a differential actually could be demonstrated between the ability of mammary tissue from pregnant and parturient rats to inactivate prolactin.

Methods:

The inguinal mammary glands were removed immediately after killing rats on the 12th to 15th, 15th to 17th, and 17th to 19th days of gestation, and from parturient rats on the 1st, 4th, 15th and 20th days of lactation. The tissues

were placed in ice-cold Ringer-phosphate solution, and homogenates were prepared with a motor-driven, ground-glass homogenizer immersed in an ice-water bath. Duplicate 100 mg. samples of tissue were placed in standard Warburg flasks containing 0.2 mg. of purified prolactin. As a control for each experiment, the same amount of prolactin and buffer were placed in Warburg flasks without any mammary homogenate. Each flask, containing a total volume of 3.0 ml., was placed in a water bath maintained at a temperature of 38 $\pm$ 0.1° C., for a period of 1 hour. Prolactin was also incubated with boiled mammary homogenate, and with boiled and fresh milk from the mammary glands of lactating rats. After incubation, the mixtures were removed and the flasks were rinsed with the buffer. The fluids were combined and diluted so that 0.10 ml. contained 0.005 mg. of the original prolactin.

The fluids were assayed for prolactin in White Carneau pigeons by the sensitive intradermal method of Reece and Turner (1937). A prolactin control solution was always injected over one crop sac and compared with an equal amount of tissue-incubated prolactin, injected over the other crop sac of the same bird. It has been stated that this technique permits the detection of differences of 20 per cent between two prolactin preparations (Hall, 1944).

In order to determine the actual amount of mammary tissue used in each experiment, a correction was made for milk content by determining the total lactose per 100 mg. of fresh tissue (Shaffer and Somogyi, 1933). A lactose

value of 2.7 per cent was used for the rat milk, a figure slightly below that given by Folley and Greenbaum (1947). This introduces some degree of error, particularly in calculating the amount of milk present in the pregnant mammary gland which is usually not normal in composition, but an even greater error would have resulted if no correction had been made. This can be seen from the fact that 60 per cent or more by weight of the fresh mammary tissue from lactating rats was found to be milk and not tissue.

Results:

The pertinent data relating to the pregnant rats are tabulated in Table 3. The amount of prolactin inactivated by tissues from rats pregnant between 12 and 15 days was 19.5 per cent; at 15 to 17 days of pregnancy, 11.5 per cent; and at 17 to 19 days of pregnancy, 19.6 per cent. The average inactivation during this period was 16.9 per cent or 0.0338 mg. of the original 0.2 mg. of prolactin. When corrected for milk content, it was calculated that 100 mg. of mammary tissue from pregnant rats could inactivate 0.0423 mg. of prolactin.

The pertinent data relating to lactating rats are tabulated in Table 4. Lactating tissue inactivated 55.3 per cent on the 1st day postpartum, 65.7 per cent on the 4th postpartum day, 64.8 per cent on the 15th postpartum day and 70.7 per cent on the 20th day of lactation. The average inactivation during this period was 64.1 per cent or 0.1282

TABLE 3. INACTIVATION OF PROLACTIN BY MAMMARY TISSUE FROM RATS IN DIFFERENT STAGES OF PREGNANCY

No. rats/ assay	Assay Results: No. pigeons/ assay	Assay Results: Av. rating/ pigeon: Control	Assay Results: Av. rating/ pigeon: Experimental	Amt. of prolactin inactivated mg.	Prolactin inactivated %	Calculated amt. of prolactin inactivated by 100 mg. of actual tissue* mg.
			12-15th Day of Pregnancy			
2	5	1.08	.80	.0518	25.9	.0648
2	5	1.08	.90	.0334	16.7	.0418
3	5	.95	.87	.0168	8.4	.0210
2	5	1.05	.80	.0476	23.8	.1595
2	5	.89	.76	.0292	14.6	.0365
1	5	.92	.90	.0044	2.2	.0055
2	5	.95	.85	.1210	10.5	.0262
2	5	1.25	.90	.0560	28.0	.0700
1	5	1.10	.80	.0546	27.3	.0683
Av.		1.05	.84	.0350	19.5	.0437
			15-17th Day of Pregnancy			
2	5	1.05	.80	.0476	23.8	.0595
2	5	.89	.76	.0292	14.6	.0365
1	5	.92	.90	.0044	2.2	.0055
1	5	.54	.60	--	none	--
1	5	.90	.87	.0066	3.3	.0082
1	10	.91	.68	.0504	25.2	.0630
Av.		.87	.77	.0230	11.5	.0287
			17-19th Day of Pregnancy			
1	5	.86	.88	--	none	--
1	5	1.01	.65	.0712	35.6	.0890
1	5	.73	.73	--	none	--
1	5	.65	.48	.0522	26.1	.0652
2	5	1.05	.70	.0666	33.3	.0832
1	5	1.10	.85	.0454	22.7	.0567
Av.		.90	.72	.0392	19.6	.0490

*Corrected for milk content.

TABLE 4. INACTIVATION OF PROLACTIN BY MAMMARY TISSUE FROM RATS IN DIFFERENT STAGES OF LACTATION

No. rats/ assay	Assay Results: No. pigeons/ assay	Assay Results: Av. rating/pigeon: Control	Assay Results: Av. rating/pigeon: Experimental	Amt. of prolactin inactivated mg.	Prolactin inactivated %	Calculated amt. of prolactin inactivated by 100 mg. of actual tissue* mg.
			1st Day of Lactation			
2	5	.90	.35	.1222	61.1	.3141
2	10	1.23	.62	.0992	49.6	.2550
Av.		1.07	.49	.1107	55.3	.2845
			4th Day of Lactation			
2	5	1.20	.25	.1584	79.2	.4072
2	5	1.05	.35	.1334	66.7	.3429
1	5	1.55	.45	.1420	71.0	.3650
2	5	.75	.11	.1706	85.3	.4385
3	5	1.08	.44	.1186	59.3	.3049
2	5	1.05	.50	.1048	52.4	.2694
2	5	1.20	.55	.1084	54.2	.2786
1	5	1.40	.68	.1028	51.4	.2643
2	5	1.25	.45	.1280	64.0	.3290
2	5	1.09	.21	.1614	80.7	.4149
Av.		1.16	.40	.1328	65.7	.3412
			15th Day of Lactation			
3	10	1.00	.31	.1380	69.0	.3547
2	5	.85	.25	.1410	70.5	.3624
1	5	1.00	.45	.1100	55.0	.2827
Av.		.95	.37	.1297	64.8	.3333
			20th Day of Lactation			
2	5	.80	.19	.1524	76.2	.3917
2	5	1.00	.10	.1800	90.0	.4627
2	10	1.06	.44	.1168	58.4	.3002
4	10	.96	.37	.1228	61.4	.3156
4	10	1.11	.36	.1352	67.6	.3475
Av.		.99	.29	.1414	70.7	.3634

*corrected for milk content

mg. of prolactin. When corrected for milk content the tissue from lactating rats inactivated 0.3300 mg. of prolactin. This is about an 8 fold difference in favor of the mammary tissue from lactating rats.

No inactivation of prolactin occurred when it was incubated with boiled mammary tissue or boiled milk. Several samples of fresh milk obtained from lactating rats inactivated about 20 per cent of the prolactin. However, this does not necessarily prove that rat milk *per se* can destroy prolactin, since the milk was obtained from cut slices of mammary gland and undoubtedly contained some mammary tissue exudates. It should also be recalled that by the prolactin assay method employed, a 20 per cent difference is of the lowest level of significance.

Conclusions:

These data demonstrate that mammary tissues from parturient rats have much greater capacity to inactivate prolactin than similar tissue from pregnant rats. The amount inactivated by tissues from pregnant rats averaged 16.9 per cent (11.5 per cent to 19.6 per cent range), while lactating tissue inactivated about 64.1 per cent (55.3 per cent to 70.7 per cent range). When corrected for milk content it was found that lactating tissue could inactivate about 8 times as much prolactin as an equal amount of tissue from pregnant rats. These data suggest that the mammary glands of pregnant rats cannot utilize prolactin to any

marked extent, and that this probably contributes to the absence of copious lactation during gestation.

EXPERIMENT III. CAN THE OVARIAN HORMONES INHIBIT THE MAMMARY RESPONSE TO PROLACTIN?

Purpose:

It is well known that the ovarian hormones during pregnancy prepare the mammary gland for lactation. Mammary growth in most animals is completed by mid to two-thirds of pregnancy, as shown by the ability of the gland to secrete milk in large quantities if pregnancy is terminated after this time (Turner, 1939). The continued presence of the ovarian hormones at increasing levels during the latter half of pregnancy leads one to suspect that they may play an equally important role in inhibiting lactation during this period.

Although there is some basis for the belief that mammary growth and lactation are antagonistic to each other (Loeb and Hesselberg, 1917; Nelson, 1934, 1936), there is also considerable evidence that mammary growth and lactation can co-exist simultaneously in the same animal, as during a simultaneous pregnancy and lactation or during the initiation of lactation with ovarian hormones in cattle or goats (see review of literature). Therefore, it was the purpose of this experiment to test whether the ovarian hormones, either individually or together, could truly render the mammary gland refractory to prolactin stimulation.

Methods:

A total of 63 young, previously unmated, New Zealand White rabbits of both sexes were used in three experimental

trials. The animals weighed from 5 to 7 pounds each at the beginning of the experiments. Intact males were used in the first experiment, orchidectomized males in the second experiment and ovariectomized females in the third experiment. A period of ten days was permitted to elapse after castration in order to remove the influence of the gonads. The animals were fed commercial rabbit pellets, and both food and water were available at all times.

All rabbits received daily subcutaneous injections for the first 25 days of 960 I.U. of estrone and 1 I.U. of progesterone in separate 0.1 ml. volumes of corn oil. Scharf and Lyons (1941) had previously shown that this combination elicited optimal mammary growth in male rabbits. From the 26th to 35th day, all except three control rabbits received 2 mg. daily of prolactin by subcutaneous injection in 0.1 ml. of distilled water, together with one or both of the steroid hormones.

In order to determine whether the mammary glands were in a state of active growth during the last 10 days of each experiment, several rabbits from each group were injected intravenously with 50 microcuries of radioactive phosphorus (P^{32}) per kg. of body weight, 4 hours prior to sacrifice. Radioautographs were prepared of individual mammary glands according to the technique outlined by Lundahl, Meites and Wolterink (1950).

After killing the rabbits on the 36th day, the mammary glands were exposed and rated visually for intensity of

lactation. Ratings of 0 to 4 were assigned each rabbit (Gardner and Turner, 1933).

Results:

The data for the 3 experiments are shown in Table 5. In the first experiment, in which intact males were used, the 3 control rabbits (group 1) which received both steroid hormones but no prolactin for the 35 days had no milk in their mammary glands. Groups 2 and 3 received both steroid hormones for the full 35 days and prolactin from the 26th to the 35th day. No milk could be seen in their mammary glands although an occasional drop could be squeezed from the nipples. Groups 4 and 5 were injected with the two ovarian hormones for the first 25 days followed by prolactin alone for the next 10 days. Considerable lactation was evident in all these rabbits (average rating of 3). The mammary glands of four representative rabbits from this experiment are shown in Figure 1.

The results of the second experiment, performed on orchidectomized rabbits, indicate that groups 1 and 2 responded the same as the similarly treated intact male rabbits of the first experiment. Group 3, which received prolactin and estrone from the 26th to 35th days, had slightly less milk in their mammary glands than the rabbits injected with prolactin alone (group 2). When progesterone was given together with prolactin during the last 10 days of the 35-day experimental period (group 4), no inhibition of lactation was evident.

TABLE 5. LACTATIONAL RESPONSES IN RABBITS RECEIVING PROLACTIN WITH OR WITHOUT ESTRONE AND/OR PROGESTERONE

Exp.	Group and No. of rabbits	Treatment		Av. lactational rating per gland
		1st to 25th day	26th to 35th day	
		Intact male rabbits		
1	1(3)	Est.[1] + Prog.[2]		0
	2(5)	Est.[1] + Prog.[2]	Prolactin[3] + Est. + Prog.	less than 1
	3(5)	Est.[1] + Prog.[2]	Prolactin[3] + Est. + Prog.	less than 1
	4(5)	Est.[1] + Prog.[2]	Prolactin[3] only	3
	5(5)	Est.[1] + Prog.[2]	Prolactin[3] only	3
		Castrate male rabbits		
2	1(5)	Est.[1] + Prog.[2]	Prolactin + Est. + Prog.	less than 1
	2(5)	Est.[1] + Prog.[2]	Prolactin only	3
	3(5)	Est.[1] + Prog.[2]	Prolactin + Est.	2
	4(5)	Est.[1] + Prog.[2]	Prolactin + Prog.	3
		Castrate female rabbits		
3	1(5)	Est.[1] + Prog.[2]	Prolactin + Est. + Prog.	less than 1
	2(5)	Est.[1] + Prog.[2]	Prolactin only	4
	3(5)	Est.[1] + Prog.[2]	Prolactin + Est.	3
	4(5)	Est.[1] + Prog.[2]	Prolactin + Prog.	4

[1]Est. = estrone, 960 I.U. daily.
[2]Prog. = progesterone, 1 I.U. daily.
[3]Prolactin, 2.0 mg. daily.

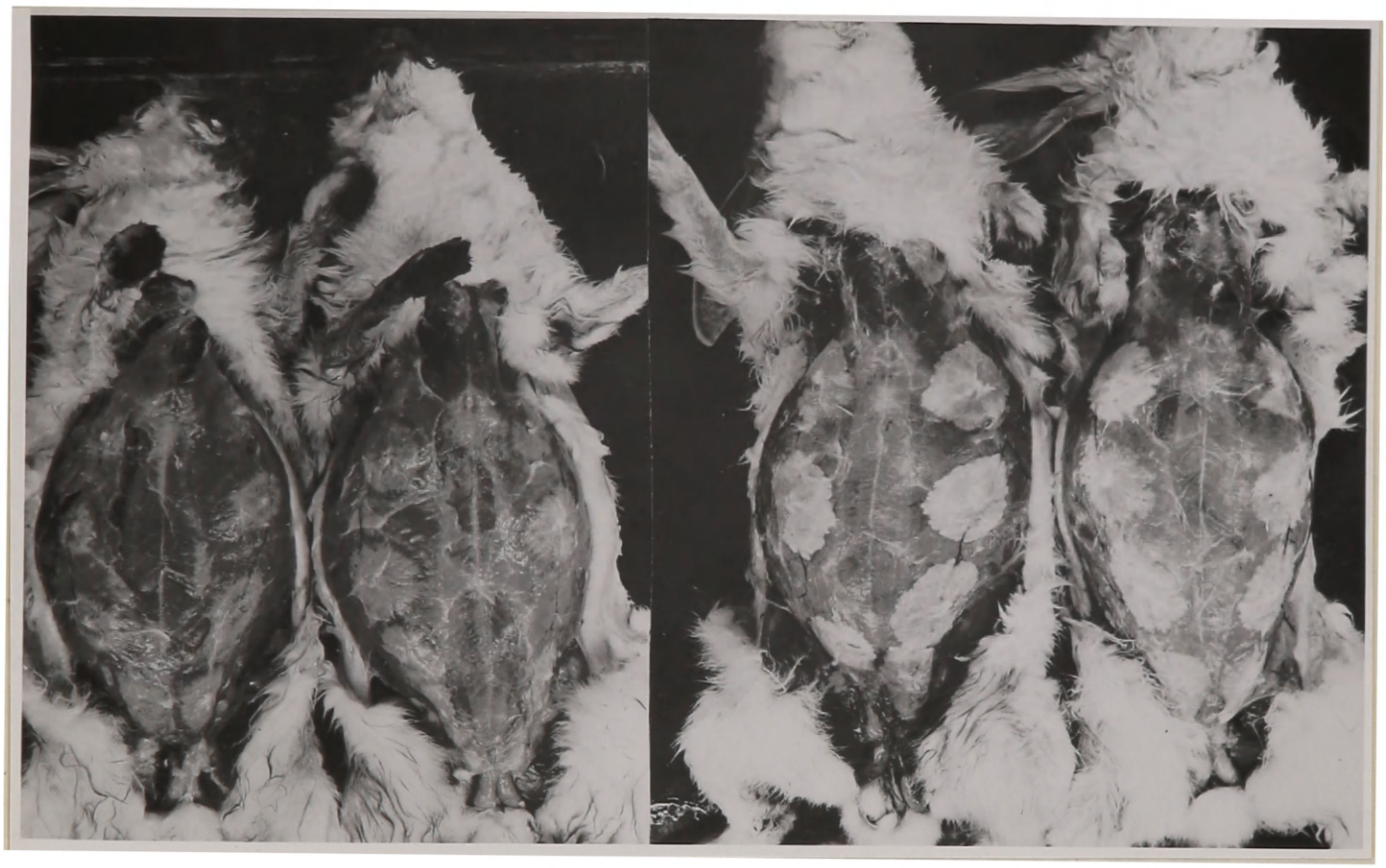

Figure 1. All 4 male rabbits were injected with estrone and progesterone for the first 25 days. From the 26th to 35th day, the two rabbits on the left were injected with prolactin, estrone and progesterone, and show no milk in their mammary glands. The two rabbits on the right were injected with prolactin only during this period and show considerable lactation.

The data from the third experiment, on ovariectomized rabbits, are essentially in agreement with those of the orchidectomized rabbits. However, the degree of mammary growth and quantity of milk secreted were greater (Figure 2) than in the male rabbits. Again estrone slightly inhibited prolactin action (group 3) while progesterone did not inhibit lactation (group 4).

Radioautographs of mammary glands from three intact male rabbits of groups 2 and 4 of the first experiment are shown in Figure 3. It can be seen that mammary growth in the two rabbits injected with both steroid hormones for the full 35 days was active and widespread, as indicated by the concentration of P^{32} in the peripheral and nipple areas (the regions of most active growth). The mammary gland of the rabbit from group 4, which received no steroid hormones from days 26 to 35, shows growth stasis as indicated by the more uniform distribution of P^{32} generally and a lesser concentration in the peripheral areas. The milk from this gland showed only a minimal amount of radioactivity as compared to that found in the mammary tissue, an observation in accord with previous findings (Lundahl et al., 1950).

Conclusions:

These results are believed to demonstrate that when estrone and progesterone are injected together in a ratio previously shown to promote optimal mammary growth in male rabbits, the mammary tissue is not responsive to prolactin

Figure 2. Both ovariectomized rabbits received estrone and progesterone for the first 25 days. From the 26th to 35th day, the rabbit above was injected with prolactin only and its mammary glands are filled with milk. The rabbit below received prolactin, estrone and progesterone, and shows complete inhibition of lactation.

Figure 3. The two glands on the left are radioautographs from two rabbits given estrone, progesterone and prolactin from the 26th to 35th day. The gland on the right is from a rabbit given prolactin alone during this period. Active growth in the two glands on the left is indicated by the concentration of P^{32} in the peripheral and nipple areas. Growth stasis in the gland on the right is indicated by the more general distribution of P^{32}, with least in the peripheral areas.

stimulation. When either was injected alone, progesterone produced no inhibition and estrone only a slight inhibition of the mammary response to prolactin. These data suggest that the action of the two ovarian hormones on the mammary gland during pregnancy render it refractory to prolactin stimulation. These results, therefore, at least partially support the hypothesis that mammary growth and lactation are antagonistic to each other. To what extent they apply to other species than the rabbit remains to be determined.

EXPERIMENT IV. EFFECTS OF ALTERING THE BALANCE BETWEEN PROLACTIN AND OVARIAN HORMONES ON THE INITIATION OF LACTATION IN RABBITS

Purpose:

In Experiment III it was shown that a moderate dose of prolactin could initiate lactation in rabbits with well developed mammary glands. This initiation of lactation could be effectively inhibited if optimal, mammary growth-promoting doses of estrone and progesterone were administered at the same time. In the absence of either one or both of the ovarian hormones, this dose of prolactin elicited a good degree of lactation. The present experiment was undertaken to determine the lactational response in rabbits to injections of (a) large doses of prolactin during treatment with a constant but optimal combination of estrone and progesterone for mammary development and (b) a moderate dose of prolactin in the presence of less than optimal amounts of the two steroid hormones.

Methods:

Thirty-six New Zealand White rabbits, weighing 5 to 6 pounds each, were ovariectomized and permitted 10 to 14 days recovery to remove the influence of the ovarian hormones. Each rabbit was then injected subcutaneously with 0.096 mg. of estrone and 1.0 mg. of progesterone daily in separate 0.1 ml. volumes of corn oil for 25 days. This combination of the two hormones was shown by Scharf and Lyons

(1941) to elicit optimal mammary development in male rabbits, and was used in the previous experiments. From the 26th to 35th days, 3 groups of 6 rabbits each were continued on the same doses of the 2 steroid hormones, together with 2, 4 or 8 mg. of prolactin daily, respectively; 3 other groups of 6 each were injected with 2 mg. of prolactin daily together with none, 1/4 or 1/2 of the amounts of the 2 steroid hormones used previously. The prolactin was injected subcutaneously in 0.1 ml. volumes of distilled water. The rabbits were killed on the 36th day and their mammary glands were exposed and rated visually for lactational response, from 0 to 4 according to the Gardner and Turner (1933) method. Commercial rabbit pellets and water were available at all times to the animals.

Results:

The findings are summarized in Table 6. The 2 mg. daily dose of prolactin initiated an abundant lactation in the absence of the two steroid hormones (group 1 and top animal in figure 2) while the same dose of prolactin was almost completely ineffective when injected together with 0.096 mg. of estrone and 1 mg. of progesterone daily (group 2 and animal number 3 in figure 4). This is in agreement with the previous observations in rabbits under identical treatment. In the presence of the above amounts of the 2 steroids, 4 mg. of prolactin daily elicited a moderate lactional response (group 3 and animal number 4 in figure 4) while 8 mg. of

TABLE 6. EFFECTS OF DIFFERENT LEVELS OF PROLACTIN, ESTRONE AND PROGESTERONE ON LACTATIONAL RESPONSE IN RABBITS

Group	Daily treatment (26th-35th days of experiment)	Av. lactational rating per gland
1	2 mg. Prolactin	4.0
2	2 mg. Prolactin + .096 mg. Est.[1] + 1 mg. Prog.[2]	.2
3	4 mg. Prolactin + .096 mg. Est. + 1 mg. Prog.	1.9
4	8 mg. Prolactin + .096 mg. Est. + 1 mg. Prog.	3.2
5	2 mg. Prolactin + .048 mg. Est. + .5 mg. Prog.	1.1
6	2 mg. Prolactin + .024 mg. Est. + .25 mg. Prog.	1.5

[1]Est. = estrone.
[2]Prog. = progesterone.

Figure 4. These five representative ovariectomized rabbits received growth stimulating levels of estrone (0.096 mg.) and progesterone (1.0 mg.) for the first 25 days. From the 26th to the 35th day they were treated as follows: the first rabbit on the left was injected only with 2 mg. prolactin daily and an abundant lactation was initiated (average rating = 4.0). The second animal from the left received 2 mg. prolactin, 0.048 mg. of estrone and 0.5 mg. of progesterone; a small amount of milk secretion was elicited (average rating = 1.1). The third animal received 2 mg. prolactin daily, 0.096 mg. of estrone and 1.0 mg. of progesterone; practically no lactation was initiated. The fourth and fifth animals received 0.096 mg. of estrone and 1.0 mg. of progesterone and either 4 mg. or 8 mg. of prolactin, respectively. In animal number four a moderate lactational response (average rating = 1.9) was elicited, while a good lactational response (average rating = 3.2 was observed in animal number five.

prolactin daily (group 4 and animal number 5 in figure 4) produced almost as much milk as in group 1. When the 2 steroid hormones were injected in reduced amounts although in the same ratio as used previously (group 5 and animal number 2 in figure 4) the 2 mg. daily dose of prolactin was sufficient to elicit at least a small amount of milk secretion. When the 2 steroids were further reduced, a much greater amount of milk secretion was detected (group 6 and animal number 1 in figure 4). This was much greater in group 2 but was less than in group 1.

Conclusions:

These data demonstrate that (a) levels of estrogen and progesterone which are optimal or near optimal for mammary development in rabbits do not completely inhibit lactation if a sufficiently large amount of prolactin is injected at the same time, and (b) when the two steroid hormones are injected in less than optimal amounts for mammary growth, even a moderate dose of prolactin can elicit at least some degree of lactation. This indicates that the antagonism between mammary growth and lactation is not absolute but is relative, depending on the balance between the levels of prolactin and the two ovarian hormones in the body. If the balance is in favor of prolactin, lactation will be initiated, while if the balance is in favor of the two ovarian hormones, lactation will be inhibited.

EXPERIMENT V. EFFECTS OF INJECTING MAMMARY TISSUE FROM PARTURIENT ANIMALS ON PITUITARY PROLACTIN CONTENT OF RATS

Purpose:

The level of the lactogenic hormone in the pituitaries of most animals remains low during pregnancy, begins to increase slightly prior to parturition and reaches its peak following parturition (Meites and Turner, 1948a, b). The latter have also shown that estrogens are capable of increasing pituitary prolactin content equivalent to postpartum levels in several species.

It seemed possible that this increased prolactin level following parturition might be mediated in part through the mammary gland itself. It was reasoned that if mammary tissue from parturient animals could inactivate increased quantities of prolactin and if it was more responsive to prolactin, it might influence the pituitary to secrete more prolactin. It was the purpose of this study to determine whether postpartum mammary tissue could alter the prolactin content of cycling and lactating female rats.

Methods:

Mammary tissue 1 to 2 days postpartum from rats and from parturient rabbits was collected separately and frozen solid. When sufficient tissue was accumulated, it was macerated in a Waring blender with ice-cold normal saline. The extraneous tissue was removed by filtration through a Buchner funnel and

the filtrate was lyophilized. One hundred mg. of dried mammary extract from rabbits and rats was suspended in 1 ml. and 1.5 ml. of normal saline respectively for injection.

Thirty mature female rats were divided into three groups of ten each. They were injected subcutaneously, daily, as follows: (1) controls, 1 ml. normal saline; (2) 1.5 ml. rat mammary extract daily; (3) 1 ml. rabbit mammary extract daily. At the end of 10 days of injections, the rats were sacrificed and weighed. The pituitaries were removed, weighed and assayed in 20 pigeons according to the Reece-Turner (1937) method.

Sixteen additional lactating rats (4 to 6 days post-partum) were isolated from their litters for a period of 8 hours. They were then divided into 2 groups of 9 and 7 each. The larger group served as controls and received only saline subcutaneously. The other group was injected with 1.5 ml. of rat mammary extract. Twenty-four hours later, both groups were killed and weighed. The pituitaries were removed and assayed for prolactin in 7 pigeons.

Results:

The findings are summarized in Table 7. The rabbit mammary extract appeared to decrease the pituitary prolactin content in mature rats by 27.7 per cent after 10 days of injection. Though the site of injection in the rat was varied every day, the area was highly inflamed in contrast to the minimum reaction seen when rat mammary extracts were used. The rat mammary extract, in contrast to the rabbit

TABLE 7. EFFECTS OF MAMMARY TISSUE FROM PARTURIENT RATS AND RABBITS ON THE PITUITARY PROLACTIN CONTENT OF CYCLING AND LACTATING RATS

Animal	No.	Treatment	Av. Body Wt. gm.	Av. Pit. Wt. mg.	Av. Pigeon Units* Per Pit.	Per mg. pit.	Per 100 gm. body wt.	% change
Mature rats	10	normal saline	190.4	12.0	1.10	.0917	.578	--
Mature rats	10	1.5 ml. rat mammary extract	189.0	10.5	1.56	.1488	.826	+41.8
Mature rats	10	1.0 ml. rabbit mammary extract	190.2	10.9	0.85	.0779	.447	-27.7
Lactating rats	9	normal saline	250.5	14.6	1.60	.1096	.639	--
Lactating rats	7	1.5 ml. rat mammary extract	255.4	14.7	1.87	.1272	.732	+16.9

*Reece-Turner units.

extract, increased the pituitary prolactin content in mature rats by 41.8 per cent after 10 days of injection. An increase of 16.9 per cent was also observed 24 hours after the rat extract was given to rats on the 4th day of lactation.

Conclusions:

On the basis of these limited data, postpartum mammary glands from rats and rabbits do not appear to influence pituitary prolactin content in mature and lactating female rats. Further work is necessary before any definite conclusion can be drawn regarding the effects of mammary tissue on pituitary prolactin secretion.

EXPERIMENT VI. EFFECTS OF PROLACTIN UPON OXYGEN CONSUMPTION OF MAMMARY TISSUE OF RATS DURING LACTATION

Purpose:

Kleiber et al. (1943) and Folley and French (1949) have reported that the oxygen consumption of the lactating mammary gland is considerably higher than that of the mammary tissue in pregnant rats. This increased metabolic activity in the mammary gland following parturition is known to be accompanied by a relatively high level of prolactin secretion by the anterior pituitary. Folley (1952) reported that the addition of prolactin in vitro to mammary tissue from parturient rats increased net respiratory activity. It was the object of this experiment to determine whether the addition of prolactin to lactating rat mammary tissue in vitro would influence oxygen consumption.

Method:

Lactating mammary glands were obtained from 18 rats of the Carworth strain. On the 4th, 5th, 8th, 9th, 10th and 12th days after parturition the animals were killed by decapitation. The inguinal mammary glands were quickly removed and rinsed in Ringer's solution and placed on moist filter paper. Slices of approximately 0.4 to 0.5 mm. in thickness were cut with a Stadie-Riggs microtome. Samples weighing approximately 200 mg. were placed in a Warburg flask containing 2.8 ml. Ringer-phosphate buffer, 0.3 per cent glucose and 0.02 M sodium acetate. The center well contained a folded filter paper wick and 0.1 ml. of 20 per cent KOH solution. Duplicate

TABLE 8. EFFECTS OF PROLACTIN UPON THE QO_2 OF MAMMARY GLANDS OF RATS DURING LACTATION

Days of lactation	No. of rats	QO_2*	QO_2* in presence of prolactin	% change	
4	2	1.74	2.19	25.9	
4	2	2.86	3.12	9.1	16.5
4	2	2.67	3.06	14.6	
5	2	1.67	2.24	34.1	
8	2	3.78	5.63	49.0	
9	2	2.20	3.44	56.3	44.9
9	2	2.78	3.71	33.5	
10	2	2.98	2.95	--	
12	2	3.04	4.90	61.2	

*QO_2 of dry tissue not corrected for milk content.

samples of mammary tissue with and without added prolactin were used. A total of 2 mg. of Squibb prolactin (20-25 I.U./mg.) were added to the former flask. All incubations were carried out at 37 ± 0.1° C., in the presence of a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide. After an equilibration period of 10 minutes, the flasks were agitated for 2 to 3 hours and the oxygen consumption was determined at intervals of 15 minutes each. Additional samples of 100 to 200 mg. of tissue were weighed and dried for 48 hours at 100° C. These were weighed again to determine the percentage dry matter of the tissue. The results are reported in terms of QO_2, i.e., the $mm.^3$ of oxygen consumed/mg. of dry tissue per hour. The QO_2 values given are averages based usually on the first eight 15-minute periods. At the end of each experiment the solutions had a milky-white color indicating the presence of milk. No correction was attempted for the milk content of the tissues.

Results:

The results are tabulated in Table 8. Oxygen consumption during lactation was increased by the addition of 2 mg. of prolactin in acetate-glucose-Ringer phosphate medium. This increase appears to be lowest early in lactation, averaging only 16.5 per cent in 6 animals on the 4th day of lactation. On the 12th day the average increase was 61.2 per cent in 2 animals. The QO_2 was relatively low compared to the figures given by Folley and French (1949) and this is

undoubtedly due to the fact that no correction was made in the present experiment for the milk present. The results in Experiment II indicate that the milk content of the mammary gland during lactation accounts for about two-thirds of the dry matter present. This indicates that these QO_2 values would be increased approximately three-fold if corrected for milk content, bringing them within the range of the figures of Folley and French (1949). However, the relative increase in QO_2 would not be altered if this additional correction was made. Therefore, these results remain valid as they are.

In one experiment involving animals on the 10th day of lactation, prolactin had no effect on QO_2. In this case the prolactin was added from the side arm of the flask rather than being present in the original solution. For some unknown reason there was no increase in QO_2 when this procedure was used. One may speculate that it may be a matter of solubility, i.e., the prolactin did not go into solution readily. However, there was no progressive increase in QO_2 as one would expect if the prolactin acted more slowly.

Conclusion:

When added *in vitro*, prolactin was capable of increasing the QO_2 of lactating mammary tissue from rats. This emphasizes the susceptibility of mammary glands from parturient rats to prolactin stimulation. Folley (1952) reported that the *in vitro* addition of prolactin to mammary tissue from

pregnant rats had no effect on respiratory activity, providing further support for the view that prolactin and the ovarian hormones are antagonistic in their actions on the mammary gland.

DISCUSSION

MAMMARY GROWTH VERSUS LACTATION

The continued presence of lactation during a subsequent pregnancy indicates that the ability of estrogen and progesterone to render the mammary gland refractory to prolactin stimulation is not absolute. Rather it depends on the balance between the amount of prolactin and the two ovarian hormones, estrogen and progesterone, in the body, as shown in Experiments III and IV. In the latter experiment the level of prolactin was increased and the two steroids were maintained at the same level, or the same level of prolactin was maintained and the doses of the two steroids were decreased. In the first series, as the level of prolactin was increased to 4 or 8 mg daily, a good lactational response was observed in contrast to little or no milk secretion with 2 mg. of prolactin daily. In the second series the greatest amount of milk was observed in the mammary glands when the two steroids were completely withdrawn and only 2 mg. of prolactin daily was administered, while the same dose of prolactin in the presence of either 1/2 or 1/4 of the two steroid hormones produced a smaller flow of milk. In view of these results, one may conclude that (1) the levels of estrogen and progesterone which are optimal or near optimal for mammary development in rabbits do not completely inhibit

lactation if sufficiently large doses of prolactin are injected at the same time and (2) when the two steroid hormones are injected in less than optimal amounts for mammary growth, even a moderate dose of prolactin can elicit at least a moderate degree of milk secretion. This indicates that the antagonism between mammary growth and lactation is relative, depending on the balance between the levels of prolactin and the two ovarian hormones in the body. If the growth stimulus exerted by the two ovarian hormones on the mammary glands is greater than the lactational stimulus of prolactin, then milk production will be inhibited, and vice versa. This does not exclude the possibility that other hormonal or non-hormonal factors may modify the lactational response.

Desclin (1952) has provided further evidence that large doses of prolactin can overcome the inhibitory action of the ovarian hormones on mammary secretion. He observed that when 30 I.U. of prolactin were injected daily into mature female rats, there was intense luetinization of the ovaries but no mammary secretion; however, when 200 I.U. of prolactin were injected daily, there was mammary secretion despite intense luteinization of the ovaries. Earlier, Nelson (1934) had reported that lactation in parturient guinea pigs was not suppressed by estrogens if a sufficient dose of prolactin was injected at the same time.

The initiation of a localized lactation in rabbits by intraductal injections of 10-20 I.U. of purified prolactin into a single nipple, without terminating pregnancy (Meites

and Turner, 1948a) is additional proof that prolactin can overcome the inhibitory influence of the ovarian hormones on the mammary tissue during pregnancy. The failure of
on the mammary tissue during pregnancy.
[illegible] lactation in pregnant animals with crude prolactin preparations, without first inducing abortion, may be due to contamination with other pituitary hormones.

It is believed that the absence of milk flow during pregnancy or the presence of abundant lactation in animals which are simultaneously pregnant and lactating, can be accounted for on the basis of the balance present between the levels of prolactin and the two ovarian hormones in the body. During a non-lactational pregnancy, the combined action of estrogen and progesterone stimulates mammary development but renders it relatively refractory to prolactin stimulation. In addition, the secretion rate of prolactin by the pituitary during a non-lactational pregnancy is too low to initiate abundant lactation (Meites and Turner, 1948a, b). Following parturition, prolactin secretion greatly increases and at the same time the reduction in ovarian hormones enhances the sensitivity of the mammary glands to prolactin stimulation, serving to initiate copious milk flow. Lactation can continue in parturient animals which subsequently become pregnant, probably because (a) the initial level of prolactin secretion is already high and remains high as a result of the continued milking stimulus (Reece

and Turner, 1937; Meites and Turner, 1942b) and (b) estrogen and progesterone are secreted at lower levels during early than in late pregnancy, and hence they would not appreciably antagonize prolactin action and decrease milk production until the latter part of gestation. Thus, in dairy cows which are simultaneously lactating and pregnant, there is no pronounced drop in milk yield until after the 5th month of gestation, following which time the decline is about 20 per cent greater than in non-pregnant cows (Hammond, 1927; Turner, 1939).

It is clear from the present work that the theory that mammary growth and lactation are antagonistic (Loeb and Hesselberg, 1917; Nelson, 1936; Folley and Kon, 1937) is not totally incorrect. However, the present work indicates that the original concept must be modified considerably. Mammary growth and lactation are antagonistic only when optimal or near optimal amounts of the two ovarian hormones are present. Even then, a large amount of prolactin can initiate some degree of milk secretion. It is obvious, therefore, that the antagonism between mammary growth and lactation is not <u>absolute</u> but is <u>relative</u>.

THE ROLE OF THE OVARIAN HORMONES

It is clear from Experiment III that progesterone <u>per se</u> does not inhibit the initiation or maintenance of lactation. This is in agreement with the observation of previous workers, who found that even large doses of progesterone

could not inhibit the initiation or the maintenance of established lactation (Walker and Matthews, 1949; Folley, 1942; Barsantini and Masson, 1947).

When only estrone was given in addition to prolactin, this resulted in only a slight inhibition of lactation. This is in general agreement with the observations of previous workers, who found that much larger doses of estrogens are required to inhibit established lactation in ovariectomized as compared to intact rats (Masson, 1948; Folley and Kon, 1938; Anselmino and Hoffmann, 1936a, b). There is evidence that estrogen in large doses may have certain toxic effects rather than specifically inhibit lactation. Edelmann and Gaunt (1941), Trentin and Turner (1941), Walker and Matthews (1949) found that large doses of estrogen caused lactating rats to lose weight. Meites and Turner (1948b) also found that the depressing effects of estrogen on lactation in goats is mediated largely through a loss of appetite.

In the guinea pig where only estrogen is necessary for complete mammary growth and for inhibiting lactation (Nelson, 1936, 1950), one must consider the possible effects of this hormone on the adrenals. It has been shown that small doses of estrogens stimulate increased steroid hormone output by the adrenal cortex of the guinea pig (Kimeldorf et al., 1947; Zondek et al., 1952). In view of the ability of the adrenal cortical hormones, such as desoxycorticosterone, cortisone and progesterone, to elicit mammary growth (Folley and Kon, 1938; Nelson et al., 1943; Selye, 1954; Johnson and

Meites, 1955), the adrenal steroids must be considered when estrogen is given to guinea pigs or to other species. For example, the inhibition of lactation with small daily doses of estrogen (5 mcg. daily) in intact rats and the observation that only simultaneous injections of progesterone and estrone inhibit lactation in ovariectomized rats (Walker and Matthews, 1949) indicates that estrogen per se is not responsible for the inhibition of lactation but that this is probably due to a synergism between estrone and progesterone. The source of the progesterone-like steroid in the case of the ovariectomized rats is probably the adrenal cortex.

The above indicates that the inhibition of established lactation is due to a synergistic action of estrogen and progesterone, and is in harmony with the results of Experiment III where it was shown that only when the two steroids were administered in doses which were optimal or near optimal for promoting mammary growth, that the moderate dose of prolactin used initiated little or no milk flow. This is also in agreement with Desclin's (1952) observations. He found that when ovariectomized rats were injected daily with 0.1 mg. of estradiol benzoate, 4 mg. of progesterone and 30 I.U. of prolactin, there was excellent mammary growth but no lactation. However, when only progesterone was injected with prolactin, or when only diethylstilbestrol was given with prolactin, abundant milk secretion was initiated.

ROLE OF THE PLACENTA

The placental theory of inhibition is not entirely incorrect in view of the endocrine functions attributed to it. It appears that the placenta in some species gradually assumes certain ovarian functions which allow gestation to continue (Newton, 1938). It has been shown in the human being, guinea pig, mare and monkey that ovariectomy at mid-pregnancy does not terminate pregnancy (Asdell, 1928; Douglass, 1931; Probstner, 1931). In the monkey and human being ovariectomy at this time does not alter urinary estrogen or pregnanediol levels (Turner, 1949). This is believed to be due to the ability of the placenta to act as an endocrine gland and secrete these hormones (Haterius, 1936; Leonard, 1945; Newton, 1938; Selye et al., 1935). Since placental tissue in the above species probably assumes the role of the major producer of estrogen and progesterone during the second half of pregnancy, it may play an important role in inhibiting lactation during gestation.

INACTIVATION OF PROLACTIN BY BODY TISSUES

The results of Experiment I on the inactivation of prolactin by body tissues in vitro show that on a fresh tissue basis, mammary, crop gland and the ovarian tissues were outstanding in their ability to inactivate prolactin. These tissues appeared to possess at least twice the ability of liver and kidney slices to inactivate this hormone. This is in agreement with Seidlin (1940) who observed that

gonadotrophic and thyrotrophic hormones are withdrawn from the blood stream by their respective target organs. He based this on the observation that thyroidectomized and gonadectomized guinea pigs excreted considerably more of the injected thyrotrophin and gonadatrophin, respectively, than did intact animals. In vitro incubation studies of these hormones, with their respective target organs, resulted in considerable inactivation of these hormones after only one hour.

Sonenberg (1951) reported that a radioactive preparation of prolactin labeled with I^{131} was concentrated in the ovary, liver, adrenal and kidney, but found no significant concentration of radioactivity in the mammary tissue of the rat under various physiological conditions. One may question the potency of this preparation since its activity was determined in only two birds, a number much too small to give an accurate indication of biologic activity. In addition, the control injections were made in different pigeons, providing an additional source of error. In contrast to these results, Cox (1951) reported that 20 minutes after intravenous injection of I^{131} labeled prolactin, the highest concentration was found in the liver and kidney, and appreciable amounts were also present in mammary tissue, milk, ovaries, and adrenals. Her results indicated that prolactin promptly left the circulation and underwent rapid breakdown. The result of the present study indicates that inactivation may

occur in numerous tissues but primarily in the mammary gland and ovary.

Meites (1940) injected prolactin subcutaneously into rabbits and followed its excretion in the urine. He was able to recover less than 1 per cent during the first 24 hours after injection, only a trace during the second 24 hours, and none during the third 24 hours. When prolactin was injected intravenously, none was recovered in the urine during the 72-hour period which followed; and when attempts were made to recover it from the bloodstream, about half was found at 5 minutes and none at 1/2 hour and 1 hour after injection.

The results of the first experiment suggested that the ability of mammary tissue to inactivate prolactin may depend on whether the animal is pregnant or lactating. In the second experiment it was found that lactating mammary tissue taken from rats on the 4th day after parturition showed about eight times as much ability to inactivate prolactin as mammary tissue taken from rats on the 12th to 15th days of pregnancy. This suggests, in agreement with the results of Experiments III and IV, that (1) mammary glands of pregnant rats are refractory to prolactin stimulation. The refractoriness is believed due to the action of the growth stimulating levels of the two ovarian hormones, and (2) mammary glands of lactating rats are sensitive to the action of prolactin because the refractory effect of the ovarian hormones is not present. The preceding suggests a possible

mechanism for the maintenance of prolactin at high levels during lactation. The gland is using and inactivating the available pituitary prolactin, and it may be withdrawing it from the circulatory system at a rate that exceeds the ability of the pituitary to produce prolactin. The pituitary, therefore, in attempting to maintain its normal secretory level, may be indirectly stimulated by the high metabolic activity of the lactating mammary gland. This possibility remains to be tested.

The possibility was also considered that the lactating gland may furnish a chemical mediator which activates the pituitary to secrete more prolactin (Experiment VI). Extracts of postpartal lactating mammary glands were made from rats and rabbits. These were injected into mature and lactating rats and their pituitaries were assayed for prolactin. The rabbit mammary extract caused a decrease in pituitary prolactin content in mature rats, while the rat mammary extract elicited an increase in pituitary prolactin content in both mature and lactating rats. The results are therefore insufficient to allow one to draw any definite conclusions regarding the presence of a chemical or hormonal mammary stimulator of pituitary prolactin secretion. This does not exclude the possibility that the parturient mammary gland may provide a nervous stimulus to pituitary prolactin secretion.

OXYGEN UPTAKE OF THE MAMMARY GLAND

In Experiment II it was shown that the lactating gland of parturient rats had more ability to inactivate prolactin than the growing mammary gland from pregnant rats. The post-partal mammary gland is more susceptible to prolactin stimulation, and apparently this is accompanied by an increase in mammary metabolic activity. This has been shown to be true by Kleiber *et al.* (1943) who conducted the first *in vitro* studies on the respiration of mammary tissue of rats. They found no difference between oxygen consumption on the 20th day of pregnancy and throughout lactation on a moist tissue basis. However, when calculated on the basis of dry matter content, they found a progressive increase in QO_2 from 0.9 at the end of pregnancy to 2.9 on the 21st day after parturition. Folley and French (1949) conducted similar studies and observed considerably higher values, due probably to their correction for milk content. Just prior to parturition the QO_2 had its lowest value of 1.5 but increased on the day following parturition (QO_2 = 4.0) and reached its peak at mid-lactation (QO_2 = 6.3). An *in vitro* effect of prolactin was first shown by Folley (1952), who observed that prolactin was capable of increasing net respiratory activity only in lactating rat mammary slices and not in mammary slices from pregnant rats. Experiment VI shows that prolactin can increase the oxygen consumption of lactating rat slices *in vitro*. It would have been

worthwhile to determine the *in vitro* effects of prolactin on oxygen consumption in mammary tissue from pregnant rats.

SUMMARY

1. In Experiment I prolactin was incubated *in vitro* for 1 hour at 38° C. with the following tissues: liver, kidney, brain and muscle of rats; mammary gland from lactating and nonlactating guinea pigs and rabbits; pigeon crop glands; corpora lutea and the remaining ovarian tissue from pseudopregnant rabbits. After incubation the prolactin was assayed in pigeons and directly compared with an equivalent amount of prolactin which had been incubated in a tissue-free solution.

On a fresh tissue basis, mammary gland, pigeon crop gland and the two ovarian tissues showed the greatest capacity to inactivate prolactin. Lactating mammary slices appeared to be more effective in this respect than nonlactating mammary slices. Liver and kidney inactivated only about half as much prolactin as the preceding tissues, while muscle and brain had no effect on the prolactin. Liver and kidney homogenates were more effective in removing prolactin than slices of these tissues. When any of the tissues were boiled for 10 minutes prior to incubation with prolactin, they lost their ability to inactivate the hormone.

2. In Experiment II the inguinal mammary glands were removed from 17 rats on the 12-15th days of gestation and from 19 rats on the 4th day postpartum. The glands were

homogenized and 100-mg. samples were incubated with 0.2 mg. of Squibb prolactin (20-25 I.U./mg.) at 38 ± 0.1° C. for 1 hour. For controls, the same amount of prolactin was similarly incubated without mammary tissue or with boiled mammary tissue. After incubation, the tissues were removed and assayed in White Carneau pigeons for prolactin activity.

The mammary homogenate from the lactating rats inactivated 65.6 per cent of the prolactin compared with 19.5 per cent for the tissue from the pregnant rats. When corrected for milk content, by lactose determinations, the former tissue showed 8 times as much capacity to remove prolactin as the latter. These data suggest that the mammary glands of pregnant rats cannot utilize prolactin to any marked extent, and that this contributes to the absence of copious lactation during gestation.

3. In Experiment III a total of 63 albino rabbits of both sexes, intact and castrate, were injected with 960 I.U. of estrone and 1 I.U. of progesterone daily for 25 days in order to induce optimal mammary development. From the 26th to 35th days, all except three rabbits were injected daily with 2 mg. of prolactin (20-25 I.U. per mg.), with or without one or both of the steroid hormones. At the end of the 35th day, the rabbits were killed and the mammary glands were exposed and rated for intensity of lactation. The extent of mammary growth induced by these treatments was determined by injecting radioactive phosphorus (P^{32}) 4 hours prior to sacrifice of the rabbits, and then making radioautographs of the mammary glands.

The rabbits given prolactin and both steroids during the last 10 days of the 35 day experimental period had practically no milk in their mammary glands (average rating of less than 1); those injected with prolactin alone had mammary glands filled with milk (average rating of 3 to 4); those given prolactin and progesterone showed no inhibition in lactational response (average rating of 3 to 4); and the rabbits injected with prolactin and estrone showed only a slight decrease in milk secretion (average rating of 2 to 3). The radioautographs indicated the presence of intensive mammary growth in the rabbits treated with both steroids throughout the entire 35 day period but not in the rabbits given both steroids only for the first 25 days. It is concluded that in the doses employed, estrone and progesterone together can effectively inhibit the milk-secreting action of prolactin on the mammary gland of the rabbit, whereas progesterone alone is ineffective and estrone alone is only slightly effective in this respect.

4. In Experiment IV 36 young rabbits were ovariectomized, and after 10 to 14 days were injected daily with 0.96 mg. of estrone and 1 mg. of progesterone for 25 days to induce optimal mammary development. From the 26th to 35th days, 3 groups of 6 rabbits each were continued on the same doses of steroid hormones together with 2, 4 and 8 mg. of prolactin daily, respectively; 3 other groups of 6 each were injected with 2 mg. of prolactin daily together with 0, 1/4 or 1/2 of the 2 steroid hormones used previously. On

the 36th day the rabbits were killed and their mammary glands were exposed and rated visually for lactational response.

In the first 3 groups lactation was almost completely inhibited when only 2 mg. of prolactin was injected daily in the presence of the 2 steroid hormones, while 4 or 8 mg. daily elicited good lactational responses. In the second 3 groups, the greatest amount of milk was secreted when 2 mg. of prolactin was given daily without the steroid hormones, while the same dose of prolactin in the presence of either 1/4 or 1/2 of the steroid hormones produced a smaller flow of milk.

It is concluded that the antagonism between mammary growth and lactation is relative, depending on the balance between the levels of prolactin and the 2 ovarian hormones in the body. When the mammary growth stimulus exerted by the 2 ovarian hormones is greater than the lactational stimulus of prolactin, milk flow will be inhibited, and vice versa. The application of these findings to explain the absence of lactation during a non-lactational pregnancy or the simultaneous coexistence of lactation and pregnancy is discussed.

5. In Experiment V, extracts of mammary tissue taken from parturient rats and rabbits were prepared and injected subcutaneously into mature and lactating rats. Thirty mature rats were divided into three groups and were injected daily for 10 days as follows: group 1 received a normal saline

solution; group 2 received 1.5 ml. rat mammary extract daily; group 3 received 1.0 ml. rabbit mammary extract daily. At the end of the experimental period the rats were killed and their pituitaries were assayed for prolactin content. The rat extract increased pituitary prolactin level by 41.8 per cent, while the rabbit extract decreased it by 27.7 per cent.

Sixteen additional rats on the 4th day of lactation were isolated for 4 hours from their litters and divided into two groups of 9 and 6, respectively. Group 1 received 1 ml. of normal saline, while group 2 received 1.5 ml. rat mammary extract in a single injection. At the end of 24 hours they were killed and their pituitaries were assayed for prolactin content. The rat mammary extract elicited a 16.9 per cent increase in prolactin content.

On the basis of these limited data it appears that mammary extracts from parturient rats and rabbits do not influence the pituitary prolactin content when injected into mature and lactating female rats. Further work is necessary before a definite conclusion can be drawn regarding the effects of mammary tissue on pituitary prolactin secretion.

6. In Experiment VI, the <u>in vitro</u> effects of prolactin on the QO_2 of mammary slices from lactating rats was determined. Eighteen rats on the 4th, 5th, 8th, 9th, 10th, and 12th day of lactation were decapitated and their inguinal mammary glands were removed. QO_2 was determined on slices in Warburg flasks containing acetate-glucose-phosphate buffer solution in the absence or presence of prolactin. Prolactin

was found to increase oxygen consumption, particularly in the latter stages of lactation. This suggests that the mammary glands from parturient rats are susceptible to prolactin stimulation.

7. Lactation is thought to be held in abeyance during pregnancy by two mechanisms: (1) the growth stimulating levels of estrogen and progesterone on the mammary gland, render it refractory to prolactin stimulation; (2) the relatively low level of prolactin secretion is insufficient to overcome the inhibitory effects of the 2 ovarian hormones on the mammary gland. At parturition, the high levels of the two ovarian hormones are greatly reduced, permitting the increased amounts of prolactin from the pituitary to initiate lactation. Lactation can continue during a subsequent pregnancy because (a) prolactin secretion is maintained at a high level by the milking act and (b) estrogen and progesterone are not secreted in sufficient quantities, particularly during the first half of pregnancy, to overcome the action of prolactin on the mammary gland. Other hormones may also influence the initiation and maintenance of lactation, but they are believed to play a secondary role to the factors mentioned above.

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