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Progesterone and Cortisol in the Hamster: Their Role in Parturition, as Well as the Growth and Lactogenesis of the Mammary Glands

Anne Christine Flook

PROGESTERONE AND CORTISOL IN THE HAMSTER: THEIR ROLE IN PARTURITION, AS WELL AS THE GROWTH AND LACTOGENESIS OF THE MAMMARY GLANDS

by

Anne Christine Flook

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INTRODUCTION

Parturition, the final event of gestation, normally results in the delivery of the fetus and the placenta. It is followed by puerperium, the period of time including lactation, during which the reproductive organs return to the non-pregnant state. Progesterone has been suggested to inhibit the premature onset of parturition through its effects on the myometrium. Also, fetally produced cortisol has been implicated in the termination of gestation (Liggins, 1968; 1969). The role of progesterone in inhibiting myometrial contraction has been studied by Schofield (1968). She has reported that strips of uterine muscle from rabbits pretreated with progesterone failed to allow the spread of waves of depolarizing impulses following stimulation. In contrast, uterine muscle from rabbits pretreated with estrogen showed complete spread of depolarization following stimulation. The role of fetal cortisol in the initiation of parturition was first suggested by Rea in 1898. He found the women carrying anencephalic monsters suffered prolonged gestation. A study of the endogenous levels of progesterone and cortisol in the hamster during late pregnancy, parturition, and early lactation could result in hormonal profiles which support the possible roles of these hormones in the termination of normal pregnancy.

The mammary gland can also be used as an index of pregnancy since lobular-alveolar development is a direct response to pregnancy titers of reproductive and adrenocortical hormones. Similarily, milk ejection and maintenance of milk production are indices of hormonal titers

during puerperium. Mammary gland development maintenance, and productivity can be studied through glandular content of RNA, DNA, total proteins, and casein-like phosphoproteins content.

It was the object of this study to determine serum levels of progesterone and cortisol in the hamster for a time period extending from the twelfth day of a 16 day pregnancy through the fourth day of lactation, with the day of delivery designated as day zero. Mammary gland DNA, RNA, total proteins, and casein-like phosphoproteins were determined for the same time period.

LITERATURE REVIEW

The Role of the Corpus Luteum in Pregnancy

In 1899, Gustav Born (Csapo-1956) proposed the theory that the corpus luteum was a secretory organ necessary for implantation and the maintenance of gestation. This theory was later substantiated by Fraenkel in 1910. He demonstrated that destruction of corpora lutea in pregnant rabbits resulted in abortion. Later, Corner and Allen (1930) reported that crude extracts of corpora lutea could facilitate implantation and maintain pregnancy in a rabbit ovariectomized 18 hours after impregnation. They isolated the biologically active fraction of corpora lutea extracts in 1929. It was purified and its structure determined by Westfall and Cobler in 1934. They named it progesterone (Csapo-1956).

The Effects of Progesterone on the Myometrium

Even before the isolation of progesterone there was evidence indicating a relationship between the corpus luteum (and hence, progesterone) and the contractile activity of the myometrium. For example, in 1906 Dale and Cushy separately showed that the myometrium of the pregnant cat (a corpus luteum dominanted phase) relaxed upon hypogastric nerve or adrenaline stimulation. In contrast, they reported that the myometrium of the nonpregnant cat contracted upon stimulation. Corpora luteal control of the myometrium was further suggested by Keye

in 1923. He found that uterine contractions in the sow were strong and infrequent during the follicular phase of the estrous cycle. However, during the corpus luteum dominant phase of the cycle the contractions were weak but frequent.

The corpus luteum was clearly demonstrated to be responsible for reduction of myometrial contractions by Vandyke and Gustaveson in 1929. They reported that corpora luteal extracts, when administered to the nonpregnant cat, produced the same myometrial relaxation found separately by both Cushy (1906) and Dale (1906) in the pregnant cat. The role of the corpus luteum was further clarified by Knaus in 1930. He found that administration of corpora luteal extracts to non-pregnant animals resulted in loss of spontaneous uterine contractions and response to oxytocin stimulation. Reynolds and Allen (1932) then found that strong infrequent myometrial contractions, characteristic of the follicular phase of the estrous cycle, were suppressed within 60 minutes of administration of corpora luteal extracts.

With the isolation of progesterone from corpora luteal extracts, it became possible to study the mechanism by which the hormone inhibited myometrial contraction. For example, Jung (1965) demonstrated a blocking of the spread of depolarization in myometrial fibers taken from a rabbit pretreated with progesterone. Schofield (1969) has confirmed these findings and further reported that strips of uterine muscle from estrogen pretreated rabbits showed no inhibition or slowing of depolarizing impulses following stimulation. The results of Jung (1965) and Schofield (1969), confirmed the findings of both Dale

(1906) and Cushy (1906). The implication is that high titers of progesterone during the corpus luteum dominanted phase of the estrous cycle, or during pregnancy, inhibit the spread of depolarizing impulses through the myometrium. Schofield (1969) has shown that the effects of progesterone are mediated through alterations in electrolyte concentrations across the cell membrane.

Plasma Concentration of Progesterone Associated with Parturition

Evidence so far indicates that progesterone reduces myometrial contractility. Consequently, one would expect an increase in the amount of progesterone available to the myometrium during gestation. This would help prevent the premature expulsion of uterine contents. Assuming an increase in available progesterone did occur, it would probably result in a detectable change in plasma concentrations of this hormone. Circulating progesterone titers have been reported in several species. Increasing progesterone secretion from the time of conception to at least mid-pregnancy has been well documented (Heap and Deasely-1966; Hoffman et.al.-1973; Stabenfeldt, et.al.-1972). However, the progesterone profile from mid-pregnancy through delivery is not as clear cut. One would anticipate a drop in available progesterone immediately prior to parturition. This would allow increased uterine activity necessary for delivery.

In fact, from species to species (or within the same species) progesterone concentrations in the peripheral plasma have been reported to increase, decrease, or remain unchanged with the onset of

delivery. For example, Labsetwar and Watson (1974) have reported that rats ovarian venous progesterone (progesterone secretion) decreased constantly from a value of 1500 ng/ml obtained on day 19 of gestation to 700 ng/ml found immediately prior to delivery (day 22). No sudden decrease was observed with the onset of parturition. During the same time period, 20-dihydroxyprogesterone increased from 1000 ng/ml to 5000 ng/ml. It remained elevated throughout parturition and the first two days of lactation. Although the physiological role of this progestin in parturition has not been reported, it may act as a less active progesterone metabolite (Weist, et.al.-1968). Hence, increased 20-dihydroxyprogesterone could indicate increased progesterone synthesis (and metabolism), but reduced circulating progesterone.

In the hamster, Joshi, Watson, and Labsetwar (1974) reported no change in peripheral plasma progesterone during the entirety of gestation, parturition, or lactation. Progesterone fluccuated only slightly between 1000 and 1500 ng/ml. However, they did note constantly increasing ovarian progesterone secretion as measured from the ovarian vein. A peak in ovarian progesterone output was reported on the fifteenth day of gestation. This was followed by a 4 fold decrease in ovarian secretion on the day of delivery. (These data were not mentioned in the text of that paper, but could be gleaned from the daily secretion values recorded in Figure 1 of that paper.) The fate of the secreted peak of progesterone is unknown.

Data similar to that inferred from the work of Joshi (et.al.-1974) had been reported by Leavitt and Blaha (1970). Sampling daily from

the posterior vena cava, just below the entrance of the ovarian and adrenal veins, they found increasing hamster plasma progesterone from day one (1.0 ng/ml) to day 14 (25.0 ng/ml) of gestation. Progesterone then dropped to 2.0 ng/ml on the day prior to delivery. The 5 fold differences in total values between these two studies may have been due to dilution of the secreted hormone by the circulation. The authors were sampling from two different regions of the venous system. Lukaszewka and Greenwald (1970) further confirm the pattern of progesterone secretion reported by Leavitt and Blaha (1970) and that obtained from the data of Joshi (et.al.-1974).

Among the farm animals, there are also discrepancies in the recorded amounts of circulating progesterone associated with parturition. For example, sampling at intervals of several days, Neher and Zarrow (1954) found that total peripheral progestons in the pregnant ewe increased 5 fold from the day of conception to the day of delivery. Following delivery, progestons then dropped to 2.0 ug/ml of plasma over a period of the first 14 days of lactation.

Stabenfeldt, et.al.-1972 have also studied peripheral plasma progesterone in the pregnant ewe. However, daily observations revealed increasing progesterone until day 126 of a 165 day gestation. This was followed by a 5 fold decrease during the last 39 days of pregnancy. A final decrease was observed immediately following delivery, probably due to the removal of the progesterone-producing placenta.

In the cow, in a very percise study, Hoffman et.al. (1973) re-

sulted in a peripheral plasma progesterone profile that would closely resemble the progesterone profile of the blood bathing the myometrium (in pattern though not in absolute concentration).

Of the animals reviewed in this section, only the hamster and the cow have been shown not to produce placental progesterone. Consequently, peripheral progesterone values of only these two species may be representative of changes in progesterone as related to myometrial 'block'. In contrast, the placenta has been shown to synthesize progesterone in the sheep and the rat (Courrier-1945). Thus, peripheral hormonal changes may not be similar to those occuring at the myometrium. Only uterine vein progesterone changes would be of significance under these circumstances. However, these animals were cited in this review not only to point out this fact, but also to give examples of the discrepancies that do exist from study to study of peripheral progesterone as related to parturition.

The Role of the Fetal Adrenals in the Induction of Parturition

There is a great deal of evidence indicating a role of the fetal adrenals in stimulating the initiation of parturition. For example, overstimulation of the fetal adrenals, as observed in Angora goats which characteristically suffer fetal adrenal hyperplasia, results in habitual abortion (VanRensberg-1967). In contrast, fetal lamb adrenalectomy causes failure of the initiation of labor (Liggins and Kennedy-1968). Control of the adrenals, and hence, parturition, by the brain (or the hypothalamus and pituitary) was first suggested in

1898 by Rea. He noted that women carrying anencephalic monsters suffered prolonged delay of delivery. Later, Liggins and Kennedy (1967) added support to this suggestion when he reported that electrocoagulation of the fetal lamb pituitary resulted in indefinite gestation. He also reported the occurence of simultaneous fetal adrenal hypoplasia, further indicating control of the adrenals by the pituitary. More recently, the hypothalmus-pituitary-adrenal axis has been well documented. In most instances, interruption of this axis in the fetus results in delay of the onset of delivery (Liggins and Kennedy-1967). This effect has been shown to be due to changes in fetal adrenal corticoid output (Basset and Thornburn-1969).

Effects of Corticoid Administration on the Induction of Parturition

Following the realization that functional fetal adrenals were necessary for normal parturition, work was begun to determine the effects of experimentally elevated glucocorticoids on the time of delivery. (Liggins, in relating unpublished data in 1971, reported that only glucocorticoids, not mineralocorticoids, had a role in parturition.) Glucocorticoids have been administered to both the fetus and the mother. This was because cortisol has been shown to cross the placenta in both directions (Anderson et.al.-1972; Dixon et.al.-1970). For the sake of clarity, the effects of experimentally elevated fetal corticoids will be discussed separately from the effects of experimentally elevated maternal corticoids.

Effects of fetal administration: Dexamethazone is a synthetic glucocorticoid which is approximately 25 times more potent than cortisol (Travis and Sayer-1965). In sheep, it has been shown that fetal infusion of 0.06 to 0.4 mg per 24 hours of dexamethazone between the 97th and 120th days of a 165 day gestation resulted in premature delivery (Liggins-1969). Bassett, Oxbarrow et.al. (1969) have reported that infusion of 50 mg of cortisol per 24 hours for 4 days will also terminate gestation. ACTH administration in sheep has been shown to have the same effect, further confirming the pituitary-adrenal axis (Liggins-1968).

Effects of maternal administration: Again in sheep, Liggins (1968) found that infusion of 4.0 mg of dexamethazone per 24 hours between the 97th and 120th days of gestation had no noticable effect on the time of delivery. However, abortion has been induced in sheep with slightly higher doses of 6.0 to 10.0 mg per 24 hours (Fyling et.al.-1973). Since dexamethazone has not been reported to cross the placenta, it is not possible to correlate effective maternal dose to effective fetal dose with this drug.

In contrast to the effects of high doses of dexamethazone, administration of ACTH to the pregnant ewe during the same time period as the above studies does not affect pregnancy (Anderson et.al.-1972). This point is significant in that it implies that the physiological levels of cortisol that would be released due to ACTH administration are not enough to induce abortion. Thus, cortisol release due to the "stress" of pregnancy would not trigger parturition. Nor would the

1.0 to 2.0 ug/100 ml of cortisol diffusion from the fetal circulation during normal pregnancy have an effect since those values are well within the physiological range of maternal cortisol concentration. The implication of this information, gathered from corticoid administration studies, is that the fetal corticoids responsible for the initiation of labor have their effect on the fetal side of the placenta. However, it was the object of this section to point out that corticoid administration to the fetus or the mother can induce parturition during late pregnancy. It is not possible at this time to determine where or how the corticoids have their effect.

Maternal Plasma Concentration of Cortisol

Through the studies of the fetal hypothalamus-pituitary-adrenal axis, it has been shown that functional fetal adrenals are necessary for normal termination of gestation. As stated in the previous section, there is indication that the fetally produced glucocorticoids act directly on the fetal side of the placenta to terminate pregnancy. However, it has been demonstrated, in sheep at least, that corticoids cross the placenta. Dixon et.al. (1970) reported that 10 to 20% of the cortisol administered systemically to the fetal lamb was recovered from the peripheral maternal circulation. Consequently, studies of the peripheral maternal cortisol concentrations should reveal a peak in cortisol just prior to the onset of labor. This peak, in large part, would be due to washover of the elevated fetal cortisol responsible for initiation of parturition.

The anticipated rise in fetal corticoids necessary for termination of gestation has been confirmed in sheep by Bassett and Thornburn (1969). Sampling daily, they found that commencing several days before birth there was an increase in fetal plasma corticosteroids; they rose from 2 ug/100 ml on day 133 to 12 ug/100 ml on day 136, the day of parturition. This rise was not due to maternal backwash since simultaneously determined maternal corticoids varied from 2.0 to 4.0 ug/100 ml plasma.

In presenting their study, Bassett and Thornburn (1969) did not suggest the presence of an increase in maternal cortisol due to fetal washover. However, re-examination of their data reveals daily flucuations in maternal corticoids. Assuming 10 to 20% washover, these flucuations could be accounted for by fetal contribution. However, they may also be due to stress as suggested in pregnant women by Adadeveh and Akinla (1971).

In the cow, frequent sampling just prior to delivery has revealed that total corticoids begin increasing 25 hours prepartum. Beginning at 5.0 ng/ml of jugular plasma, they rise to 10.0 ng/ml at 10 hours prepartum. They then drop continuously for the next 35 hours, peaking slightly again 25 hours after the initiation of lactation (Hoffman et.al.-1973). Adams and Wagner (1970) have reported similar results in the same species.

Although the source is unknown, the maternal peripheral plasma of some species reveal small peaks of corticoids just prior to the initiation of parturition. There is indication that the source of

these peaks is the fetal adrenals. This is suggested by the fact that similar increases have been observed two days prior to parturition in the adrenalectomized rat (Kamoun and Stutinsky-1968). Also, the magnitude of the peaks has been shown to be within the range of possible contribution by fetal washover.

Mammary Gland Growth and Differentiation

Mammary gland growth prior to pregnancy, resulting from estrogen stimulation, is characterized by ductal elongation and thickening. The addition of increasing progesterone titers during pregnancy results in end bud and alvedar formation in most mammalian species. Exceptions are the cow, goat, dog, and guinea pig. The presence of growth hormone, prolactin, thyroid hormones, insulin, and glucocorticoids allows full lobular-alveolar development in which the distal end buds become non-secreting alveoli. The alveoli develop secretory activity following a drop in progesterone associated with parturition, and an increase in prolactin stimulation. Secretory capability is characterized by milk-protein synthesis and secretion (Sulman-1970).

Effects of Progesterone on Mammary Gland Growth

The first proof that mammary gland development was controlled by ovarian hormones was reported by Fellner in 1913. He found that injections of extracts of ovarian tissue, containing corpora lutea and follicles, into castrated female rabbits induced mammary gland ductal

growth. The role of progesterone became more obvious with the isolation and purification of this hormone. For example, Gardner and Hill (1936) demonstrated extensive ductal growth in castrate female mice following administration of the purified hormone.

The direct effects of progesterone on mammary gland development, as opposed to the systemic effects, have also been studied. Mishkinsky et.al.-1967, found that it was necessary to add 1.0 ug/ml of progesterone to cultures of nulliparous mouse mammary glands (pretreated with estradiol) in order to achieve complete alveolar development. Hahn and Turner (1966) have reported similar results. Using progesterone embedded paraffin implants in estradiol-pretreated castrate female mice, they could demonstrate full lobular-alveolar development only in those glands in direct contact with the paraffin pellet. The reader will recall from the introduction of this section that other endogenous hormones are responsible for full lobular-alveolar development. Progesterone causes end but development. However, the work of Hahn and Turner (1966) was an in vivo study where the necessary hormones were present for development beyond the end bud stage. Consequently, although lobular-alveolar development was used as the measurable end point in their study, the major effects of progesterone may only have been to induce the precursor end bud stage.

Effect of Progesterone on Mammary Gland Secretion

In addition to facilitating mammary gland lobular-alveolar development by promoting end bud growth, progesterone has been implicated

in the inhibition of milk protein synthesis. The mechanism of inhibition is considered to be on both the local level, with progesterone affecting the alveoli directly, and systemically by decreasing prolactin release from the pituitary (Mietes-1959). The lack of prolactin would result in no formation of milk proteins.

The direct effects of progesterone on alveolar secretion have been studied in organ culture. For example, Barnawell (1967) found that the addition of progesterone to virgin dog mammary gland cultures, previously stimulated to lobular-alveolar development with subcutaneous injections of estradiol benzoate and progesterone, resulted in inhibition of secretion of milk proteins normally induced by the addition of prolactin to the organ culture.

The systemic effects of progesterone on mammary gland secretion have been stressed by Folley (1952). He suggested that estrogen stimulates the release of pituitary prolactin. However, the synergism of increasing progesterone during gestation results in an inhibition of prolactin secretion. The decrease in progesterone associated with parturition would result in stimulation of prolactin release, and mammary gland lactogenesis. In support of this theory, David et.al. (1972) established that infusion of physiological doses of progesterone into ovariectomized 14 to 16 day pregnant rats suppressed milk formation as determined by mammary gland RNA and milk protein content. However, casein synthesis did occur in comparable animals that received physiological doses of estradiol. This indicates that estrogen was not responsible for inhibition of milk protein snythesis.

Although there was no way to separate the local effects of progesterone from the systemic effects in the study of Davis et.al. (1972), the fact remains that physiological doses of progesterone, or resultant progesterone metabolites, suppressed milk protein formation.

Effects of Cortisol on Mammary Gland Development and Lactogenesis

The necessity of cortisol in mammary gland development is not well understood. For example, it has been shown that 100 to 200 ug/day of cortisol given subcutaneously for 21 days to 3 week old ovariectomized virgin mice inhibited estrogen-stimulated mammary gland ductal growth (Flux-1954). However, Nandi (1959) reported that 125 ug/day of cortisol given to adrenalectomized, ovariectomized mice resulted in maintenance of the ductal system, but lack of end bud maintenance. Nandi (1959) also reported that similarily operated animals treated with estrogen and progesterone exhibited extensive ductal branching complete with end buds, but no alveoli. Consequently, these data combined with other data not reported in this review, suggest that no definite role of cortisol in mammary gland growth and differentiation has been determined. However, cortisol has been shown to be essential for mammary gland epithelial cell proliferation and differentiation when added to insulin-maintained organ cultures. Furthermore, the addition of cortisol was necessary before prolactin could stimulate RNA synthesis (Turkington and Ward-1969). These data suggest a general 'maintenance' role for cortisol which is most likely related to its metabolic glucocorticoid characteristics.

The relationship between cortisol and lactogenesis has been suggested by Nandi and Bern (1961) and Talwalker et.al. (1963). They have indicated that an increase in prolactin at parturition in mice, rats, and rabbits may not be sufficient for lactogenesis. They have suggested that increasing glucocorticoids may also be necessary. Further, it was indicated that high levels of reproductive steroids may inhibit the lactogenic effects of glucocorticoids. Hence, the decrease in sex steroids at parturition, as well as a possible increase in glucocorticoids may result in complete lactogenesis.

In conclusion, with the onset of parturition, one would expect to find a decrease in maternal peripheral plasma progesterone. This decrease would allow an increase in myometrial contraction. At the same time, a decrease in progesterone may allow an increase in the release of pituitary prolactin which would stimulate mammary gland lactogenesis. The effects of the increased prolactin would combine with any local effects high titers of progesterone would have on the developing mammary glands resulting in complete lactation.

Also with the onset of parturition, one would expect to see a small peak in maternal peripheral plasma cortisol. This would be due to washover of the peak of fetal cortisol responsible for the initiation of labor. The effects of the changing plasma levels of cortisol on the developing mammary glands is unknown. The effects of cortisol on lactogenesis are also unknown but have been suggested to be positive.

It was the object of this investigation to measure maternal

peripheral plasma progesterone and cortisol in the hamster for the last 4 days of pregnancy, the day of parturition, and the first 4 days of lactation. At the same time, mammary gland DNA, RNA, total proteins, and casein-like phosphoproteins were determined in an attempt to correlate the changes in progesterone and cortisol associated with parturition to mammary gland growth, differentiation, and lactogenesis.

MATERIALS AND METHODS

Daily serum levels of progesterone and cortisol were studied in the hamster for a time period extending from the 12th day of a 16 day pregnancy through the 4th day of lactation, with the day of parturition called day zero. Cortisol was assayed by competitive protein-binding radioassay following methylene chloride extraction from alkaline serum. Progesterone was assayed by double radioimmunoassay following purification by petroleum ether extraction and thin layer chromatography.

At the same time, the right abdominal-inguinal mammary glands of each hamster were analyzed for RNA, DNA, and total protein content. RNA and DNA were isolated and separated by selective protein percipitation followed by acid extraction. Nucleic acid content was determined by ultraviolet absorbance. Protein concentrations were determined by the standard Lowry method.

Casein-like phosphoprotein content of the left abdominal-inguinal glands of each hamster was determined by absorbance at 750 mu following ultracentrifugation and rennin precipitation.

Sample Collection

Mature virgin Lakeshore hamsters were mated the night of proestrous as determined by post estrous discharge. The presence of sperm the following day was used as an indication that mating had occurred, and this day was referred to as day one of a 16 day pregnancy. Fifty sperm positive animals were separated into 9 groups of approximately

5 per group. All the animals of one group were sacrificed each day beginning on the 12th day of gestation and continuing through the 4th day of lactation, with the day of parturition designated as day zero. The presence of fetuses was used to confirm pregnancy in those animals killed prior to delivery. The number of pups per dam was adjusted to 5 for those animals killed postpartum. Adjustment was done on the first day of lactation. Immediately following unanesthetized decapitation, the blood of each dam was collected separately and allowed to clot at room temperature for 4 hours. It was then centrifuged at 2000 rpm for 10 minutes and the serum was drawn off and stored at -20°C until the extraction of progesterone and cortisol. At the same time, the right abdominal-inguinal mammary glands of each hamster were removed, quick frozen on dry ice, and stored at -20°C until assay for DNA, RNA, and total protein. The glands of the left sides were similarly removed and stored until they could be assayed for casein-like phosphoprotein content. All progesterone, cortisol, and mammary gland DNA, RNA, total protein, and casein-like phosphoprotein content were determined for each animal separately.

All animals were maintained on standard lab chow (Ralston Purina) and water and libitum, with occasional apples as supplement. The lighting cycle was maintained at 14 hours light, 10 hours dark.

Extraction and Purification of Progesterone

Serum lipids were extracted by washing 0.2 ml thawed serum three times with 3.0 petroleum ether each time. The pooled extracts of

each sample were evaporated to dryness at room temperature with nitrogen filtered through glass wool. The lipids were washed from the sides of the tubes three times with 0.1 ml ethyl alcohol each time. Following each washing, the entire sample was spotted onto a silica-gel thin-layer chromatography plate (Uniplate, 250 u gel thickness) that had been previously washed two times in methanol and dried. Three samples were spotted onto each plate, separated by progesterone reference standards. The spots were dried with nitrogen at room temperature and the progesterone was separated from the other lipids by placing the plates in an all glass chromatographic chamber containing approximately 3.0 cm in depth of a 2:1 mixture of diethyl ether and benzene. When the solvent was within 2.0 cm of the top of the gel, the plates were removed and allowed to dry at room temperature. They were exposed to ultraviolet light, and those areas corresponding to a horizontal line represented by the visible progesterone standards were scraped from the plates with a razor blade and transferred to conical centrifuge tubes. The progesterone was removed from the silica by the addition of 0.5 ml of water, followed by extraction two times with 3.0 ml petroleum ether each time. To avoid the contamination of the organic layer with water, the aqueous layer was frozen by partially submerging the tubes into a slurry of dry ice and acetone. The organic layers were then decanted and the combined washing of each sample were evaporated to dryness at room temperature with nitrogen. The progesterone was brought into solution by the addition of 0.5 ml of 0.89 M tris-HCL buffer, pH 8.0. One tenth milliliter of the

progesterone containing buffer was used in the radioimmunoassay for progesterone.

Radioimmunoassay for Progesterone

The radioimmunoassay, as generally described by Yalow and Berson (1960) is based on competition between radioactive and non-radioactive steroids for binding sites on the gamma globulin produced specifically against that steroid. The concentration of the gamma globulin immunoserum used is dependant upon the desired sensitivity of the assay; the more dilute the antiserum, the more sensitive the assay. The addition of a second antibody, produced specifically against the first gamma globulin, results in the formation of an insoluble complex. Following centrifugation, it is possible to determine the amount of radioactive steroid remaining in the supernatant by scintillation counting. Standard curves, run with each assay, are used to relate the measured radioactivity to the amount of steroid added to the assay. Due to the cross reactivity of the antibodies to steroids other than progesterone, the samples were appropriately extracted with solvents to eliminate all the steroids except progesterone.

The exact radioimmunoassay procedure used in this study is as follows: 0.1 ml of the progesterone containing tris-HCL buffer was added to 10 by 75 mm tubes already containing 0.1 ml of 6,7-H-progesterone (New England Nuclear) which had been diluted previously with 0.89 M tris-HCL buffer to approximately 3000 counts per minute in 0.1 ml. Five tenths milliliter of sheep anti-progesterone gamma

globin (obtained from the UpJohn Co.), diluted 1:10,000 with 0.89 M tris-HCL was added to each tube, including standard tubes already containing progesterone in concentrations varying from 12 to 20,000 pg in 0.2 ml tris buffer. Following a brief mixing, 0.1 ml of rabbit anti-sheep antiserum, diluted 1:30 with tris buffer, was added to each tube. This was followed by another brief mixing. Blank tubes, used to determine the total added radioactivity, were also prepared. They contained 0.5 ml of tris buffer, 0.1 ml of anti-sheep antiserum, and 0.1 ml of the diluted tritiated progesterone. The total binding capacity of the antibodies was determined by the addition of 0.1 ml of tritiated progesterone to 0.5 ml of anti-progesterone antiserum, followed by the addition of 0.1 ml anti-sheep antiserum. Enough tris buffer was added to each tube to bring the total volume to 0.8 ml. The amount of binding occurring in this tube was referred to as 100% binding. All tubes were stored in the dark overnight at 4°C. The next day, the tubes were centrifuged at 3000 rpm in a model PR-6 International Centrifuge for 20 minutes at 4°C. Five tenths milliliter of the supernatant of each tube was transferred to a Wheaton Scintillation vial to which was later added 14.0 ml of scintillation fluid containing 10% Beckman BIO-SOLV (formula BBS-3) and 4.2% Liquifour in toluene. The vials were shaken and counted in a refrigerated Packard Tri-Carb Liquid Scintillation Spectrometer (model 2425). All progesterone concentrations, as read from the standard curve, were multiplied by 2.5 to correct for the initial dilution prior to extraction, and then adjusted for percent recovery following extraction and plating.

Extraction of Cortisol

Cortisol was extracted by washing 0.1 ml of thawed serum three times with 3.0 ml of methylene chloride each time. The serum had previously been made alkaline by the addition of 0.05 ml of 1 N sodium hydroxide and 1.0 ml of water. After each extraction, the bottom organic layer containing the cortisol was carefully drawn off and transferred to a conical centrifuge tube. The sodium hydroxide was washed from the combined extracts of each sample twice with 2.0 ml of water each time. The top aqueous layer was discarded after each washing. The extracts were evaporated to dryness at 37°C with nitrogen. During the drying process, the sides of the tubes were washed two times with 0.1 ml of methylene chloride each time. The samples were assayed by a modification of the competitive protein binding radio-assay as described by Murphy (1967).

Competitive Protein Binding Radio-assay for Cortisol

The competitive protein binding radio-assay (cPBR) is based on a competitive principle similar to that used in the radioimmunoassay. The non-radioactive steroid displaces the radioactive steroid from the binding sites on a naturally occurring corticosteroid binding globulin (CBG). Once displacement had occurred, an absorbant is added, in this case Florisil, to remove the unbound steroids from the supernatant. The supernatant is then counted and the amount of bound steroid is determined from a standard curve.

More specifically, 1.0 ml of diluted male rabbit plasma was added to each tube containing the evaporated cortisol extracts. The diluted plasma had been previously prepared by the addition of 0.75 ml male rabbit plasma to a flask in which enough tritiated cortiocosteroid had been evaporated to result in a final radioactivity of approximately 3000 cpm per ml upon the addition of 100 ml of distilled water. Immediately following the addition of the diluted plasma, the tubes were mixed briefly and placed in a 45°C water bath for exactly 5 minutes. They were then removed, quickly mixed again, and placed in an ice bath. Exactly 10 minutes later, 80.0 mg of washed Florisil (60-80 mesh) was added to each tube. The tube was mixed for exactly 30 seconds and returned to the ice bath. This step was repeated for all the tubes in the order that the diluted plasma had been added. Finally, 0.5 ml of the supernatant was carefully drawn off each tube after the Florisil had settled, and was transferred to a scintillation vial. Fifteen milliliters of scintillation fluid was added to each vial. The radioactivity of each sample was determined in the same method as stated for the progesterone radioimmunoassay. Hamster serum cortisol concentrations were determined from a standard curve that had been prepared using the same solution of diluted rabbit plasma as had been used in the assay of these samples. Since time is critical in this assay, no more than 8 samples were assayed at one time. All cortisol concentrations, as read from the standard curve, were adjusted for percent recovery following extraction.

Mammary Gland Analysis

Casein-like phosphoprotein content: Casein-like phosphoprotein content of the total abdominal-inguinal glands collected from the left side of each hamster was determined by a modification of the method of Tan et.al. (1972) which measures the phosphate content of rennin precipitated phosphoproteins. The glands were thawed, weighed, and homogenized with a motor-driven hand-held glass homogenizer containing 11.0 ml imidazole buffer, pH 6.6. The buffer was composed of potassium chloride (0.15 M), sodium diphosphate (0.004 M), and imidazole (0.01 M). Following homogenization, the samples were ultracentrifuged for one hour at 105,000 rpm in a Schuman Ultracentrifuge. The upper solid fat layer was then carefully lifted off and the supernatants were stored at -20°C. Upon thawing, the casein-like phosphoproteins were precipitated by the addition of 0.1 ml of 1.44 M calcium chloride and 0.1 ml of a solution containing 1.0 mg per ml rennin (Miles-Servac) to 0.2 ml of the supernatant. The mixtures were maintained at 37°C for one hour to allow complete precipitation. Following centrifugation at approximately 1000 rpm for 10 minutes at room temperature, the precipitates were washed once with 2.0 ml of 1% trichloroacetic acid (TCA) and twice with 2.0 ml of 5% TCA. The acid washings solublized and removed the rennin from the precipitates. The washings were discarded. The remaining pellets were digested by the addition of 1.0 ml of 1.5 N sodium hydroxide. The digestion mixtures were placed in a boiling water bath for 10 minutes. Proteins, nucleic acids, and phospholipids were precipitated and removed by the addition

of 0.5 ml of 85% perchloric acid (PCA), followed by centrifugation at 1000 rpm for 10 minutes at room temperature. The supernatant was added to tubes containing 0.5 ml of concentrated sulfuric acid, 0.5 ml of 10% ammonium molybdate solution, and 3.0 ml of a 1:1 mixture of isobutanlo:benzene. The tubes were shaken vigorously. After the solutions had settled, 2.0 ml of the phosphate containing upper layer was drawn off and added to tubes containing 3.0 ml of 3.2% sulfuric acid in absolute alcohol (v/v). This was followed by the addition of 0.5 ml stanneous chloride solution freshly prepared by mixing one part of 10% stanneous chloride in concentrated hydrochloric acid to 199 parts of 0.5 N concentrated sulfuric acid. Absorbancy due to phosphate content was determined with a Beckman Spectrophotometer (Model 2400) one hour after the addition of the stanneous chloride solution. A standard curve was prepared using purified casein (National Biochemical). It was digested in 1.5 N sodium hydroxide and treated the same as the other samples. All data are reported as casein-like phosphoproteins as per the suggestions of Tan et.al. (1972). This is due to the tendency of rennin to precipitate phosphoproteins other than casein. Casein-like phosphoprotein values reported in this study have been corrected for percent recovery.

Mammary gland DNA, RNA, and total protein content: DNA, RNA, and total protein content was determined from the mammary glands collected from the right side of each hamster. Nucleic acids and total proteins were simultaneously precipitated during the removal of the acid soluble phosphates, polysaccarides, nucleotide coenzymes, free

nucleotides, and phospholipids. The precipitated RNA, DNA, and protein were separated from each other by selective acid solubilization.

The specific assays used for DNA, RNA, and total protein in this study are as follows: The thawed, weighed glands were homogenized in a motor-driven hand-held glass homogenizer containing 8.5 ml of cold 6% PCA. One milliliter of each homogenate was transferred to a clean homogenizer containing an additional 7.5 ml of cold 6% PCA. After more complete homogenization, the solutions were evenly divided, allowing duplicate assays, and transferred to separate conical centrifuge tubes. Following centrifugation in a refrigerated Sorval at approximately 1000 rpm for 4 minutes, the supernatants were discarded and the precipitates were washed two times with 3.0 ml of cold 3% PCA. They were then washed once with 6.0 ml of cold 95% ethanol saturated with sodium acetate, and once with 6.0 ml of 95% ethanol alone. Each washing was followed by a refrigerated centrifugation at 1000 rpm for 4 minutes, and the supernatants were discarded. The precipitates, containing DNA, RNA, and proteins, were resuspended by the addition of 3.0 ml of sodium hydroxide (0.3 N), followed by repeated swirlings while incubating at 37^{o}C for one hour. RNA was extracted after the solutions had been cooled to room temperature and 0.6 ml of 12% PCA had been added to bring the pH down to approximately 1.0. Following centrifugation, the supernatants were collected. The precipitates were washed two times with 9.7 ml of 0.1% PCA. The washing were added to the RNA fractions giving a total volume of 20.0 ml.

DNA was extracted from the remaining precipitates following re-

suspension with 8.0 ml of 6% PCA. The solutions were incubated at 70°C for 20 minutes. They were then cooled on ice and centrifuged for 4 minutes at approximately 1000 rpm. The supernatants were collected for DNA determinations. Another 8.0 ml of hot (70°C) 6% PCA was added to each precipitate and the procedure was repeated, making the total volume of the DNA fractions 16.0 ml. The absorbances of the nucleic acid fractions were determined at 260 mu using a Beckman Spectrophotometer, (Model 2400). Absorbance of the solutions due to protein contamination was eliminated by subtracting the absorbance at 286 mu (due primarily to the tyrosine and tryptophane content of protein) from those absorbances obtained at 260 mu (Fleck and Munro-1962).

The protein content of DNA and RNA fractions (extracted as stated above) was determined by the Lowry method (Lowry et.al.-1951). To 0.2 ml of the RNA extract was added 1.0 ml of alkaline copper reagent. This reagent was prepared by the addition of 0.25 g of copper sulfate to 50.0 ml of a 1% sodium tartrate solution. One milliliter of the copper sulfate solution was then added to 50.0 ml of a 2% sodium carbonate solution prepared in 0.1 N sodium hydroxide, resulting in the alkaline copper reagent referred to above. After 10 minutes, 0.1 ml of 1 N Folin-Ciocalteau reagent (Fisher Scientific) was added. Following a 30 minute color development period, absorbancy was determined at 750 mu using a Beckman Spectrophotometer (Model 2400). Absorbancy was due to the reduction of the phosphomolybdicphosphotungstic complex of the Folin reagent by the copper-protein complex of the sample.

The protein content of the DNA solutions was determined in a

similar manner. To 0.2 ml of the extracted DNA solutions was added 0.055 ml of a 10% sodium hydroxide solution and 0.045 ml of water. This step was necessary to neutralize the 6% PCA used in the extraction process. This was followed by the addition of 1.5 ml of the alkaline copper reagent, and 0.15 ml of the Folin-Ciocalteau solution in the manner described for protein content of the RNA extracts. Absorbancy due to the protein content of the DNA extracts was also determined at 750 mu.

The protein content of the precipitate remaining after nucleic acid isolation was also assayed. The precipitate was digested at room temperature for 4 hours with 0.5 ml of 1 N sodium hydroxide. After digestion, 4.0 ml of non-alkaline copper reagent was added. This reagent was prepared in the manner described for the above mentioned alkaline copper reagent except that the 2% sodium tartrate was diluted in water instead of 0.1 N sodium hydroxide. Ten minutes later, 0.4 ml of Folin-Ciocalteau solution was added. The absorbancy was determined as described for RNA and DNA after dilution of the protein solution with 14.6 ml of water. Protein concentrations were determined from a standard curve which had been established using recrystallized bovine serum albumin (Pentex, Miles Laboratories). RNA and DNA concentrations were determined from standard curves prepared from yeast RNA and calf thymus DNA (Worthington Biochemical), respectively. The protein concentrations, as read from the curve, were multiplied by 15 since the final volume of the protein solutions was 15 times the initial volume of 1.3 ml. Similarly, the RNA concentrations as read from the

standard curve were multiplied by 20, and the DNA concentrations by 120. To correct for the initial dilution, prior to extraction, all values were then multiplied by 8.5. Since RNA, DNA, and total protein determinations were assayed in duplicate, the values reported for these parameters represent the mean of the duplicate determinations. All values are reported as total mg contained in the sample assayed, and as mg per mg wet, undefatted tissue assayed.

RESULTS

Serum Levels of Progesterone and Cortisol

Progesterone has been suggested to inhibit both myometrial contractility, as well as synthesis of milk proteins by the mammary glands. At the same time, it has been shown to facilitate full mammary gland lobular-alveolar development when synergized with other hormones. Consequently, one would expect to observe increasing or constantly elevated titers of progesterone preceeding parturition. Just prior to the onset of delivery, one would anticipate a fall in progesterone output to allow uterine contraction.

Cortisol, on the other hand, appears to have the opposite effect on parturition. Fetally produced corticoids have been suggested to initiate termination of gestation. Consequently, one would expect to see a small peak of cortisol appear in the maternal circulation before parturition. This peak might preceed the anticipated drop in progesterone. This suggestion is based on the fact that experimentally induced elevation of fetal corticoids in sheep caused reduction of placental production of progesterone (Liggins-1969).

Maternal peripheral serum progesterone levels were measured from the twelfth day of pregnancy through the fourth day of lactation in order to correlate a change in progesterone with the onset of parturition. As is shown in Table 1, Figure 1, progesterone increased three fold from a value of 38.5 ± 8.8 ng/ml on the twelfth day of pregnancy, to a value of 141.9 ± 48.6 ng/ml on the day of delivery (day zero).

The slight decrease from 71.1 ± 14.5 ng/ml on day 13 to 49.7 ± 10.3 ng/ml on day 14 was followed by a 4 fold increase to 130.0 ± 47.1 ng/ml for the last day prior to parturition. Immediately following the day of parturition, progesterone decreased to 24.2 ± 4.1, 20.8 ± 7.7, 12.8 ± 2.0, and 19.5 ± 9.3 ng/ml for the first 4 days of lactation, consecutively. None of these changes in maternal peripheral serum progesterone were shown to be statistically significant at the p=.05 level of significance as determined by the Student t test. The importance of this lack of statistical significance will be discussed later in this section.

Peripheral maternal serum cortisol levels were also measured from the twelfth day of gestation through the fourth day of lactation. Maternal cortisol (Table 1, Figure 2) decreased from a concentration of 62.9 ± 2.0 ng/ml obtained on day 12, to a value of 50.2 ± 4.6 ng/ml on the following day. A slight increase to 58.8 ± 1.6 ng/ml was seen on day 14. Following this, peripheral cortisol then decreased to 32.1 ± 12.9, 22.4 ± 12.5, and 15.7 ± 7.7 ng/ml for the last day prepartum, the day of parturition, and the first day of lactation, respectively. Cortisol then increased two fold to 30.6 ± 10.0 ng/ml on day two of lactation. This was followed by a decrease to 17.0 ± 5.8 and 3.2 ± 1.8 ng/ml for days 3 and 4. In general, there was a 20 fold decrease in serum cortisol from day 12 of pregnancy through day 4 of lactation. There was no evidence of a peak in maternal cortisol (possibly of fetal origin) on the day of delivery. However, slight increases were observed on day 14 of pregnancy and day 2 of lactation.

Table I. Maternal Peripheral Serum Concentration of Progesterone and Cortisol During Late Pregnancy, Parturition, and Early Lactation

Day Gestation-Lactation***	Animals**	Progesterone ng/cc serum*	Cortisol ng/cc serum*
12	4,4	38.5 ± 8.8	62.9 ± 2.0
13	7,4	71.1 ± 14.5	50.2 ± 4.6
14	7,5	49.8 ± 10.3	58.8 ± 1.64
15	5,6	130.0 ± 47.1	32.1 ± 12.6
parturition (zero)	6,5	141.9 ± 48.6	22.4 ± 12.5
1L	5,5	23.9 ± 4.1	15.7 ± 7.7
2L	5,4	20.8 ± 7.2	30.6 ± 10.9
3L	5,8	12.8 ± 2.0	12.0 ± 5.8
4L	5,3	19.5 ± 9.3	3.19 ± 1.2

* mean value ± standard error of the mean.

** the first number is the number of animals for the progesterone determination, the second number is the number of animals for the cortisol determinations.

*** day one of pregnancy was desginated as the day vaginal sperm were detected.

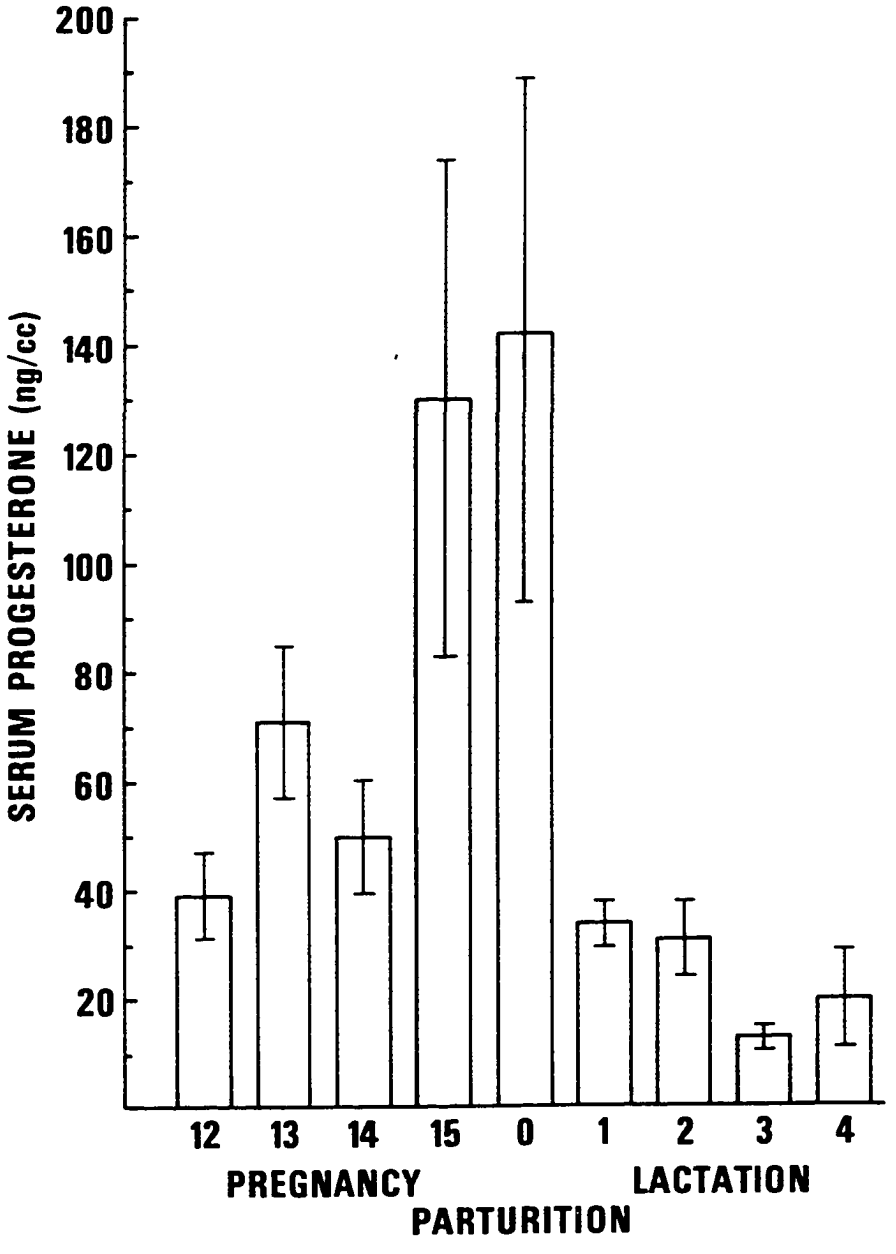

Figure 1. Hamster serum progesterone levels during late pregnancy, parturition, and early lactation (mean ± S.E.M.).

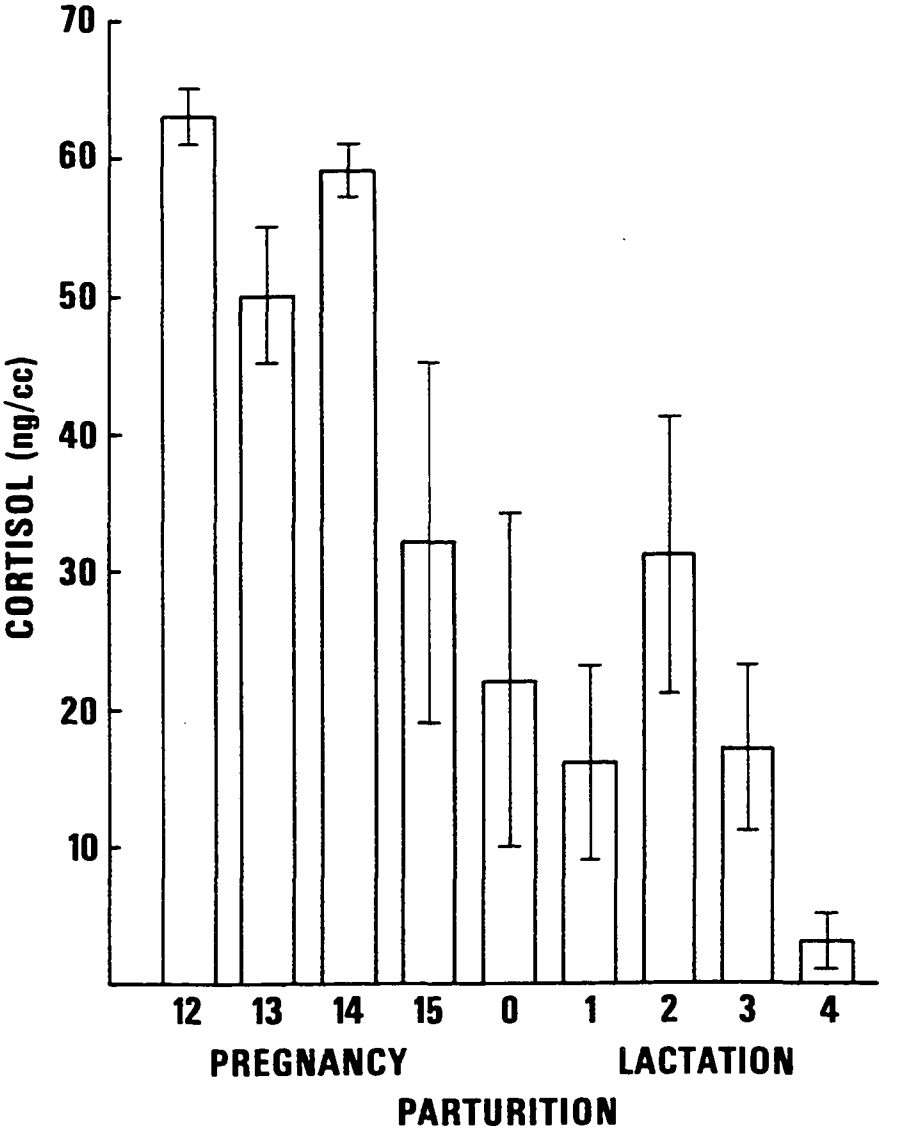

Figure 2. Maternal hamster serum levels of cortisol as measured during late pregnancy, parturition, and early lactation (mean ± S.E.M.).

The possible importance of these increases will be pointed out in the discussion section. Statistically, only the decrease in cortisol between days 12 and 13 of gestation was shown to be significant on the p=.05 level as determined by the Student t test.

Analysis of Mammary Gland RNA, DNA, and Total Protein

To correlate blood levels of progesterone and cortisol to mammary gland development, mammary gland DNA, RNA, and total proteins were determined for the wet, undefatted abdominal-inguinal glands collected from the right side of each hamster during late pregnancy, parturition, and early lactation. Total RNA, DNA, and protein content will be reported here. This allows comparison with other data for the hamster reported in this fashion (Sinha, et.al.-1970). Nucleic acid and protein content per mg wet, undefatted tissue analyzed will also be reported. However, as will be pointed out in the discussion, variation in the amount of glandular water and fat during pregnancy and lactation tend to mask changes in RNA, DNA and protein concentration.

Total RNA content: Total RNA (Table 2) remained unchanged for days 12 and 13 of gestation, with values of 8.1 ± 0.5 and 8.5 ± 0.7 mg, respectively. RNA then began to increase steadily to 12.0 ± 0.9, 13.8 ± 2.0, and 18.7 ± 1.0 mg for the last two days prepartum and the day of parturition (day zero). Those changes occurring between days 13 and 14, and days 15 and day zero, were shown to be statistically significant on the p=.05 level.

Following parturition, RNA dropped slightly to 17.8$\pm$ 0.1 mg for the first day of lactation. It continued to decrease to 14.2$\pm$ 0.6 and 12.7$\pm$ 0.9 mg for days two and three of lactation. This was followed by a significant (p=.05) two fold increase to 21.3$\pm$ 2.2 mg for the fourth day of postpartum.

RNA content per mg wet, undefatted tissue is also summarized in Table 2. Changes in RNA concentration were not as evident as changes in total RNA. For example, RNA concentration, beginning at 15.7 ug/mg on day 12, decreased slightly to 13.9 and 13.3 ug/mg for days 13 and 14 of gestation. This was followed by an increase to 18.4 ug/mg on the last day prepartum. RNA concentration then dropped to 10.8 ug/mg on day zero. Slight increases to 11.9 and 15.9 ug/mg were observed on the first two days of lactation. This was followed by a small decrease to 11.4 ug/mg on day 3 of lactation. RNA rose again on day 4 to 16.1 ug/mg. As stated before, the variation in these data is due more to variations in mammary gland tissue weights rather than variations in RNA content.

Total DNA content: Total DNA content is summarized in Table 3. Total mammary gland DNA as determined individually from the right abdominal-inguinal glands of each hamster, increased significantly (p=.05) from 2.1$\pm$ 0.3 mg on day 12 to 6.4$\pm$ 0.2 mg on the next day. DNA remained elevated for days 14 and 15 (with values of 7.0$\pm$ 0.6 and 6.6$\pm$ 0.5 mg respectively). On day zero, total DNA again increased two fold to 12.6$\pm$ 1.5 mg (significant at the p=.05 level). This is a value almost 6 times that seen on day 12 of gestation. Following parturition, DNA

Table 2: RNA Content of Mammary Tissue Collected from the Right Sides of the Hamster During Late Pregnancy, Parturition, and Early Lactation.

Day Gestation-Lactation***	Animals	RNA		
		Gland Wt. (mg)**	Total (mg)*	ug/mg Tissue
12	4	416.4	8.1 ± .5	15.7
13	4	621.7	8.5 ± .7	13.9
14	4	896.9	12.0 ± .9	13.3
15	4	749.6	13.8 ± 2.0	18.4
parturition (zero)	5	1722.8	18.7 ± 1.0	10.8
1L	3	1493.3	17.8 ± .1	11.9
2L	4	888.2	14.2 ± .6	15.9
3L	4	1098.5	12.6 ± .9	11.4
4L	4	1318.8	21.2 ± 2.2	16.1

* Mean ± standard error of the mean

** Mean wt. of glands analyzed

*** Day was designated as the day vaginal sperm were detected.

content remained high for the first day of lactation (with a value of 13.2^{+}_{-} 2.3 mg) and then dropped two fold to 6.6^{+}_{-} 0.4 mg on the second day postpartum. This drop was statisically significant at p=.05. Glandular DNA then increased two fold again to 11.4^{+}_{-} 2.9 mg for the third day of lactation. This was followed by another decrease (insignificant at p=.05) to 8.5^{+}_{-} .03 mg on the fourth day postpartum. In general, total mammary gland DNA increased 4 fold during the final days of gestation. In contrast, during lactation, small decreases in total DNA were observed.

DNA concentrations per mg wet, undefatted tissue are also presented in Table 3. DNA concentrations do not show the 6 fold increase observed in total DNA. In fact, there was no clearly evident change in DNA concentrations beyond an initial increase from 4.2 ug/mg on day 12 to 10.3 ug/mg on day 13. For the other days of pregnancy, day zero, and the days of lactation, DNA concentrations per mg tissue flucuated between values of 6.5 and 10.4 ug/mg with no discernible pattern. Also, no correlation can be found between these concentrations and those reported by Sinha (et.at.-1970). The primary reason for this lack of correlation is based on the fact that Sinha (et.al.-1970) reported DNA concentrations per mg dry, fat-free tissue. This indicates, as stated with RNA concentrations, that variations in nucleic acid and protein concentrations in this study are due to changes in tissue weights.

Ratio of RNA to DNA: The ratio of RNA to DNA (presented in Table 4) reflects changes in protein synthesis of the secretory cells of the

Table 3: DNA Content of Mammary Tissue Collected from the Right Sides of the Hamster During Late Pregnancy, Parturition, and Early Lactation.

Day Gestation-Lactation*	Animals	DNA		
		Gland wt. (mg)**	Total (mg)***	ug/mg Tissue
12	4	416.4	2.8 ± .3	4.2
13	4	612.7	6.1 ± .2	10.3
14	4	896.9	7.0 ± .6	7.8
15	4	749.6	6.6 ± .5	8.8
parturition (zero)	5	1722.8	12.6 ± 1.5	7.3
1L	3	1493.3	13.2 ± 2.3	8.8
2L	4	888.2	6.6 ± .4	7.4
3L	4	1098.5	11.4 ± 2.9	10.4
4L	4	1318.8	8.5 ± .3	6.5

* day one was designated (as the day vaginal sperm were detected.)

** mean gland weight analyzed

*** mean $\pm$ standard error of the mean

Table 4: Hamster Mammary Gland Total RNA per Total DNA

Day Gestation-Lactation*	Animals	Total mg	NA/Total mg DNA**
12	4		2.88
13	4		1.33
14	4		1.70
15	4		1.50
parturition (zero)	5		1.35
1L	3		1.35
2L	4		2.16
3L	4		1.11
4L	4		2.49

* day one was designated as the day vaginal sperm were detected

** mean mg RNA/ mean mg DNA

alveoli. An increase in this ratio would indicate an increase in protein synthesis (i.e.: milk proteins) assuming the number of cells (represented by a change in DNA) did not increase at the same time. During this study, there was little variation in the RNA/DNA ratio. An exception to this was the decrease between days 12 and 13, with values of 2.88 and 1.33, respectively. Also, RNA/DNA increased to 2.16 on day two of lactation, and again on day 4 to 2.49. The ratio of RNA/DNA varied slightly between 1.11 and 1.70 for the other days of pregnancy and lactation. These results are in close agreement with those presented by Sinha (et.al.-1970).

Total proteins: Increasing mammary gland total proteins (Table 5) are an index of increase in cellular proliferation, as well as an increase in synthesis of milk proteins. In this investigation, total proteins remained unchanged for days 12 and 13 of pregnancy with values of 48.4 ± 3.8 and 48.4 ± 14.1 and 66.0 ± 12.1 mg for days 14 and 15. Protein content continued increasing to 99.1 ± 12.7 mg at day zero. This was followed by a slight decrease to 84.4 ± 11.3 and 67.4 ± 7.0 mg for the first two days postpartum. Protein content then increased two fold to 112.4 ± 17.6 mg on day 3 of lactation decreasing again on the fourth day postpartum to 71.0 ± 2.3 mg. In general, there was a slight overall increase in total proteins from the twelfth day of pregnancy through the fourth day of lactation.

Mammary gland protein concentrations per gram wet, undefatted tissue assayed are also summarized in Table 5. Protein concentrations increased and fell sporadically between values of 95.0 ± 10.0 mg/g

Table 5: Protein Content of Mammary Tissues Collected During Late Gestation, Parturition, and Early Lactation.

Day Gestation-Lactation*	Animals	PROTEIN Gland wt.(mg)**	Total (mg)***		mg/g Tissue
12	4	516.8	48.4	3.8	95.0
13	4	621.7	48.4	1.6	60.0
14	4	896.9	63.5	14.1	87.0
15	4	749.6	66.0	12.1	94.0
parturition (zero)	5	1722.8	99.1	12.7	60.0
1L	3	1493.3	84.4	11.3	57.0
2L	4	888.2	67.4	7.0	77.0
3L	4	1098.5	112.4	17.6	82.0
4L	4	1318.8	71.0	2.3	53.0

* day one was designated as the day vaginal sperm were detected.

** mean gland weight

*** mean $\pm$ S.E.M.

tissue, observed on day 12 of gestation, and 53.0±6.0 mg/g observed on day 4 of lactation. Due to the large standard errors both here and in the total protein values, none of these changes are significant at the p=.05 level.

Mammary Gland
Casein-like Phosphoprotein Content

Mammary gland milk protein content (or in this case, casein content) is an expression of protein synthesis within the alveoli. However, casein-like phosphoprotein (CLPs) will be reported here due to the difficulty in isolating pure casein (Tan et.al.-1972). Casein-like phosphoproteins were measured in the abdominal-inguinal glands collected from the left side of each hamster during late gestation, parturition, and early lactation. As shown in Table 6, total CLPs increased 6 fold from the twelfth day of pregnancy (with a value of 0.88±.03 mg) to the day of parturition (with a value of 5.06±1.11 mg). A slight increase was observed on day 13, to 1.45±.41 mg, increasing again the next day to 3.05±1.26 mg. This was followed by a sudden return to 0.88±.22 mg on day 15. This decrease was significant at the p=.05 level. On the day of parturition, CLPs increased to 5.06±1.11 mg (significant at p=.05) and remained elevated for the first 4 days of lactation with values of 4.30±.61, 3.92±.64, 4.42±1.20, and 3.31±.50 mg, respectively. These values are 4 to 5 times that observed on day 12 of gestation.

Casein-like phosphoprotein concentrations per mg wet, undefatted tissue followed a similar pattern to that of total CLPs in that there

was a first wave of phosphoprotein increase from day 12 to day 13. This remained elevated for the fourteenth day of gestation. On day 15 there was a slight insignificant ($p=.05$) decrease which preceeded a second wave of increasing phosphoproteins beginning on day zero. Unlike total phosphoproteins, CLPs concentrations continued to increase through the first day of lactation. They remained elevated for days 2 and 3 of lactation. On the fourth day postpartum, CLPs concentrations decreased about 1.5 times, returning to values observed on days 13 and 14 of pregnancy. With exception to this decrease, the concentrations of CLPs during lactation were from 2 to 4 times greater than the concentrations observed during pregnancy. These data are reported in Table 6.

In brief summary, during the final days of gestation, there was a large increase in peripheral serum progesterone. This increase began with a slight peak on day 13. At the same time, there was evidence of an increase in total mammary gland DNA, CLPs, and total proteins. Total RNA did not show an increase until day 14 of pregnancy. Cortisol was constantly decreasing. Just prior to parturition, progesterone increased 4 fold. Coinciding with this increase, there was a two fold rise in total RNA. Total DNA remained unchanged, as did total proteins. CLPs decreased 3 fold. With the onset of delivery, progesterone remained elevated. Total proteins, DNA, CLPs, and RNA all increased. Following delivery, progesterone decreased 7 fold. However, CLPs, RNA, total proteins, and for the most part, DNA, all remained elevated for the first 4 days of lactation. Cortisol, on the other hand,

Table 6: Casein-like Phosphoprotein Content of Mammary Tissue Collected During Late Gestation, Parturition, and Early Lactation

		CASEIN****		
Day Gestation-Lactation*	Animals	Gland wt. (mg)**	Total Casein (mg)***	mg/g Tissue
12	3	771.2	.88 ± .03	1.14
13	4	486.0	1.45 ± .41	2.98
14	4	1021.9	3.05 ± 1.26	2.98
15	4	770.9	.88 ± .22	1.14
parturition (zero)	4	1707.3	5.06 ± 1.11	2.96
1L	5	969.7	4.30 ± .61	4.43
2L	5	923.6	3.92 ± .64	4.24
3L	5	1046.8	4.42 ± 1.20	4.22
4L	5	1143.0	3.31 ± .50	2.89

* day one was designed as the day vaginal sperm were found

** mean gland weight

*** mean ± S.E.M.

**** casein-like phosphoproteins

continued decreasing with exception to slight peaks observed on day 14 of pregnancy and day two of lactation.

The Importance of Statistical Significance

With rare exception, the flucuations in hormone titers, as well as changes in mammary gland RNA, DNA, total proteins, and CLPs, were insignificant at the p=.05 level as determined by the Student t test. The lack of significance was due to the large standard errors. There are two likely causes of the wide range of values. The first is experimental error. However, this was not the case in this investigation since large standard errors were not observed on all days studied, and the experimental conditions were constant.

The second cause of a broad range of values is individual variation since not all animals undergo biological changes at the same time. Consequently, it can be assumed that the lack of statistical significance does not indicate the lack of a biological change. For this reason, the data obtained in this investigation will be discussed as representative of biological variations despite lack of statistical significance at the p=.05 level.

DISCUSSION

During the final stages of pregnancy, the mammalian mother is involved in two major endeavors related to gestation. The first is the control of the time of the onset of parturition, insuring that delivery is neither premature nor delayed. The second is the preparation of the mammary glands for lactation, at the same time preventing lactation prior to delivery. Both endeavors are hormonally controlled. Progesterone and fetally-produced cortisol have been implicated in the regulation of the time of delivery. Progesterone has been suggested to inhibit myometrial contraction, thus preventing premature expulsion of uterine contents. Fetal cortisol has been suggested to initiate the onset of labor at the end of gestation.

At the same time, both hormones have been shown to be necessary for mammary gland development and initiation of lactogenesis. Although the precise role of progesterone in mammary gland development may vary from species to species, it can generally be said to be necessary for completion of lobular-alveolar development. Further, progesterone has been implicated in the inhibition of production of milk proteins (lactogenesis) prior to parturition. During pregnancy, parturition, and lactation, cortisol is necessary for maintenance of normal metabolism.

It has been the purpose of this investigation to determine peripheral serum progesterone and cortisol concentrations during late pregnancy, parturition, and early lactation. The object was to relate

changes in the concentrations of these hormones to the onset of parturition, as well as the development and function of the mammary gland.

Progesterone and Parturition

Previously, this paper has been concerned with pointing out the changes in peripheral progesterone throughout pregnancy. This was to support the role of progesterone in 'blocking' myometrial contractions. However, the major emphasis in this study was the role of progesterone (and cortisol) in the control of parturition, not pregnancy maintenance. Hence, this study was concerned only with changes in progesterone (and cortisol) immediately before, after, and during delivery. As a consequence, it is not within the scope of this study to discuss the possible hormonal profile throughout pregnancy in the hamster. The most that can be gleaned from these data reported here concerning progesterone during pregnancy is that the peripheral progesterone concentration during days 12 and 14 of pregnancy were as low as those found on days 1 through 4 of lactation, during which time the corpora lutea have regressed (Leavitt and Blaha-1970). This suggests that there was no overall rise in peripheral progesterone during gestation in the hamster.

Low peripheral progesterone concentrations on days 12 and 14 of gestation are not in agreement with data reported by Leavitt and Blaha (1970) or Lukaszewka and Greenwald (1973). However, Leavitt and Blaha (1970) report values of progesterone from blood collected from the posterior vena cava, just below the entrance of the ovarian and adrenal

veins. This may be considered to be a measure of ovarian secretion rather than peripheral progesterone. Lukaszewka and Greenwald (1973) collected blood from the abdominal aorta. Their data should more closely parallel those obtained in this study wherein blood was collected following decapitation.

A comparison of the results reported here to those of Lukaszewka and Greenwald (1973) reveals several important differences. Although there is excellent agreement between the two studies for progesterone values obtained on days 12 and 14 of gestation, (values varying slightly between 35 and 45 ng per ml), there is little agreement of data found on days 13 and 15. Those researchers found decreasing progesterone concentrations for those days, with a value of approximately 25 ng per ml for both days. In contrast, the hamsters used in this study were found to have increased peripheral progesterone on days 13 and 15 (with values of 63 and 130 ng per ml, respectively). These concentrations are two to five times those found by Lukaszewka and Greenwald (1973). This author can find no clear cut reason for these differences. The lack of overlap of their standard errors of the daily values ($p=.05$) would seem to indicate a physiological (rather than experimental) basis for the decrease in progesterone observed for days 13 and 15 by those researchers. Lukaszewka and Greenwald (1973) did not report peripheral progesterone concentrations for the day of delivery or the first days of lactation. This is unfortunate in that those data would have allowed further comparison which may have shed light on the above discrepancies.

A more recent study, by Joshi, Watson, and Labsetwar (1974) also addressed itself to progesterone titers as related to pregnancy and parturition in the hamster. They could detect no change in peripheral progesterone concentration (as measured from an unmentioned region of the posterior vena cava) during all of pregnancy, parturition, and lactation. However, there are two interesting points regarding their study. First, they report peripheral progesterone concentrations ranging from 1000 to 1500 ng per ml blood. These values are 50 times greater than those reported by Lukaszewka and Greenwald (1973) and 10 times greater than those found in this study. In the same paper, Joshi (et.al.-1974) determined ovarian secretion of progesterone from one ovary (as measured from the ovarian vein) to be 100 times that found by Leavitt and Blaha (1970) for both ovaries. These discrepancies cannot be attributed to differences in assay sensitivity since all assays were capable of detecting concentrations as low as 10 ng.

The second important point in discussing the data presented by Joshi (et.al.-1974) concerns the pooling of daily samples. They report the progesterone concentration of serum taken from a pool of blood collected on days 13, 14, 15, and 16. Under these circumstances, it is difficult to consider their data as having any relationship to changes in progesterone titers as associated with parturition. As a consequence, the information presented by those researchers will not be considered for comparison in this discussion. Their work was mentioned at this point to avoid any comparisons by a reader who may be familiar with the work of Joshi (et.al.-1974).

As pointed out previously in this paper, one would anticipate a drop in progesterone just prior to parturition. However, as shown in Figure 1 and Table 1, there was a 3 fold rise in peripheral serum progesterone between days 14 and 15 of gestation. Progesterone concentrations remained elevated on the day of delivery and then decreased 5 fold between the day of delivery and the first day of lactation. This rise in progesterone immediately prior to parturition is contradictory to previously reported values during the same time period in most laboratory species. It has not been observed in the rat (Labsetwar and Watson-1973), the mouse (Murr, et.al.-1974), or previous studies in the hamster (Leavitt and Blaha-1970).

In contrast to the above mentioned findings in laboratory animals, a slight peak in peripheral progesterone during delivery has been found in some farm animals. The occurence of this peak appears to be independent of whether or not the animal produces placental progesterone. This suggests that the source of the progesterone is not necessarily the placenta. For example, in the pig, which produces placental progesterone, Baldwin and Stabenfeldt (as related in personal communication with Stabenfeldt) noted a small peak in total progestens during the last 12 hours of farrowing. However, this rise was no where near the magnitude of the one observed in hamsters in this study.

Similarily, in the cow, which does not produce placenta progesterone, Hoffman (et.al.-1973) noted a peak in progesterone beginning 7 hours before delivery. Again, the magnitude of the peak was not as high as the 3 fold increase observed in this study.

There are 4 possible sources of progesterone during gestation, the corpora lutea, the ovarian interstitium, the placenta, or the adrenals. Since Leavitt and Blaha (1970) have shown that total ovarian secretion of progesterone (including interstitial hormone secretion) begins decreasing by day 15 of gestation in the hamster, and that the magnitude of this decrease is only 20 ng per ml, it is unlikely that the ovaries are the source of the peripheral progesterone increase observed on days 15 and zero in this study.

The placenta presents another possibility. The rabbit, which does not normally produce placental progesterone (Courrier-1945), can be induced to produce enough hormone to maintain pregnancy when under duress (Csapo and Lloyd-Jacob-1962). If this were the case in the hamster, one would have to question if delivery were enough of a stressful event to induce placental production of progesterone. And, if so, what would be the physiological need for high progesterone titers at that time? The same question can be asked of any progesterone produced by the adrenals due to stress. Since, in this study, the hamsters were undisturbed until decapitation, it is unlikely that extraneous stress caused increased adrenal output.

Thus, on the basis of what is known, none of the above potential sources of progesterone would have contributed the total peak of progesterone observed just prior to delivery. The other possibility is that all of these sources may have contributed in part. This theory, however, still leaves the question of physiological significance unanswered.

Cortisol and Parturition

The influence of fetal cortisol on the time of parturition has been studied extensively in sheep (Liggins-1968, 1969) and the rabbit (Nathanielsz and Abel-1972). The ability of fetal cortisol to cross ("spillover") the placental barrier to the maternal circulation, at least in sheep, has been established by Malinowska (et.al.-1972) and Dixon (et.al-1970). However, the contribution of the fetal cortisol to the circulating maternal cortisol titers at the time of parturition is unknown. The object of this part of the investigation was to determine if a detectable peak in maternal cortisol, due to fetal 'spillover', could be found on, or immediately before, the day of delivery.

Hamster maternal peripheral serum cortisol concentrations dropped 20 fold from the twelfth day of gestation through the fourth day of lactation. Slight, but insignificant ($p=.05$), peaks were observed on day 14 of pregnancy and day 2 of lactation. There was no evidence of an increase in maternal cortisol on the day of delivery, due either to fetal 'spillover' or the stress of delivery. However, the large variation in cortisol values obtained on day zero may easily mask any slight rises in individual values. A reduction in that variation, possibly by more frequent sampling, or by sampling at the onset of delivery, might make any changes in cortisol more apparent.

A similar pattern of overall decrease during pregnancy was found in the cow (Adams and Wagner-1970). They also observed a slight peak in maternal cortisol on the day of delivery. They suggested this peak

to be of fetal origin. However, there is every possibility that the source of that small peak was the maternal adrenal due to the stress of delivery.

Contributions of the maternal adrenal during parturition have been further studied in the rat. Kamoun and Stutinsky (1968) have found a slight increase in maternal corticoids two days prior to delivery in the adrenalectomized rat. The most obvious source of this increase would be the fetal adrenals. A similar rise in maternal cortisol was found in this study in the intact hamster two days prior to parturition. This suggests that the fetal contribution to the initiation of delivery may occur earlier than the day of parturition.

Finally, a second rise in cortisol was found in the hamster on day two of lactation. A similar peak was observed in the cow at the same stage of lactation (Adams and Wagner-1970). The cause of this second peak, as well as the physiological significance, is unknown.

Effects of Progesterone and Cortisol on Mammary Gland Development and Lactogenesis

Mammary gland development during pregnancy: As discussed previously, hamster peripheral serum progesterone increased for the last 3 days of gestation. Peripheral serum cortisol decreased during the same time period, with a slight peak on day 13. Concomitant with these hormonal changes, mammary gland total DNA, an index of cellular proliferation, increased 4 fold from the twelfth day of gestation to the day of delivery. These data are in accord with those reported for the hamster by Sinha (et.al.-1970). However, the absolute values reported by those

researchers are twice those found in this study. This is because Sinha (et.al.-1970) determined DNA content of the mammary glands of both sides of the hamster. DNA content was determined only from the right mammary glands in this study. A similar wave of cellular proliferation just prior to parturition has been observed in the rat by Lewin and Lewin (as indicated in personal conversation with Greenbaum and Glater-1970). Since progesterone stimulates lobular-alveolar development (Fellner-1913, Folley-1952), it would seem possible that the increased progesterone just prior to parturition might facilitate this final wave of mitosis.

The role of cortisol in mammary gland development during pregnancy is less defineble. Cortisol has been shown to be necessary for maintenance of mammary gland growth and differentiation (Anderson and Turner-1962). The drop in cortisol observed in this study just prior to parturition does not appear to have prevented the final wave of cellular proliferation discussed above.

Total mammary gland RNA also increased during the final stages of gestation. There was evidence of a significant ($p<.05$) rise in RNA between days 13 and 14 of gestation. A second significant ($p=.05$) increase was observed between day 15 and the day of delivery. Similar results have been reported by Sinha (et.al.-1970). They found an increase in total RNA between day 15 and the day of parturition (referred to by them as the first day of lactation) parallel to that observed in this study. However, they did not include days 12, 13, or 14 of pregnancy in their study. Consequently, there is no confirmation

of the increase in RNA observed between days 13 and 14 in this investigation.

Changes in mammary gland RNA represent changes in the synthetic activity of the glands (Sinha, et.al.-1970). There are two phases of protein synthesis as the mammary glands change from virgin to lactating (Greenbaum and Slater-1957). The first phase occurs during early pregnancy and is due to the synthesis of structural proteins. Sinha (et.al.-1970) have reported a 4 fold rise in hamster mammary gland RNA occurring prior to the twelfth day of gestation. This first phase of protein synthesis, represented by increased RNA, would not be observed in this study.

The second wave of protein synthesis, which includes milk protein production, occurs with the onset of parturition (Griffith et.al.-1959). Hence, one would expect to see a rise in total RNA closely associated with the onset of delivery. The two small peaks in RNA observed prior to delivery in this investigation may represent the initiation of the synthesis of milk proteins. Although it has been suggested that high titers of progesterone inhibit milk protein synthesis (David et.al.-1970), inhibition may occur after the formation of the template RNA necessary for milk protein production. Consequently, the glands have prepared for milk protein production, but will not produce milk proteins on a large scale until the decrease in progesterone associated with parturition.

The ratio of RNA to DNA, interpreted as reflecting changes in protein synthetic activity, are also in agreement with data reported

by Sinha (et.al.-1970). The values reported in both studies are fairly constant. This would be expected since there was an increase in total RNA (possibly due to an increase in milk protein templates) accompanied by an increase in total DNA (due to a final wave of cell proliferation). This index was included in this investigation primarily to allow comparison with data reported in other studies. In fact, it is a poor index of changes in synthetic activity since, as evidenced above, both RNA and DNA may change for different reasons while the ratio of the two remains constant, falsely indicating no change in synthetic activity.

The 3 fold increase in total glandular DNA, as well as the doubling of the RNA during late pregnancy, indicate the potential for increased total protein synthesis. As presented in Table 5, there was a small two fold increase in total proteins from the twelfth day of gestation through the day of delivery. This represents only a small increase in total proteins when considering the amount of cellular proliferation that occurs as the glands go from the virgin state to the lactating state. However, as stated previously, most of the proliferation of structural proteins would have occurred prior to the twelfth day of pregnancy. The two fold increase in total proteins observed between day 12 and day zero in this study most likely represents a combination of the mammary gland cellular proliferation during the final stages of pregnancy and the beginning of milk protein synthesis.

Early milk protein synthesis was indicated in this investigation

by the constant increase in casein-like phosphoproteins (CLPs) are an index of glandular secretory activity. The increase in CLPs during late pregnancy suggest that the high titers of progesterone observed during the same time period do not entirely inhibit milk protein production. Also, the elevated RNA found during late pregnancy suggests that the secretory cells may already have the potential for milk protein production prior to delivery. Limited prepartum synthesis seems feasible since it would allow immediate postpartum lactation.

Parturition and lactogenesis: With the sudden fall in progesterone at parturition one would anticipate the onset of lactogenesis to be accompanied by a sudden increase in glandular milk protein production. This increase would be detected by a rise in mammary gland total proteins and casein-like phosphoproteins. There would be no need of an increase in total RNA assuming that the prepartum rise represented the production of template RNA necessary for milk protein synthesis. Total DNA would also be expected to remain constant since the final wave of cellular proliferation (as suggested by Lewin and Lewin 1970) occurred during the final stages of gestation.

In fact, total glandular RNA did remain constant during lactation with exception to the fourth day postpartum. On that day, total RNA was found to have increased almost two fold. A similar increase was not reported by Sinha (et.al.-1970), for the same day (referred to by them as day 5 of lactation). This increase was not accompanied by a rise in DNA, total proteins, or CLPs. The cause for the rise in RNA on the fourth day of lactation is unknown and cannot be determined

from the data obtained in this study.

Total DNA also did not remain constant during lactation. It was found to decrease to prepartum values on days 2 and 4 postpartum. Sinha (et.al.-1970) did not report a similar decrease on day 4 of lactation (referred to by them as day 5 of lactation). The cause of these decreases in total DNA is unknown.

Casein-like phosphoproteins doubled between the day of parturition and the first day of lactation. The fact that this increase coincides with the drop in peripheral progesterone adds further weight to the argument that progesterone inhibits mammary gland milk protein synthesis (Davis et.al.-1970). The drop in CLPs observed on the fourth day of lactation is unexplainable. It may have been caused by depletion of stored milk due to nursing just prior to killing of the dam. However, if this were the case, it is interesting that similar decreases were not observed on other days for the same reason.

Mammary gland total protein content remained elevated for the 4 days of lactation studied. However, the elevated values were accompanied by extremely wide ranges of variation. These ranges may also have been caused by lactation. This is possible since casein-like phosphoproteins alone make up approximately 5 mg per g of tissue analyzed. Add that to the contributions made by other milk proteins (lactalbumins), and one may find that the flucuations in total proteins may also be attributed to removal of stored milk proteins by nursing.

Finally, as indicated throughout, total RNA, DNA, and proteins

were reported in this investigation. Although it seems more valid to report concentration, it appears that total values were sufficient in this study. This is indicated by the fact that changes in glandular nucleic acids did not parallel changes in mammary gland weights. The variation in tissue weights may have been due to changes in mammary gland water and fat content. In the rat, the percentage of water increases 30% from the day of parturition to the first day of lactation. At the same time, the fat decreases 15% (Kuhn and Powenstein-1967). Consequently, although the weight of the glands may change, the nucleic acid and protein content remains constant (unless changing for other reasons).

SUMMARY

The involvement of progesterone and cortisol in the control of parturition has been well documented. In this investigation, peripheral serum progesterone increased immediately prior to delivery and then dropped between the day of parturition and the first day of lactation. From these data it is impossible to determine if the drop in progesterone preceeded or followed the initiation of delivery. If it had been possible to show that drop had occurred prior to parturition, it would have added further weight to the theory that high titers of progesterone block the onset of delivery.

Peripheral serum cortisol was also studied from the twelfth day of gestation through the fourth day of lactation. It has been suggested by other researchers that fetal cortisol initiates the onset of delivery. It was the intention of this part of the study to determine if there was a detectable increase in maternal cortisol associated with the onset of parturition. Such an increase might be due to spillover of the peak of fetal cortisol that facilitates termination of gestation. There was no evidence of a peak of cortisol in the maternal circulation detected on the day of parturition in this investigation. However, a slight rise in cortisol was observed two days prior to delivery. A similar rise has been observed in the rat. It was suggested to be of fetal origin. The role of that peak in the termination of gestation was not determined.

Progesterone and cortisol also affect mammary gland growth and

lactogenesis. Stimulated by progesterone, and maintained by cortisol, the abdominal-inguinal glands showed evidence of a final wave of cellular proliferation just prior to termination of gestation. This was indicated by a 4 fold increase in total DNA occurring between the twelfth day of pregnancy and the day of parturition. There was also evidence of increased protein synthesis during late pregnancy. This was suggested by a two fold increase in total RNA, as well as increases in both total proteins and casein-like phosphoproteins. Although the most significant rise in CLPs occurred with the decrease in peripheral progesterone, there was still evidence of increasing CLPs prior to delivery. This prepartum increase in CLPs represents the synthesis of milk proteins despite the high titers of progesterone. The rise in total RNA at the same time indicates the potential for increased milk protein production.

Hence, just prior to the onset of parturition, there was an increase in serum peripheral progesterone. Although the physiological role of this increase is unknown, it may have facilitated a final wave of mammary gland cellular proliferation. At the same time it failed to completely inhibit mammary gland milk protein synthesis as suggested by Davis (et.al.-1970). With the onset of parturition, and the decrease in progesterone, milk protein production increased significantly. There were no further increases in RNA, DNA, or total proteins during lactations.

LITERATURE CITED

Adadevoh, B.K. and Akinla, O. 1971. Plasma cortisol (11-hydroxycorticosteroid) during and after pregnancy. J. Obst. Gynec. B. Comm. 78:712.

Adams, W.M. and Wagner, W.C. 1970. The role of corticoids in parturition. Biol. Rep. 3:223.

Anderson, A.B. M., Pierrepoint, C.G., Griffiths, K., and Turnbull, A.G. 1972. Steroid metabolism in the adrenals of fetal sheep in relation to natural and corticotropin induced parturition. J. Reprod. Fert. Suppl. 16:25.

Anderson, R.R. and Turner, C.W. 1962. Effect of adrenalectomy and corticoid replacement on mammary gland growth in rats. Proc. Exp. Biol. Med. 109:85.

Barnawell, E.B. 1967. A comparative study of the responses of mammary tissues from several mammalian species of hormones in vitro. J. Exp. Zool. 160:189.

Bassett, J.M. and Thornburn, G.D. 1969. Foetal plasma corticosteroids and the initiation of parturition in sheep. J. Endocr. 44:285.

Bassett, J.M., Oxbarrow, T.J., Smith, I.D., Thornburn, G.D., 1969. The concentration of progesterone in the peripheral plasma of the pregnant ewe. J. Endocr. 45:449.

Corner, G.W. and Allen, W.M. 1930. Physiology of the corpus luteum. VII. Maintenance of pregnancy in rabbit after very early castration, by c. l. extracts. Proc. Soc. Exp. Biol. Med. 27:403.

Courrier, R. 1945, Endocrinologie de la gestation, Masson, Paris.

Csapo, A., 1956. Progesterone 'block'. Amer. J. Anat. 98:273.

Csapo, A. and Lloyd-Jacob, M.A. 1962. Placenta, uterine volume, and their control of the pregnant uterus in rabbits, Amer. J. Obstet. Gynec. 83:1073.

Cushy, A.R. 1906. On the movement of the uterus. J. Physiol. 35:1.

Dale, H.H. 1906. On some physiological effects of ergot. J. Physiol. 34:163.

Davis, J.W., Wikman-Coffelt, J., Eddington, C.L. 1972. The effects of progesterone on biosynthetic pathways in mammary tissue. Endocr. 91:1011.

Dixon, R., Hyman, A., Gurpie, E., Dyrenfurth, I., Cohen, H., Bowe, E., Engel, T., Daniel, S., James, S., Vandewiele, R. 1970. Feto-maternal transfer and production of cortisol. Steroid. 16:771.

Fellner, O.O. 1913. Experimentelle untersuchunger uber die Wirkung von gewebsextrakten aus der plazent und den weiblichen sexualoganen auf das genitale. Arch. Gynak. 100:641.

Fleck, A. and Munro, H.N. 1962. The precision of ultraviolet absorption measurements in the Schmidt-Thannhauser procedure for nucleic acid estimation. Biochem. Biophys. Acta. 55:571.

Flux, D.S. 1954. The effect of adrenal steroids on growth of the mammary glands, uteril, thymus, and adrenal glands of intact, ovariectomized, and oestrone-treated ovariectomized mice. J. Endocrin. 11:238.

Folley, S.J. 1952. In The Superadrenal Cortex. p. 85. Ed. Yoffey, J.M. London. Butterworths.

Freankel, L. 1910. Neue experimente zur function des corpus luteum. Arch. F. Gyn. 91:705.

Fyling, P., Sjaastad, O.V., Velle, W. 1973. Mid-term abortion induced in sheep by synthetic corticoids. J. Reprod. Fert. 32:305.

Gardner, W.U. and Hill, T. 1936. Effect of progestin upon mammary glands of the mouse. Proc. Soc. Exp. Biol. Med. 34:718.

Greenbaum, A.L. and Slater, T.F. 1957. Studies of the particulate components of rat mammary gland 2. Changes in the levels of nucleic acids of the mammary glands of rats during pregnancy, lactation, and mammary gland involution. Biochem. J. 66:155.

Griffith, R.D. Turner, C.W. 1959. Normal growth of rat mammary glands during pregnancy and early lactation. P. Soc. Exp. Biol. Med. 122: 619.

Hahn, D.W. and Turner, C.W. 1966. Effect of estrogen and progesterone on mammary gland DNA and feed intake in hypophsectomized female rats. Proc. Soc. Exp. Biol. Med. 122:183.

Heap, R.B. and Deasley, R. 1966. Progesterone in systemic blood and placentae of intact and ovariectomized pregnant guinea pigs. J. Endocr. 34:417.

Hoffman, B., Schams, D., Gimenez, T., Ender, M. L., Herrman, C., Karg, H. 1973. Changes in progesterone, total estrogens, corticosteroids, prolactin, and LH in bovine peripheral plasma around parturition with special reference to the effects of exogenous corticoids and a prolactin inhibitor, respectively. Acta Endocr. 73:385.

Joshi, H.S., Watson, D.J., Labsetwar, A.R. 1974. Relationship between progesterone secretion and peripheral plasma levels of lutenizing hormone during pregnancy and postpartum period in hamsters. Biol. Repro. 10:39.

Jung. H. 1965. Zur physiologie und klinik der hormonalen uterusregulation. Bibliotheca Gynaecol. 33:1.

Kamoun, A. and Stutinsky, F. 1968. Role de la surrenale foetale dans 1' augmentation de la corticosterone plasmatique chez le ratte a la fin la gestation. J. Physiol. Paris. 6:475.

Keye, J.D. 1923. Periodic variations in spontaneous contractions of uterine muscle in relation to the estrous cycle and early pregnancy. Bull. Johns Hopkins Hosp. 34:60.

Knaus, H. 1930. Zur physiologie des corpus luteum. Arch. Gynak. 141:374.

Kuhn, N. J. and Lowenstein, J. M. 1967. Lactogenesis in the rat. Changes in metabolic parameters at parturition. Biochem. J. 105: 995.

Labsetwar, A.P. and Watson, D.J. 1974. Temporal relationship between secretory patterns of gonadotropins, estrogens, progestins, and prostaglandin-F in periparturient rats. Biol. Repro. 10:103.

Leavitt, W.W. and Blaha, G.C. 1970. Circulating progesterone levels in the Golden Hamster during the estrous cycle, pregnancy and lactation. Biol. Repro. 3:353.

Liggins, G.C. 1968. Premature parturition after infusion of cortisol into foetal lambs. J. Endocr. 42:323.

Liggins, G.C. 1969. Premature delivery of foetal lambs infused with glucocorticoids. J. Endocr. 45:515.

Liggins, G.C., Kennedy, P.C., Holm, L.W. 1967. Failure of the initiation of parturition after electrocoagulation of the pituitary of the foetal lamb. Am. J. Obstet. Gynec. 98:1080.

Liggins, G.C. and Kennedy, P.C. 1968. Effects of electrocoagulation of the foetal lamb hypophysis on growth and development. J. Endocr. 40:333.

Lowry, O.H., Rosenbrough, N.J., Fane, A.L., Fane, R., Rose, J. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193:256.

Lukaszewka, J.H. and Greenwald, G.S. 1970. Progesterone levels in the cyclic and pregnant hamster. Endocr. 86:1.

Malinowska, K.W., Hardy, R.N., Nathanielsz, P.W. 1972. Plasma adrenal-corticosteroid concentrations immediately after birth in the rat, rabbit, and guinea pig. Experientia. 28:366.

Meites, J. 1959. Mammary growth and lactation. In Reproduction in Domestic Animals. Vol. I. Eds. Cole, H.H. and Cupps, P.T. New York-London. p. 539. Academic Press.

Mishkinsky, J., Dikstein, L., Ben-David, M., Azeroual, J., Sulman, F.G. 1967. A sensitive in vitro method for prolactin determination. Proc. Exp. Biol. Med. 125:360.

Murphy, B.E.P. 1967. Some studies of the protein binding of steroids and their application to routine and ultra-micro measurement of various steroids in body fluids by competitive protein binding radioassay. J. Olin. Endocr. 27:973.

Murr, S.M., Stabenfeldt, G.H., Bradford, G.H., Geschwind, I.I. 1974. Plasma progesterone during pregnancy in the mouse. Endocr. 94:1209.

Nandi, S. 1959. Hormonal control of mammiogenesis and lactogenesis in the C3H/He Crgl mouse. U of Cal. Publications in Zoology Vol. 65 1:128.

Nandi, S. and Bern, H.A. 1961. The hormones responsible for lactogenesis in BALB/cCrgl mice. Gen. Comp. Endocr. 1:195.

Nathanielsz, P.W. and Abel, M. 1972. Cortisol induced parturition in the rabbit. J. Endocr. 54:345.

Neher, G.M. and Zarrow, M.X. 1954. Concentration of blood progestin in the non-pregnant, pregnant, and postpartum ewe. J. Endocr. 11:323.

Portman, K. 1934. De 1' action de 1' hormone du corps jaune sur la duree de la gestation chez la lapine. C.R. Soc. Biol., Paris. 115:89.

Rea, C. 1898. Prolonged gestation, acrania monstrosity and apparent placental praevia in one obstetrical case. J. Amer. Med Assoc. 30: 1166.

Reynolds, S.R.M. and Allen, W.M. 1932. The effect of progestin containing extracts of corpora lutea on uterine mitility in the unanesthetized rabbit with observations on pseudopregnancy. Amer. J. Physiol. 102:39.

Schofield, B.M. 1968. Parturition. In Advances in Reproductive Physiology. Vol. 3. Ed. McLaren, A. Logos Press Limited.

Schofield, B.M. 1969. Parturition in the rabbit, J. Endocr. 43:673.

Short, R.V., Wagner, G. Fuchs, A.R., Fuchs, F. 1965. Progesterone concentrations in the uterine venous blood after intra-amnionic injection of hypertonic saline in mid-pregnancy. Amer. J. Obst. Gynec. 91:132.

Sinha, K.N., Anderson, R.R., Turner, C.W. 1970. Growth of the mammary glands of the Golden Hamster, Mesocricetus auratus. Biol. Reprod. 2:185.

Stabenfeldt, G.H., Drost, M., Franti, C.E. 1972. Peripheral plasma progesterone levels in the ewe during pregnancy and parturition. Endocr. 90:144.

Sulman, F.G. 1970. Physiological regulation of lactation. In Hypothalamic Control of Lactation. Monographs on Endocrinology. Springer-Verlag. New York.

Talwalker, P.K., Nicoll, C.S., Meites, J. 1963. Induction of lactation in pregnant rabbits with prolactin, cortisol acetate, or both. Endocr. 73:261.

Tait, S.A., Okanoto, M., Tait, J.F. 1967. Superfusion studies of steroid output in vitro. Fed. Proc. 26:488.

Tan, W.C., Goldsmith, I. J., Young, S. 1972. The content of casein in the lactating mammary glands of the rat. Acata Endocr. 69:413.

Thornburn, G.D., Nicol, D.H., Basset, J.M., Shutt, D.A., Cox, R.I. 1972. Parturition in the goat and sheep: Changes incorticoids, progesterone, cestrogens, and prostaglandin-F. J. Fert., Suppl. 16-61.

Travis, R.H. and Sayer, G. 1965. Adreno corticotropic hormones: Adrenocortical steroids and their synthetic analogs. In The Pharmacological Basis of Therapeutics. 3rd ed. p. 1634. Eds. Goodman, L. and Gilman, A. New York, MacMillian.

Turkington, R.W. and Ward, O.T. 1969. Hormonal stimulation of ribonucleic acid polymerase in mammary gland in vitro. Biochem. Biophys. Acta. 124:291.

VanDyke, H.B. and Gustavson, R.G. 1929. On pregnancy response of cat. J. Pharm. Exp. Therp. 37:379.

VanRensberg, S.J. 1967. Gestation in sheep after foetal adrenalectomy and cortisol acetate administration. J. Endocr. 38:83.

Weist, W.G., Kidweil, W.R., Bologh, K. 1968. Progesterone cataboltis in the rat ovary: A regulatory mechanism for progestational potency during pregnancy. Endocr. 82:844.

Yalow, R.S. and Berson, S.A. 1960. Immunoassay of endogenous plasma insulin in am. J. Olin. Invest. 39:1157.