Ph. D.

MAX JAMES PAAPE

EFFECTS OF PERSISTENCE AND DRY PERIOD
ON GERMINATION OF THE OAT

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
MAX JAMES PAAPE
1961

INFLUENCE OF PREGNANCY AND DRY PERIOD ON LACTATION IN THE RAT

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
MAX JAMES PAAPE
1967

This is to certify that the

thesis entitled

INFLUENCE OF PREGNANCY AND DRY PERIOD

ON LACTATION IN THE RAT

presented by

MAX JAMES PAAPE

has been accepted towards fulfillment
of the requirements for

PhD degree in Dairy

H. Gilbert Tucker
Major professor

Date November 7, 1967

ABSTRACT

INFLUENCE OF PREGNANCY AND DRY PERIOD
ON LACTATION IN THE RAT

by Max J. Paape

The influence of pregnancy on various biochemical parameters of the secretory and connective tissue components of lactating and post-lactational involuting mammary glands was studied. In addition, the influence of 0-, 4-, 8-, 12-, and 16-day dry periods on the carry over of secretory and connective tissue into a second lactation was also studied. The parameters measured were DNA (cell numbers), RNA and RNA/DNA (protein synthetic activity), hydroxyproline (collagen), hexosamine (ground substance) and crude lipid.

Concurrent pregnancy from the 8th to the 24th day of lactation reduced ($P < 0.01$) daily litter weight gain, DNA, RNA, and RNA/DNA ratio of the mammary gland, relative to lactating, non-pregnant (LNP) controls by 32.1, 7.7, 20.5, and 15.0 per cent respectively.

Following weaning on the 8, 12, 16, or 20th day of lactation, pregnancy retarded DNA declines after the 12th day of gestation. On the 8th day of the dry period, following weaning on the 8th or 12th day of lactation, DNA, RNA, and RNA/DNA ratios of pregnant animals gradually increased until parturition.

Hydroxyproline of the mammary gland for lactating, pregnant (LP) animals was constant from the 8th to the 20th day of lactation, but then decreased 32.8 per cent between the 20th and 24th day. On the 24th day, hydroxyproline for LP animals was 32.3 per cent less than corresponding LNP controls.

From the 8, 12, 16, and 20th day of lactation to the 4th day after weaning, hydroxyproline content for both pregnant and non-pregnant animals showed changes ranging from a 3.8 per cent increase to a 18.7 per cent decrease. Unlike DNA, whose loss was retarded after the 12th day of pregnancy, hydroxyproline losses were not retarded until the 16th day of pregnancy.

Hexosamine of the mammary gland for LP and LNP animals increased 19.7 and 31.0 per cent respectively, from the 8th to the 16th day of lactation but decreased 42.6 and 17.7 per cent, respectively, from the 16th to the 24th day. These quadratic curves ($P < 0.01$) suggest that net synthesis of hexosamine by the mammary gland occurred at the peak of lactation. Following weaning, pregnancy effectively reduced the loss of hexosamine after the 12th day of gestation, but did not promote net increases until the 20th day of pregnancy.

In the second lactation, dry period length did not influence total litter weight gain from the 3rd to the 8th day, but from the 8th to the 16th day those animals given

a 4-day dry period produced 22.0 and 9.3 per cent heavier litters than animals given a 0-day or longer (8-, 12-, 16-day) dry periods, respectively.

On the first day of the second lactation, animals given an 8- or 0-day dry period contained approximately 13 per cent more DNA than rats given 16-, 12-, or 4-day dry periods. On the 8th and 16th day of the second lactation mammary glands of animals given 0- or 16-day dry periods contained approximately 14 per cent less DNA than animals given a 4- or 8-day dry period.

The various dry periods had no effect on RNA on the first day of the second lactation ($P > 0.05$). However, on the 8th day, mammary glands of rats given an 8-day dry period contained on the average 15.8 per cent more RNA than glands which received 16- or 0-day dry periods. By the 16th day these 16- and 0-day dry period groups contained 14.3 and 22.1 per cent less RNA than rats given the 4-day dry period. Length of the dry period had no effect on RNA/DNA ratios. Thus, the dry period effects subsequent lactation by causing loss of cells or preventing mitosis during lactation rather than loss of synthesis per cell.

During the second lactation rats which received the 0- or 16-day dry period contained less hydroxyproline, than rats which received the 4-, 8-, or 12-day dry periods.

Length of the dry period did not effect hexosamine on the 1st or 8th day of the second lactation ($P > 0.05$). However, by the 16th day, a linear increase ($P < 0.05$) with increasing length of the dry period was observed which was related to a linear increase in weight of the adrenal gland.

INFLUENCE OF PREGNANCY AND DRY PERIOD ON LACTATION IN THE RAT

By

Max James Paape

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Dairy

1967

BIOGRAPHICAL SKETCH

Max J. Paape was born at Port Chester, New York, on December 26, 1936. He received his elementary education in Our Lady of Mercy Parochial School, and was graduated from Port Chester Senior High School in June, 1954.

In September, 1954, he enrolled at The Long Island Agricultural and Technical Institute, Farmingdale, New York, majoring in Animal Husbandry. He was graduated in June, 1956, with the degree of Applied Arts and Science.

From September, 1956 to June 1959, he completed his undergraduate requirements leading to a Bachelor of Science in Agriculture Extension at Michigan State University.

In January, 1960, he enrolled in the graduate school of Michigan State University. He received the Master of Science degree in March, 1963 and Doctor of Philosophy degree in September 1967, in the Dairy Department with a major in Mammary Gland Physiology.

ACKNOWLEDGMENTS

The author wishes to express his gratitude and thanks to his major professor, Dr. H. Allen Tucker, for his guidance and help throughout the Ph. D. program.

The assistance of the author's past and present colleagues, Drs. C. Desjardins, Y. N. Sinha, K. T. Kirton, K. L. MacMillan and W. W. Thatcher are gratefully acknowledged. Special thanks go to Mrs. Helga Hulkonen and Miss Susan Jeffries for their assistance with the tedious chemical assays.

Sincere appreciation is extended to Drs. L. D. McGilliard, P. E. Reineke, A. J. Morris, and J. L. Gill for their help in preparing this manuscript, and to Dr. H. D. Hafs for his assistance and encouragement during the author's graduate study.

The author is grateful for the financial support provided by Michigan State University through a Graduate Research Assistantship and Research Instructorship.

Gratitude is extended to Dr. C. A. Lassiter, Chairman of the Department of Dairy, for making available the facilities and animals.

The sacrifice, patience, and encouragement of the author's wife, Susan, during the course of this study is deeply appreciated.

TABLE OF CONTENTS

Page

RESULTS AND DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Several species of mammals may become pregnant during lactation. Although one can imagine that such a condition would present physiological stress on the mammary gland and milk synthesis, little is actually known about the influence of pregnancy on the secretory and connective tissue components of the lactating mammary gland. However, pregnancy in cows reduces milk production, especially as gestation progresses. Such an effect lowers the financial return to the dairyman. A basic knowledge of the biochemical changes taking place in the lactating mammary gland, as a result of concurrent pregnancy, may contribute to understanding how pregnancy lowers milk yield. Thus, a primary objective of this study was to determine the influence of pregnancy on various biochemical parameters of the secretory and fat pad stromal components of the mammary gland during lactation.

During the past three decades, a large body of evidence has accumulated which suggests that a dry period (a period of nonlactation between lactations) is necessary for maximal milk production in the cow. Absence of a dry period will decrease the milk yields of the subsequent lactation. However, the reasons for the necessity of a dry period still

remain obscure. To omit a dry period without causing a deleterious effect on subsequent lactation would probably increase yearly production by at least 15 per cent. The changes taking place within the mammary gland during the dry period and how length of the dry period affects the mammary gland during the subsequent lactation might suggest a method whereby the dry period could be reduced or eliminated. Thus, a second objective of this study was to investigate the changes taking place in the secretory and connective tissue components of the mammary gland during the dry period, and during the adjacent lactation following different dry period lengths.

The biochemical parameters studied in the parenchymal-stromal portion of the mammary gland were deoxyribonucleic acid (DNA), ribonucleic acid (RNA), crude lipid, hydroxyproline, and hexosamine. The DNA was used to estimate total cell numbers; RNA was used as an indicator of protein synthetic activity; and lipid, hydroxyproline (a measure of collagen content), and hexosamine (a measure of ground substance content) were used as measures of the various components of connective tissue stroma. In addition, the extraparenchymal mammary fat pad, removed from the periphery of the mammary gland, was analyzed for total DNA, lipid, and hydroxyproline to provide a better understanding of the changes in stromal elements in the absence of parenchymal cells during mammary gland growth and involution.

The rat was chosen as the experimental animal and may serve as a model for future experiments in cows.

REVIEW OF LITERATURE

Development of the Mammary Gland During Pregnancy and During Lactation

DNA levels of the mammary gland as a measure of total mammary cells, have been extensively studied in the rat (Harkness and Harkness, 1956; Greenbaum and Slater, 1957; Shimizu, 1957; McLean, 1958; Smith and Richterich, 1958; Shimizu and Ugami, 1959; Griffith and Turner, 1959, 1961a; Slater, 1961; Moon, 1962, Tucker and Reece, 1963a, 1963b; Munford, 1963; and Baldwin and Milligan, 1966), and in the mouse (Lewin, 1957; and Brookreson and Turner, 1957). Such studies show that growth of the mammary gland continues throughout pregnancy, reaches a maximum about the 10th day of lactation, remains relatively constant to about the 20th to the 24th day and then decreases. Tucker and Reece (1963a, 1963b) reported a 261.1 per cent increase in total DNA from the first to the 20th day of pregnancy and a 24.8 per cent increase during lactation. Traurig (1967) using tritiated thymidine, reported a wave of mammary epithelial cell proliferation during early lactation, which confirmed the total mammary DNA data.

The level of total mammary RNA and the RNA:DNA ratio has been used to indicate the state of metabolic activity of mammary tissue with particular reference to protein

synthesis (Kirkham and Turner, 1953; Kuretani, 1957; Shimizu, 1957; Slater, 1961; Tucker and Reece, 1963a; and Baldwin and Milligan, 1966). Total mammary RNA, similar to DNA, increased progressively throughout pregnancy and early lactation, remained constant until the 24th day, and then decreased. The RNA:DNA ratios were less than 1 during the greater part of pregnancy, indicating little protein synthesis per cell. Ratios increased steadily during the last few days of pregnancy and during lactation to a level of 2 to 3 between the 10th and 20th day of lactation but then subsequently decreased.

There is little information concerning the status of the connective tissue stroma during pregnancy and lactation. It has been suggested that the connective tissue actually determines the form of mammary development. In tissue culture, mammary duct cells grow in irregular sheets in the absence of fibroblasts; but an early organization of the epithelium into alveolar-like structures was discernible if fibroblasts were added to the tissue culture media (Lasfarques, 1957). Harkness and Harkness (1956) determined that mammary collagen (hydroxyproline) levels were relatively constant throughout pregnancy and lactation, whereas the lipid content increased slightly in late pregnancy but declined during lactation. On the other hand, Wrenn et al. (1965) reported a continuous decrease in lipid content throughout pregnancy and lactation.

Development of the Lactating Mammary Gland During Concurrent Pregnancy

Rats and mice usually exhibit estrus the night following parturition (Blandau and Soderwall, 1941). If these animals are mated at this time, blastocyst implantation is usually delayed (Lataste, 1891; and Enzmann et al., 1932). The length of this delay is dependent on the number of suckling young (Enzmann et al., 1932; and Mantalenakis and Ketchel, 1966). Four or less suckling young will delay implantation two days, whereas five or more suckling young will delay implantation four or more days(Mantalenakis and Ketchel, 1966). A subcutaneous injection of a 0.3-10µg of estrogen on day 5 of lactation will cause implantation at that time and thus prevent the delay in implantation normally encountered (Yoshinaga and Hosi, 1958).

Several studies have been made of mammary development in animals lactating while pregnant. Mammary glands of rats simultaneously lactating and pregnant (LP) do not undergo appreciable involution (Reece, 1958). Reece and Warbritton (1953) observed that mammary mitotic activity for LP animals was greater on the 21st day than on the 15th day of lactation. Furthermore, the mitotic activity was much less for the LP animal than for the nonlactating, pregnant animal. When the authors removed litters on the 17th day of lactation in animals simultaneously pregnant, no increase in mitotic activity could be detected 4 days later.

In the lactating mouse, Mizuno (1960a, 1961) studied involution of mammary glands following unilateral ligation of the inguinal galactophores with continued suckling of

contralateral glands. Mammary involution, on the basis of histology, respiratory quotient (R. Q.), and nucleic acid content was markedly retarded by concurrent pregnancy.

In another experiment, Mizuno (1960b) reported no significant differences in mammary oxygen consumption, R. Q., or RNA/DNA ratio between lactating, non-pregnant (LNP) and LP mice on the 14th or 19th day of lactation. However, total DNA and RNA were greater in the LP groups. The author concluded that in the mouse, advancing pregnancy enhanced lactation, and the conditions for mammary growth may coexist with those for synthesis of milk.

Tucker and Reece (1964) studied the influence of concurrent pregnancy on lactation between the 18th and 28th day of lactation without regulating implantation time. Pregnancy caused increases in total mammary DNA and RNA on the 18th day of lactation when compared with LNP controls. On the 21, 24, and 28th day concurrent pregnancy had no effect, significant reduction, and no effect, respectively, on nucleic acid content. Within days 24 and 28, total nucleic acid content declined with advancing stages of concurrent pregnancy. When these authors standardized gestation length, they found that rats with seven or more fetuses contained less mammary DNA and RNA than LNP rats. Low numbers of fetuses (six or less), however, caused enhancement of mammary nucleic acid content.

Stanley and Reece (1967) did not observe any significant difference in litter weight gain for the LP rat as compared with LNP controls. Similarly, no significant differences could be found for total DNA, RNA, or RNA/DNA ratio of the mammary gland.

Meites and Turner (1948) reported that concurrent lactation and pregnancy in the rabbit did not alter pituitary prolactin content. The authors concluded that pregnancy does not interfere with lactation as long as demands for nutrients by both the fetuses and mammary glands are met.

Bruce (1961) observed in the rat that estrus recurred every 18 days during prolonged lactation, and when mated on the 75th day of lactation, normal pregnancy ensued. Toward the end of pregnancy, lactation always failed despite continuous suckling. This failure to maintain continuous lactation until parturition is in contrast with the results obtained by Mayer (1956). He was able to maintain lactation by frequent exchanges of litters. There is the possibility, as pointed out by Bruce (1961), that the eyes of the older litters of Mayer were open by the time the litters from the post-partum matings were born. Thus, the young were no longer wholly dependent on the mother for food, and whether milk secretion was contributing to litter weight gain just prior to parturition is questionable.

There are a few studies showing the effect of pregnancy on lactation in the bovine. Ragsdale et al. (1923), and Gaines (1926) reported that pregnancy did not influence lactation until the 5th month, after which time lactation was depressed. Cows pregnant 8 months produce 20 per cent less milk than non-pregnant cows (Gaines, 1926). The total reduction may amount to 400-800lb of milk if cows are bred during the early months of lactation (Ragsdale et al., 1923).

Status of the Mammary Gland During Post-Lactational Involution

Morphological studies of mammary involution following weaning are well known for the rat (Myers and Myers, 1921; Kuramitsu and Loeb, 1921; and Maeder, 1922); for the mouse (Turner and Gomez, 1933a; Cole, 1933; Fekete, 1938; Hooker and Williams, 1940; and Williams, 1942); for the guinea pig (Hasselberg and Loeb, 1937); and for the goat (Turner and Reineke, 1936).

According to Turner and Reineke (1936), the mode of regression of mammary glands among species is similar. Upon failure to withdraw the products of secretion, the glands become distended with milk which increases the intramammary pressure. This engorgement persists for several days then gradually recedes. The epithelial cells lining the alveoli collapse and disintegrate, many of them being cast into the

lumina, the lobules gradually disappear, and finally the point is reached were only the ducts and gland stroma remain.

The primary difference among species during involution is the length of time required for the mammary glands to revert to the virginal state. In the mouse, complete regression occurred within 13 days after weaning (Cole, 1933; and Williams, 1942). In the rat, Maeder (1922) stated that the normal virginal condition was approached by the 9th day; but Myers and Myers (1921) reported that 2 to 3 weeks were necessary. Complete involution of the lobule-alveolar system was noted in the guinea pig after 35 days (Turner and Gomez, 1933b) and in the goat after 75 days (Turner and Reineke, 1936). Generally, the duct system of the involuted mammary gland is more ramified than that of a nulliparous animal (Reece, 1958).

Histological studies (Emmel et al., 1946; and Silver, 1956) showed that in the early stages, when the alveoli are grossly distended with milk, the capillaries are collapsed, suggesting a reduced blood flow. Cross and Silver (1956) further suggested that the engorgement inhibited or restricted the transport of precursors and metabolites for milk synthesis. Silver (1956) also found that lactation could be reinitiated in rats weaned early in lactation provided the suckling stimulus was resumed within 4 to 5 days. After this time irreversible changes in the capillary blood supply to the alveoli prevented the re-establishment of mammary function.

Changes in the nucleic acid content of the mammary glands of rats and mice during involution after cessation of suckling have been investigated by several workers (Kirkham and Turner, 1953; Oshima and Goto, 1955; Greenbaum and Slater, 1957; Griffith and Turner, 1961b; Mizuno, 1961; Anderson and Turner, 1963; Munford, 1963; Tucker and Reece, 1963d; and Ota, 1964). In general. these studies show that both DNA and RNA delcine after the suckling stimulus is removed. However, Slater (1962), reported an increase in rat mammary DNA content during the first 48 hours of involution, which may be partially accounted for by a leucocytic invasion (Okada, 1956). Decreases in DNA then occurred after the 48-hour interval.

Williams (1942), Gibson (1930), Fekete (1938), and Mayer and Klein (1961) reported that post-lactational involution of the mammary gland consisted of atrophy and necrosis of the alveoli and small ducts and their replacement by fatty, areolar connective tissue. Harkness and Harkness (1956) reported that non-collagenous protein and DNA decreased, whereas collagen and lipid content of the mammary gland did not change during 21 days of involution.

Influence of the Dry Period on Subsequent Mammary Gland Performance

The effects of length of the dry period on the next lactation in cows have been reported by several investigators (Sanders, 1928; Dix and Becker, 1936; Dickerson and Chapman, 1939; Klein and Woodward, 1943; Johansson, 1962; and Ackerman et al., 1967). Such studies indicate that dry periods of less than 6 weeks decreased the milk yield during the next lactation; but dry periods longer than 8 weeks produced only slight increases in subsequent lactation yields. More recently, Swanson (1962, 1965) reported that cows milked throughout pregnancy produced 30 per cent less milk in the following lactation than their twins which received dry periods of 2 months.

Johansson (1962) showed that cows which, on an average have long dry periods are poor producers. Dickerson and Chapman (1939) compared production records following dry periods of different lengths with those of the first lactation. They found that low-producing cows received greater benefit from a long dry period than high producing cows. These workers also found that short dry periods depressed the milk yield of the next lactation more in herds fed below average than in those fed at above-average levels. Swanson (1965) reported that when adequate nutrition was provided to meet the stress of concurrent lactation and pregnancy, twin cows with no dry periods still produced less than their

twins which received 60-day dry periods. The author concluded that the role of nutrients during the dry period is probably minor.

Smith et al. (1966) suspended milking in two of four udder quarters in each of two cows 10 weeks before parturition. After parturition, when all four quarters were milked, yield of the two quarters given a dry period showed an increase in milk yield over that of the two quarters not given a dry period. These authors also concluded that the effect of the dry period on subsequent milk yield cannot be due to nutritional factors but appears to be related to changes which originate within the mammary gland itself and take effect before parturition. In a more extensive sutdy, however, Ackerman et al. (1967) suspended milking in two of four udder quarters in each of several cows 10 weeks before parturition. They reported comparable milk yield between quarters given a dry period and those receiving no dry period.

Gorman and Swanson (1967) reported that cows which received oxytocin injections during a normal dry period produced less milk than animals which received a dry period. These results suggested that oxytocin may be involved in lactation deficiencies following short dry periods.

Altman (1947) reported that a dry period was necessary to allow rapid regeneration of the secretory epithelium

before the next lactation. More recently, however, Pardue and Swanson (1963) showed that on the first day of the second lactation, total DNA content of mammary glands receiving no dry period was equal to the total DNA content of mammary glands receiving a 2-month dry period, suggesting that continuous milking may reduce the function of the mammary gland rather than its structure.

MATERIALS AND METHODS

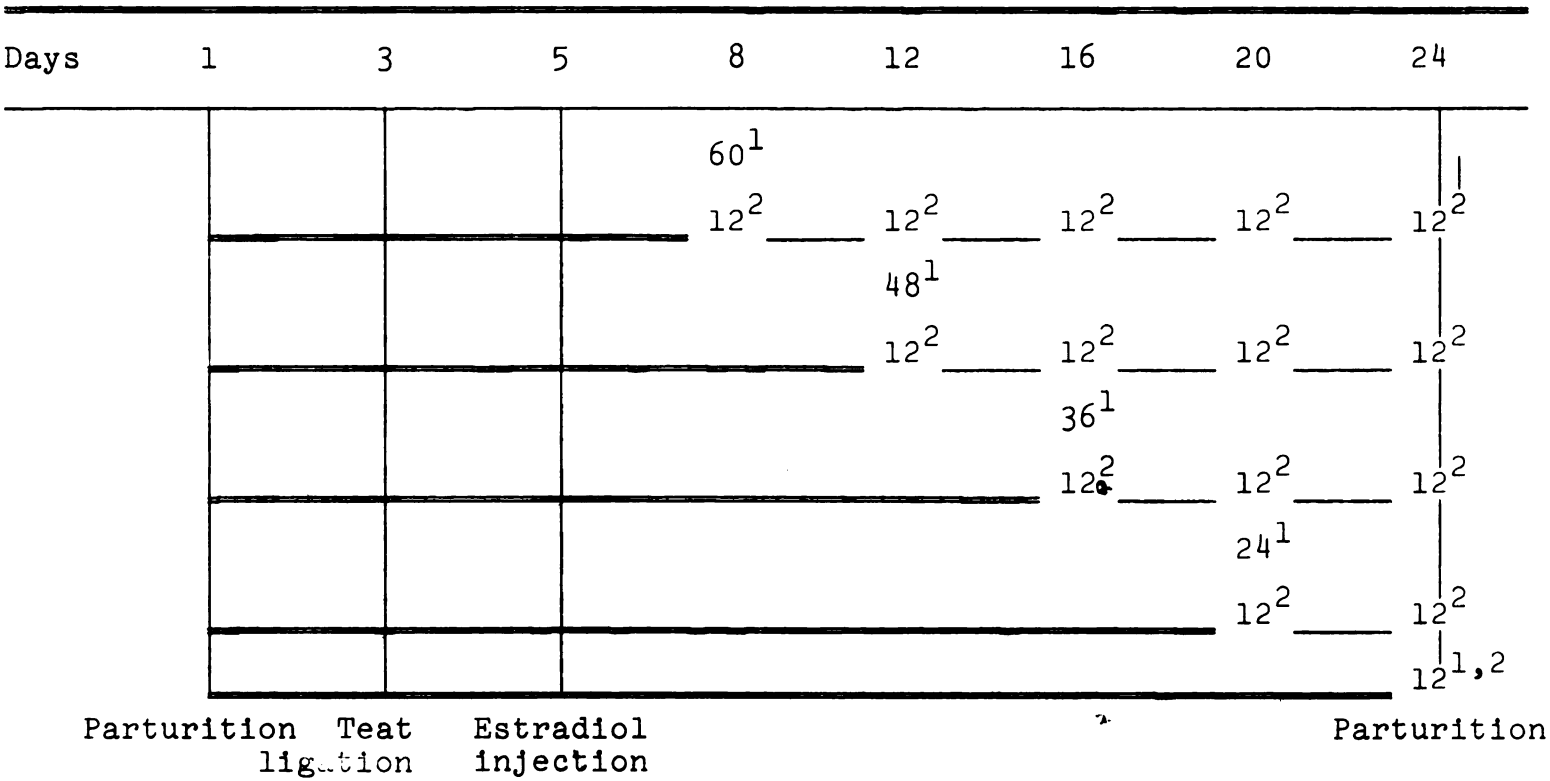
Experimental Animals and Design

Experimental animals were rats of the Sprague-Dawley strain. They were maintained at 24± 1°C and subjected to illumination between 5 a.m. and 7 p.m. All rats received a specially prepared 21.2 per cent protein diet (Appendix I) and water ad libitum

Influence of Pregnancy on Mammary Gland Development During Lactation and During Post-Lactational Involution

Primiparous pregnant female rats in advanced gestation were co-habited with male rats. Females were separated from males the day following parturition (second day of lactation). On the third day, teats of the thoracic mammary glands were ligated, litter size adjusted to six pups, and the mothers initial body weight recorded. To control blastocyst implantation time, female rats received 0.2 µg estradiol-17-B (Tucker and Reece, 1964) subcutaneously on the fifth day of lactation. Pregnancy was determined at autopsy. If the animals were pregnant, they were placed into one of the experimental groups shown in Figure 1. If the animals were not pregnant, they served as controls and were placed at random into one of the control groups shown in Figure 2.

Figure 1. Experimental design used to study the influence of pregnancy on mammary growth during lactation and during post-lactational involution.



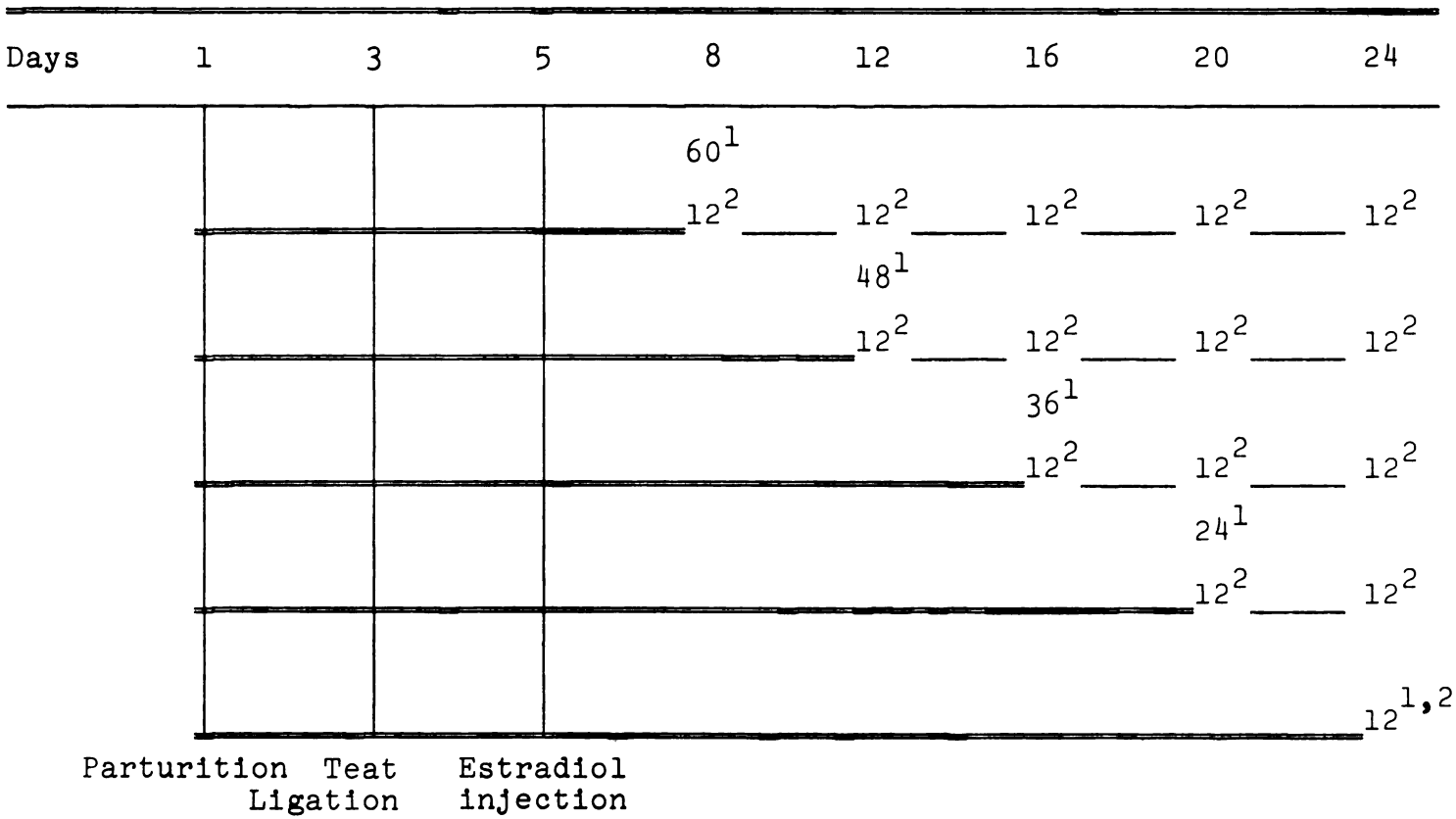
Lactating interval

Non-lactating interval

[1]Denotes number of lactating, pregnant rats which had their litters removed.

[2]Denotes number of rats killed.

Figure 2.--Experimental design used for the control of the experiment shown in Figure 1.



Lactating interval.

Non-lactating interval.

[1]Denotes number of non-pregnant, lactating rats which had their litters removed.

[2]Denotes number of rats killed.

Any lactating, pregnant rats which did not deliver their second litter by the 24th ± 1 day after parturition were discarded.

Litter weights were recorded on the 8, 12, 16, 20 and 24th day of lactation. In order to maintain a strong suckling stimulus, whenever a litter reached 16 days of age, they were replaced with a new 12-day-old foster litter. The foster litter weights were recorded, at the beginning (12th day) and end (16th day) of the 4-day suckling period.

The routine at the time of killing was as follows:

1. An animal was stunned by a blow to the back of the head and then killed by cervical dislocation. The animal was weighed.
2. If the animal was pregnant, the fetuses and uteri were removed and weighed, and the weight subtracted from the body weight. Fetus size at autopsy was compared with fetuses of known age taken from rats killed on the 8, 12, 16, and 20th day of normal pregnancy. If the fetuses were not of the expected size, indicating delayed implantation, the animal was discarded.
3. The six abdominal-inguinal mammary glands were removed within 10 minutes after stunning, dissected free of extraparenchymal fat and connective tissue, and weighed. The glands were covered with ice-cold

0.25 M sucrose and stored at -20°C for subsequent analysis. The extraparenchymal fat (fat pad), trimmed from the periphery of the mammary glands, was weighed, covered with sucrose and stored at -20°C.

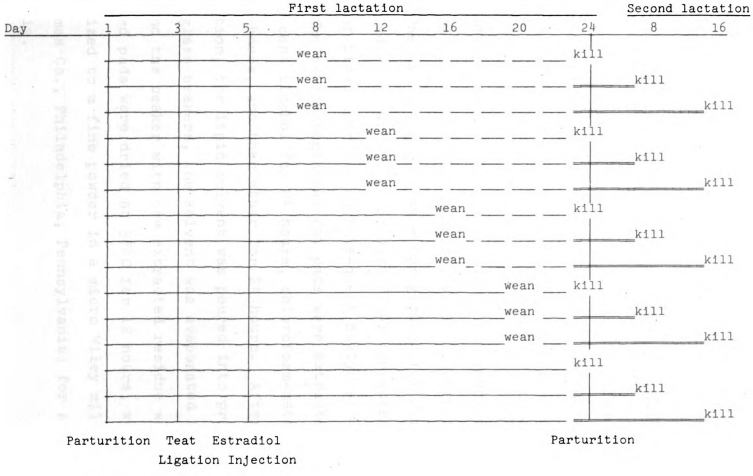
4. The adrenal glands were removed within 15 minutes after stunning, dissected free of fat and connective tissue and weighed.

Influence of Length of the Dry Period on Subsequent Lactation in the Rat

Primiparous pregnant female rats in advanced gestation were co-habited with male rats. Females were removed the day following parturition. On the third day, teats of the thoracic mammary glands were ligated, litter size adjusted to six pups, and the mothers initial body weight recorded. On the fifth day after parturition female rats received 0.2 µg estradiol-17-B. During the first lactation simultaneously lactating, pregnant rats were given dry periods of 0, 4, 8, 12 and 16 days (Figure 3).

Litter weights were recorded on the 8, 12, 16, 20 and 24th day of the first lactation. The suckling stimulus was maintained by exchanging all 16-day-old litters for new 12-day-old litters. Rats which did not deliver their second litter by the 24th ± 1 day after parturition were discarded. On the third day of the second lactation, litter size was adjusted to six pups. Litter weights were recorded daily

Figure 3.--Experimental design used to study the influence of length of the dry on subsequent lactation.



starting on the third day of the second lactation. For each of the various dry periods, 12 rats each were killed on the 1, 8, and 16th day of the second lactation.

At the time of killing, the same autopsy routine was followed as outlined for the previous experiments.

Biochemical Parameters Measured in the Mammary Glands and Mammary Fat Pads

The biochemical parameters measured in the mammary gland and extraparenchymal fat pad were the concentrations of DNA, hydroxyproline, and ethanol, chloroform-methanol, ether (ECME) extract. The level of RNA and hexosamine were measured in the mammary glands only. The samples, which had been stored in 0.25M sucrose at -20°C, were thawed at 4°C. The mammary glands were homogenized for 2 minutes in distilled water at 4°C to produce final concentrations of 50 mg of tissue per ml for subsequent analysis.

The extraparenchymal fat pads were extracted with 95 per cent alcohol for 24 hours, chloroform-methanol (2:1) for 24 hours, and then ether for 24 hours. After each extraction, the lipid solvent was poured into pre-weighed 50 ml glass beakers, the solvent was evaporated in a fume hood and the beaker with the extracted residue weighed. The defatted pads were dried at 37°C for 12 hours, weighed, and pulverized to a fine powder in a micro Wiley mill (Arthur H. Thomas Co., Philadelphia, Pennsylvania) for subsequent analysis.

The analytical procedure for nucleic acids was based on modifications of the Schmidt Thannhauser (1945) procedure described by Tucker (1964):

1. Mammary gland homogenate, containing 100 mg of tissue, was pipetted into a 16 ml plastic centrifuge tube. Ten ml of 95 per cent ethyl alcohol was added, the tube capped and shaken at room temperature for 12 hours.
2. The tube was then centrifuged at 17,000 rpm for 15 minutes and the supernatant fluid was poured into a pre-weighed 50 ml glass beaker. Ten ml of methanol:chloroform (2:1) was added to the sample which was shaken for 24 hours, centrifuged for 15 minutes at 17,000 rpm, and the supernatant fluid was poured into the same beaker.
3. At this point the nucleic acid procedure was started for the mammary fat pad. Twenty mg of the finely-ground fat pad was placed into a 16 ml plastic centrifuge tube and 10 ml anhydrous ether added to both tubes. Tubes were shaken for 12 hours at room temperature and centrifuged for 15 minutes at 17,000 rpm. The supernatant fluid from the homogenate was again poured into the glass beaker. The supernatant fluid from the fat pad sample was discarded.

4. The glass beaker containing the extracted residue of the mammary gland homogenate (EMCE fraction) was placed in a fume hood, the solvent evaporated, and the beaker with the residue weighed.
5. Five ml of ice-cold 10 per cent tricholoracetic acid (TCA) was added to the lipid-free residues of the mammary gland or fat pad portions, mixed, centrifuged for 15 minutes, and the supernatant fluid discarded. This step was repeated.
6. Five ml of ice-cold 95 per cent ethanol saturated with sodium acetate was added, mixed, centrifuged at 17,000 rpm for 15 minutes and the supernatant fluid discarded. Two ml of 1N potassium hydroxide (KOH) was pipetted into each sample, the tubes capped and placed in a 37°C oven for 15 hours.
7. The tubes were cooled in ice water, 0.5 ml of ice-cold 10 per cent perchloric (PCA) added, mixed, centrifuged for 15 minutes at 17,000 rpm and the supernatant fluid (RNA fraction) of the mammary homogenate saved. This supernatant fluid from the fat pad sample was discarded.
8. Five ml of ice-cold 5 per cent PCA was added to both precipitates, mixed, centrifuged at 17,000 rpm for 15 minutes and the supernatant fluid for the mammary gland sample saved. The supernatant fluid for the fat pad sample was again discarded. This step was repeated.

9. The combined supernatant fluids (RNA fraction) of the mammary homogenates were brought up to 20 ml with 5 per cent PCA and mixed. One ml of the RNA fraction was then pipetted into a clean 16 ml test tube containing 2 ml of 5 per cent PCA and 3 ml of freshly prepared orcinol reagent (Appendix II) was added. The tube was then capped and boiled for 30 minutes. The color development was then read in a Beckman DB spectrophotometer after 15 minutes at 670 mμ. The RNA content of the sample was calculated from a standard curve derived from pure yeast RNA (Worthington Biochem. Corp.).
10. Five ml of ice-cold 5 per cent TCA was then added to the precipitates of both mammary gland or fat pad samples from step 8, mixed, incubated at 70°C for 15 minutes, cooled to 5°C, centrifuged for 15 minutes at 17,000 rpm, and the supernatant fluids (DNA fraction) poured into separate 25 ml graduated test tubes.
11. Five ml of ice-cold 5 per cent PCA was added to the precipitate, mixed, centrifuged at 17,000 rpm for 15 minutes, and the supernatant fluids poured into their respective tubes. This step was repeated.

12. The graduated test tube containing the supernatant fluid (DNA) from the mammary gland homogenate was brought up to 25 ml with 5 per cent PCA. The fat pad sample was brought up to 20 ml with 5 per cent PCA. The optical densities were read in a Beckman DB spectrophotometer at 268 mμ, and the DNA content of the sample was calculated from a standard curve derived from pure highly polymerized DNA (Worthington Biochemical Corporation).

The analytical procedure for hydroxyproline analysis, described by Prockop and Udenfriend (1960), was modified as follows:

1. Mammary gland homogenate, containing 25 mg of tissue, was pipetted into a 16 ml culture tube and 3.5 ml of $HCl:H_2O$ (2.0:1.5) added. For fat pads, 5 mg of ground fat pad was placed into a 16 ml culture tube and 4.0 ml of $HCl:H_2O$ (1:1) added. The tubes were capped tightly and autoclaved at 15 lb pressure for 15-24 hours.
2. The tubes were cooled and the hydrolyzed contents poured into 25 ml graduated test tubes. The culture tubes were rinsed with distilled water to remove any adhering hydrolysate, and the volume in the graduated test tubes adjusted to 8 ml for the mammary gland sample or to 12 ml for the fat pad sample.

3. One ml of resin-charcoal preparation (Appendix III) was added to the hydrolyzed samples, stirred on a Vortex mixer, poured into 16 ml plastic centrifuge tubes and centrifuged at 17,000 rpm for 10 minutes.
4. One ml (mammary gland) or 0.5 ml (fat pad) was pipetted into 100 ml culture tubes. One drop of 1 per cent phenolpthalein in 95 per cent ethyl alcohol was added and the pH adjusted to light pink with 1N KOH and then 0.1N KOH.
5. The tubes were adjusted to approximately 8 ml with distilled water and saturated with an excess of potassium chloride. Readjustments to a light pink were made with 0.1 N KOH if necessary.
6. Two ml of borate buffer and one ml of 10 per cent alanine solution (Appendix III) were added.
7. The reaction mixture was then oxidized with 2.0 ml of a freshly-prepared solution of 0.2 M chloramine T in 2-methoxy-ethanol for 20 minutes. The reaction was stopped by the addition of 6 ml of a 3.6 M solution of sodium thiosulfate in water. The mixture was again saturated if necessary with potassium chloride.
8. Ten ml of toluene was added and incorporated into the reaction mixture by stirring on a Vortex mixer. The two phases were allowed to separate

and the top phase (toluene layer) was removed by suction and discarded.

9. Tubes were capped and placed in a boiling water bath for 30 minutes.
10. After cooling, 10 ml of toluene was added and incorporated into the reaction mixture by use of a Vortex mixer. The two phases were allowed to separate and 5 ml of the toluene phase was pipetted into clean 16 ml glass test tubes.
11. Two ml of sulfuric Ehrlich's reagent (Appendix III) was immediately mixed into the sample. The color reaction was then read in a Beckman DB spectrophotometer after 15 minutes and before 60 minutes at 560 mμ. The hydroxyproline content of the sample was calculated from a standard curve, derived from pure hydroxy-L-proline (Calbiochem). The standard curve was obtained by taking different dilutions of standard through the same steps as the unknown samples, starting at step 2.

The analytical procedure for hexosamine determinations, described by Boas (1953), was modified as follows:

1. Mammary gland homogenate, containing 0.2 gm of tissue, was pipetted into a 10 ml graduated test tube. One ml of 2N HCl was added, the tube was capped with a marble, and hydrolyzed at 100°C for 15 hours.

2. The tube was cooled, made up to 10 ml with distilled water, poured into a 16 ml plastic centrifuge tube and centrifuged at 17,000 rpm for 10 minutes.
3. Five ml of clear supernatant fluid was added to another 16 ml plastic centrifuge tube containing Dowex-50, 250-500 mesh resin (Appendix IV), stirred on a Vortex mixer, centrifuged for 5 minutes at 17,000 rpm, and the supernatant fluid discarded.
4. The Dowex-50 was washed twice with two 5 ml volumes of distilled water, centrifuged for 5 minutes at 17,000 rpm and the supernatant fluids discarded.
5. The hexosamine was eluted with four 2 ml washes of 2N HCl, centrifuged at 17,000 rpm for 5 minutes and the supernatant fluids poured into a 25 ml graduated test tube. The volume was made up to 10 ml and then poured into a 16 ml plastic centrifuge tube, and centrifuged at 17,000 rpm for 5 minutes.
6. Three ml of the clear supernatant fluid was pipetted into a clean 25 ml graduated test tube. Two drops of 0.5 per cent phenolpthalein in 15 per cent ethyl alcohol was added, and the pH adjusted to a pink color with 4N sodium hydroxide

(NaOH). 0.5N HCl was added until the indicator color just disappeared.

7. One ml of freshly prepared acetylacetone reagent (2 per cent solution, volume per volume, of acetylacetone in freshly prepared 1N sodium carbonate) was added to the sample.
8. The sample was capped and placed in a water bath at 89° to 92°C for 45 minutes.
9. After cooling, 2.5 ml of absolute ethyl alcohol was added, the sample mixed, and 1 ml of hydrochloric acid Ehrlich's reagent (Appendix IV) added. The tube was made up to 10 ml with absolute ethyl alcohol and thoroughly mixed.
10. One hour after the addition of the Ehrlich's reagent, the optical density was read at 530 mμ in a Beckman DB spectrophotometer. The hexosamine content of the sample was calculated from a standard curve derived from pure D (+) glucosamine hydrochloride (Matheson, Coleman and Bell). The standard curve was obtained by taking different dilutions of standard through the same steps as the unknown sample, starting at step 3.

Statistical Analysis

Analysis of variance was used to analyze the influence of pregnancy on mammary gland development during lactation. Within the LP or LNP groups, orthogonal polynomial response curves (Ostle, 1964) were made for the five stages of lactation studied. In some cases, orthogonal contrasts (Ostle, 1964) were performed within LP and LNP animals for the five stages of lactation.

Analysis of variance was also used to analyze the influence of pregnancy on involution of the mammary gland during the dry period, and the influence of length of the dry period on subsequent lactation. Orthogonal contrasts (Ostle, 1964) were made between days of lactation, and orthogonal polynomial response curves (Ostle, 1964) were made for dry periods within a stage of lactation.

RESULTS AND DISCUSSION

Influence of Pregnancy During Lactation

Body Weight

The influence of concurrent pregnancy and lactation on body weight is listed in Table 1. Analysis of variance showed that body weights of lactating, pregnant (LP) animals (246 g) were less ($P < 0.08$) than lactating, non-pregnant (LNP) controls (254 g). LP animals weighed significantly less ($P < 0.05$) than corresponding controls only on the 24th day of lactation (235 g versus 256 g, respectively). These results on the 24th day are not in agreement with those of Tucker and Reece (1964). These authors reported that body weights of LP rats were about the same as LNP controls from the 18th to the 28th day of lactation. A possible explanation for this discrepancy is that pregnancy was delayed in Tucker and Reece's experiment and the rats were not as advanced in gestation as were the rats used in the present study. Another explanation could be that the hooded Norway rats used by Tucker and Reece responded differently than the Sprague-Dawley rats used in this present experiment.

TABLE 1.--Influence of pregnancy on body weight of lactating rats.

Day of lactation	Final body weight[a] (g)	
	Lactating, pregnant	Lactating, non-pregnant
8	252 ±7	248 ±5
12	245 ±8	258 ±10
16	245 ±6	256 ±8
20	250 ±5	250 ±9
24	235 ±5[b]	256 ±8
Overall mean	246[c]	254

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.05$) than lactating, non-pregnant controls.

[c]Significantly less ($P < 0.08$) than lactating, non-pregnant controls.

The depression in body weight that I observed on the 24th day could be attributed to water loss associated with parturition and/or to advancing pregnancy. If the latter, then no weight loss could be detected during the early stages of pregnancy because the embryos were small in comparison to the weight of the mother, and, therefore, the nutrients required were insignificant. After the 20th day, however, the fetuses developed sufficiently to require appreciable amounts of nutrients for growth and maintenance. If the animal was lactating, an additional nutrient

requirement would be produced and the animal would probably use body reserves in order to meet the requirements of both pregnancy and lactation.

Weight of the Mammary Gland and Extraparenchymal Fat Pad

The influence of concurrent lactation and pregnancy on weight of the mammary gland is shown in Table 2. Weight of the mammary gland of LP animals (9.85 g) was less ($P < 0.01$) than LNP controls (11.65 g). The interaction between day of lactation and pregnancy state was also significant ($P < 0.01$).

TABLE 2.--Influence of pregnancy on weight of the mammary gland of lactating rats.

Day of Lactation	Mammary gland weight[a] (g)	
	Lactating, pregnant[b]	Lactating, non-pregnant[b]
8	9.73±0.65	9.39±0.54
12	11.64±0.59	12.44±0.83
16	11.96±0.81	12.94±1.04
20	9.83±0.78	11.99±0.94
24	6.09±0.36	11.50±0.48
Overall mean	9.85[c]	11.65

[a]Mean and standard error of mean.

[b]Significant quadratic regression ($P < 0.01$) among days of lactation.

[c]Significantly less ($P < 0.01$) than lactating, non-pregnant controls.

Weight of the mammary gland for LP and LNP animals increased 18.6 and 27.4 per cent, respectively, from the 8th to the 16th day but then decreased 49.0 and 11.0 per cent, respectively, from the 16th to the 24th day of lactation. These changes during lactation represented ($P < 0.01$) quadratic response curves for both LP and LNP groups. On the 20th and 24th day of lactation, mammary glands of LP rats weighed 18 and 47 per cent less, respectively, than LNP rats. This decline in mammary gland weight from the 16th to the 24th day of lactation for the LP animals preceded the decline in body weight by 4 days. The decline in mammary gland weight with advancing pregnancy has been reported previously for the rat (Tucker and Reece, 1964).

Weight of the extraparenchymal fat pad of LP animals (0.91 g) were not different ($P \simeq 0.90$) from LNP controls (0.90 g) (Table 3). Weight of the fat pad for either LP or LNP animals did not change ($P \simeq 0.75$) from the 8th to the 24th day of lactation. Thus, weight of the mammary fat pad did not parallel the quadratic regression curves observed for mammary gland weight. Linzell (1959) suggested that the mammary gland grows into the fat pad during pregnancy but regresses out of it during involution. In view of this, it was anticipated that weight of the fat pad would decrease during the increase in mammary gland weight and increase during the decrease in mammary gland weight. Since this was not the case, it is possible that the mammary gland did

grow into and regress out of the fat pad, but that the extraparenchymal fat pad showed a compensatory growth and regression, thereby maintaining a constant weight. It is also possible that the mammary gland did not grow into or regress out of the fat pad between the 8th and 24th day of lactation, and the change in mammary gland weight during lactation was due to an increase or decrease in mammary gland thickness rather than an increase in length and breadth.

TABLE 3.--Influence of pregnancy on weight of the extra-parenchymal fat pad of lactating rats.

Day of lactation	Fat pad weight[a] (g)	
	Lactating, pregnant	Lactating, non-pregnant
8	0.85±0.11	1.04±0.25
12	1.02±0.15	0.80±0.14
16	0.99±0.17	0.83±0.12
20	0.84±0.09	0.93±0.10
24	0.86±0.13	0.90±0.10
Overall mean	0.91	0.90

[a]Mean and standard error of mean.

Daily Litter Weight Gain

Litter weight gains per day, a measure of milk production, from the 8th to the 12th, 12th to the 16th, 16th to the 20th, and 20th to the 24th day of lactation are listed in Table 4. Daily gain in litter weight for LP animals (6.93 g) was less ($P < 0.01$) than LNP controls (10.23 g). The interaction between pregnancy state and day of lactation was also significant ($P < 0.01$). Daily litter weight gains for LP and LNP animals increased 7.4 and 2.7 per cent, respectively, from the 8-12 to 12-16 day period and decreased 119.5 and 24.3 per cent, respectively, from the 12-16 to 20-24 day period. These changes were ($P < 0.01$) quadratic responses in both groups. These results are not in agreement with Mizuno (1960b) and Stanley and Reece (1967) who reported that pregnancy did not effect litter weight gain for the first 20 days of lactation. However, the LP mice used by Mizuno were nursing 6 pups per 10 mammary glands and the LP rats used by Stanley and Reece were nursing 8 pups per 12 mammary glands. In the present study I adjusted litter size to 6 pups per 6 mammary glands. The nursing young in Mizuno and Stanley and Reece's experiments had an extra milk supply from the additional mammary glands. Thus, the experimental design used by these experimentors may not have met the requirement set forth by Cowie and Folley (1947), that the validity of using litter weight gain as a quantitative measure of lactation rests upon there being

TABLE 4.--Influence of pregnancy on daily litter weight gain of lactating rats.

Day of lactation	Daily litter weight gain[a] (g)	
	Lactating,[b] pregnant	Lactating,[b] non-pregnant
8-12	10.0±0.5	10.8±0.7
12-16	10.8±0.6	11.1±0.4
16-20	8.7±0.6	10.6±1.0
20-24	-1.7±0.5	8.4±0.7
Overall mean	6.9[c]	10.2

[a]Mean and standard error of mean.

[b]Significant quadratic regression ($P < 0.01$) among days of lactation.

[c]Significantly less ($P < 0.01$) than lactating non-pregnant controls.

sufficient young to use all of the milk secreted by the mother. Also, the eyes of rat and mice pups open at approximatley 16 days of age, and the pups will start to eat solid food and drink water. Therefore, daily gain in litter weight after 16 days of lactation is not a good measure of milk production. To avoid this problem I switched 16-day old litters for 12-day old litters. Mizuno and Stanley and Reece did not. Their litter gain from the 16th to the 20th day may not represent weight gains attributable solely to milk production.

My results in rats agree with results obtained in the cow by Ragsdale et al. (1924). These authors showed that pregnancy depressed milk yields after the fifth month of lactation.

Nucleic Acid Content of the Mammary Gland and Extraparenchymal Fat Pad

The influence of concurrent pregnancy and lactation on DNA, RNA and RNA/DNA ratio of the mammary gland, relative to LNP controls is given in Table 5. Analysis of variance showed that total DNA of the mammary gland of LP animals (29.0 mg) was less ($P < 0.01$) than LNP controls (31.5 mg). The interaction between day of lactation and pregnancy state was also significant ($P < 0.01$). DNA of the mammary gland for LP and LNP animals increased 7.3 and 7.8 per cent, respectively, from the 8th to the 16th day, decreased 10.0 and 8.7 per cent, respectively, from the 16th to the 20th day and from the 20th to the 24th day decreased 23.3 per cent for the LP animals but increased 7.9 per cent for the LNP animals. These changes between the 8th and 24th day of lactation represented quadratic ($P < 0.01$) and cubic ($P < 0.05$) curves for the LP and LNP animals, respectively.

Total RNA of the mammary gland of LP animals (148.3 mg) was less ($P < 0.01$) than LNP controls (186.5 mg) (Table 5). The interaction between day of lactation and state of pregnancy was also significant ($P < 0.01$). Total mammary gland RNA for LP and LNP animals increased 37 and 47 per cent, respectively, from the 8th to the 16th day, decreased 25.3

TABLE 5.--Influence of pregnancy on nucleic acid content of mammary glands of lactating rats.

Day of lactation	Lactating, pregnant			Lactating, non-pregnant		
	Total DNA[a,c] (mg)	Total RNA[a,c] (mg)	RNA/DNA[a,c]	Total DNA[a,d] (mg)	Total RNA[a,d] (mg)	RNA/DNA[a,c]
8	30.2 ±1.7	134.3 ±11.5	4.4 ±0.2	30.0 ±1.4	128.8 ±9.0	4.3 ±0.2
12	30.7 ±1.2	173.9 ±9.5	5.7 ±0.3	32.6 ±1.6	189.6 ±12.6	5.8 ±0.2
16	32.6 ±1.0	213.0 ±11.6	6.5 ±0.3	32.6 ±1.1	229.8 ±12.5	7.0 ±0.2
20	29.3 ±1.2	159.2 ±10.5	5.3 ±0.4	29.8 ±1.4	185.4 ±13.2	6.2 ±0.3
24	22.5 ±1.2	61.1 ±4.2	3.1 ±0.2	32.3 ±0.9	199.0 ± 9.3	6.1 ±0.2
Overall mean	29.0[b]	148.3[b]	5.0[b]	31.5	186.5	5.9

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than lactating, non-pregnant controls.

[c]Significant quadratic regression ($P < 0.01$) among days of lactation.

[d]Significant cubic regression ($P < 0.05$) among days of lactation.

and 19.3 per cent, respectively, from the 16th to the 20th day, and from the 20th to the 24th day decreased 61.6 per cent for the LP animals but increased 7.9 per cent for the LNP animals. The response curves were quadratic ($P < 0.01$) and cubic ($P < 0.01$) for the LP and LNP groups, respectively. The results for nucleic acid for the LNP mammary glands are in general agreement with previous reports (Harkness and Harkness, 1956; McLean, 1958; Moon, 1962; and Tucker and Reece, 1936b). Such reports showed that cell numbers (DNA) reached a maximum on the 8th to the 12th day of lactation and remained constant until the 16th to the 20th day and then declined; mammary RNA increased to the 16th day of lactation and then subsequently declined. There is, however, one discrepancy between my results and those of others for total mammary gland DNA and RNA. I observed an 8.7 and 19.3 per cent decrease, respectively, from the 16th to the 20th day and a 7.9 and 7.8 per cent increase, respectively, from the 20th to the 24th day. The reason for this significant fluctuation in DNA and RNA is uncertain. Normally a mother rat will nurse her litter for 21 days. But as previously stated around the 16th day of lactation the eyes of the pups open and the suckling stimulus decreases because the pups start eating solid food. In response to this decrease in suckling stimulus prolactin hormone content in the pituitary also declines (Meites and Turner, 1950). Since prolactin is a major galactopoietic

hormone in the rat (Meites, 1966), it is reasonable to expect this fall in total mammary gland RNA and DNA after the 16th day. However, in the present study, a strong suckling stimulus was maintained by exchanging 16-day-old litters for 12-day-old litters. Thus the pups were always dependent on the mother for food. It is possible that this strong suckling stimulus checked the fall in pituitary prolactin content and prevented the further decline that was observed for DNA and RNA on the 20th day and further caused DNA and RNA to increase between the 20th and 24th day.

The results for nucleic acid content obtained between the 16th and 20th day of lactation for the simultaneously pregnant and lactating animals are not in agreement with the results of Tucker and Reece (1964). These authors reported that pregnancy enhanced mammary gland DNA and RNA on the 18th day of lactation by 12 and 11 per cent, respectively. They did, however, show that pregnancy depressed DNA and RNA on the 24th day by 9.7 and 16.0 per cent, respectively, which agrees with the results of the present study. Mizuno (1960b) reported that concurrent pregnancy in the mouse enhanced mammary gland DNA and RNA on the 19th day of lactation by 14 and 10 per cent, respectively. However, Mizuno failed to show, in spite of increased RNA synthesis, any increase in litter weight gain. Although no effort was made by these workers to control implantation of the blastocyst, size of the fetus did not influence the

results of Tucker and Reece (1964) until after the 24th day. In another experiment, Tucker and Reece (1964) noted that with six or fewer fetuses mammary gland DNA and RNA were not different from LNP controls. With seven or more fetuses, DNA and RNA were less than controls on the 19th and 20th day of lactation. More recently, Stanley and Reece (1967) reported no difference between LNP and LP rats for total DNA, total RNA, or litter weight gain on the 21st day.

Mammary gland RNA/DNA ratio for LP animals (5.0) was less ($P < 0.01$) than LNP controls (5.9) (Table 5). The interaction between day of lactation and the pregnancy condition was also significant ($P < 0.01$). Mammary gland RNA/DNA ratios for LP and LNP animals increased 32.9 and 39.1 per cent, respectively, from the 8th to the 16th day but decreased 52.5 and 12.4 per cent, respectively, from the 16th to the 24th day [quadratic response curves ($P < 0.01$)].

The low RNA/DNA ratio of the mammary gland on the 24th day of lactation for the simultaneously pregnant and lactating rat supports the work of Bruce (1961) who observed that conception during prolonged lactation in the rat eventually caused the failure of lactation despite continued suckling. It appears from our results that pregnancy may be exerting its inhibitory effect on lactation in two ways: (1) between the 20th and 24th day of lactation it may reduce the amount of synthesis per cell; (2) by the 24th

day it is causing a further decrease in total milk synthesis within the mammary gland by reducing the total number of mammary cells present. In this respect, it appears that rats are similar to dairy cows because in the latter it is well known that milk yields are reduced after the fifth month of concurrent pregnancy. Whether a similar loss of mammary cells occurs in dairy cattle as occurred in the rats of the present study is not known, but the beneficial effects of a non-lactating period of two months (dry period) on subsequent lactation in dairy cattle are well established.

The influence of concurrent lactation and pregnancy on total DNA of the extraparenchymal fat pad relative to LNP controls is shown in Table 6. Total DNA of the fat pad of LP animals (0.62 mg) was greater ($P < 0.05$) than LNP controls (0.54 mg). On the 8th, 12th, 16th, 20th and 24th day of lactation, pregnancy increased DNA of the fat pad 9.6, 30.7, 6.3, 13.6 and 3.7 per cent, respectively, relative to LNP controls. The reason for this apparent increase in cell number is unknown because, as described later, none of the other parameters measured in the fat pad were influenced by pregnancy.

On the 8th, 12th, 16th, 20th and 24th day of lactation, extraparenchymal fat pads for the LNP animals accounted for 1.8, 1.3, 1.6, 1.9 and 1.8 per cent, respectively, of the total mammary DNA (mammary gland DNA plus extraparenchymal DNA), and 2.0, 2.0, 1.7, 2.3 and 2.6 per cent, respectively,

of the total mammary DNA for the LP animals. It has been previously reported (Nicoll and Tucker, 1965) that the entire mammary fat pad (parenchymal plus extraparenchymal fat pad) of mice will account for 11 per cent of the total mammary DNA. Since only extraparenchymal fat pad was included in the present study our percentage values are proportionately lower. Furthermore, it is possible that a mechanism exists which controls the number of cells in the extraparenchymal fat pad, so that a constant percentage relative to the total gland is maintained.

TABLE 6.--Influence of Pregnancy on DNA Content of the Extraparenchymal Fat Pad of Lactating Rats.

Day of lactation	Total DNA[a] (mg)	
	Lactating, pregnant	Lactating, non-pregnant
8	0.61±0.06	0.55±0.05
12	0.64±0.06	0.44±0.06
16	0.57±0.07	0.54±0.07
20	0.68±0.09	0.59±0.07
24	0.62±0.04	0.59±0.03
Overall mean	0.62[b]	0.54

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.05$) than lactating, non-pregnant controls.

Ethanol, Chloroform:Methanol, Ether (ECME) Extract of the Mammary Gland and Extraparenchymal Fat Pad

The influence of pregnancy on lactating mammary gland (ECME) extract is listed in Table 7. Analysis of variance showed that total ECME extract of the mammary gland of LP animals (3.80 g) was not different ($P < 0.75$) from LNP controls (3.87 g). A linear decrease ($P < 0.01$) was obtained for total ECME extract of the mammary gland for LP animals from the 8th to the 24th day of lactation. This decrease parallels the decrease in both DNA and weight of the mammary gland from the 12th to the 24th day of lactation and may indicate that advancing pregnancy caused a decrease in fat cells of the mammary gland. Total ECME extract of the mammary

TABLE 7.--Influence of pregnancy on ECME extract of the mammary gland of lactating rats.

Day of lactation	Total ECME extract[a] (g)	
	Lactating,[b] pregnant	Lactating, non-pregnant
8	4.08±0.18	3.79±0.20
12	4.36±0.40	4.21±0.22
16	3.86±0.42	3.99±0.27
20	3.45±0.27	3.64±0.28
24	3.26±0.28	3.71±0.33
Overall mean	3.80	3.87

[a]Mean and standard error of mean.

[b]Significant linear decrease ($P < 0.01$) among days of lactation.

gland of LNP animals did not change throughout lactation. These results for the LNP controls are in agreement with Harkness (1956) and Wrenn et al. (1965).

ECME extract of the extraparenchymal fat pad of LP animals (0.69 g) was not different ($P \approx 0.05$) from LNP controls (0.64 g) (Table 8). Response curves for total ECME extract of the mammary fat pad during lactation were not obtained for either LP or LNP animals. The constant amount of fat pad ECME extract during the 8th to the 24th day of lactation for both LP and LNP animals parallel total DNA and weights of the fat pad. This implies that fat cells in the fat pad are probably maintained throughout lactation.

TABLE 8.--Influence of pregnancy on ECME extract of the extraparenchymal fat pad of lactating rats.

Day of lactation	Total ECME extract[a] (g)	
	Lactating, pregnant	Lactating, non-pregnant
8	0.66±0.08	0.60±0.08
12	0.79±0.11	0.76±0.09
16	0.70±0.13	0.66±0.12
20	0.68±0.07	0.61±0.09
24	0.64±0.08	0.56±0.05
Overall mean	0.69	0.64

[a]Mean and standard error of mean.

Hydroxyproline Content of the Mammary Gland and Extraparenchymal Fat Pad

There was no difference ($P \simeq 0.75$) in hydroxyproline content of the mammary gland between LP (21.12 mg) and LNP (21.43 mg) animals (Table 9). The interaction between day of lactation and pregnancy state was significant ($P < 0.01$). For the LP and LNP animals, total hydroxyproline of the mammary gland followed quadratic ($P < 0.05$) and cubic ($P < 0.07$) regression curves, respectively, from the 8th to the 24th day of lactation. For the LNP animals, hydroxyproline increased 21.5 per cent from the 8th to the 12th day, decreased 13.3 per cent from the 12th to the 20th day, and increased 14.8 per cent from the 20th to the 24th day

TABLE 9.--Influence of pregnancy on hydroxyproline content of the mammary gland of lactating rats.

Day of lactation	Total hydroxyproline[a] (mg)	
	Lactating, pregnant[b]	Lactating, non-pregnant[c]
8	21.96±2.65	18.26±1.14
12	23.40±2.16	23.27±2.42
16	20.44±1.33	21.74±1.96
20	23.82±1.76	20.18±1.63
24	16.00±0.92	23.69±1.49
Overall mean	21.12	21.43

[a]Mean and standard error of mean.

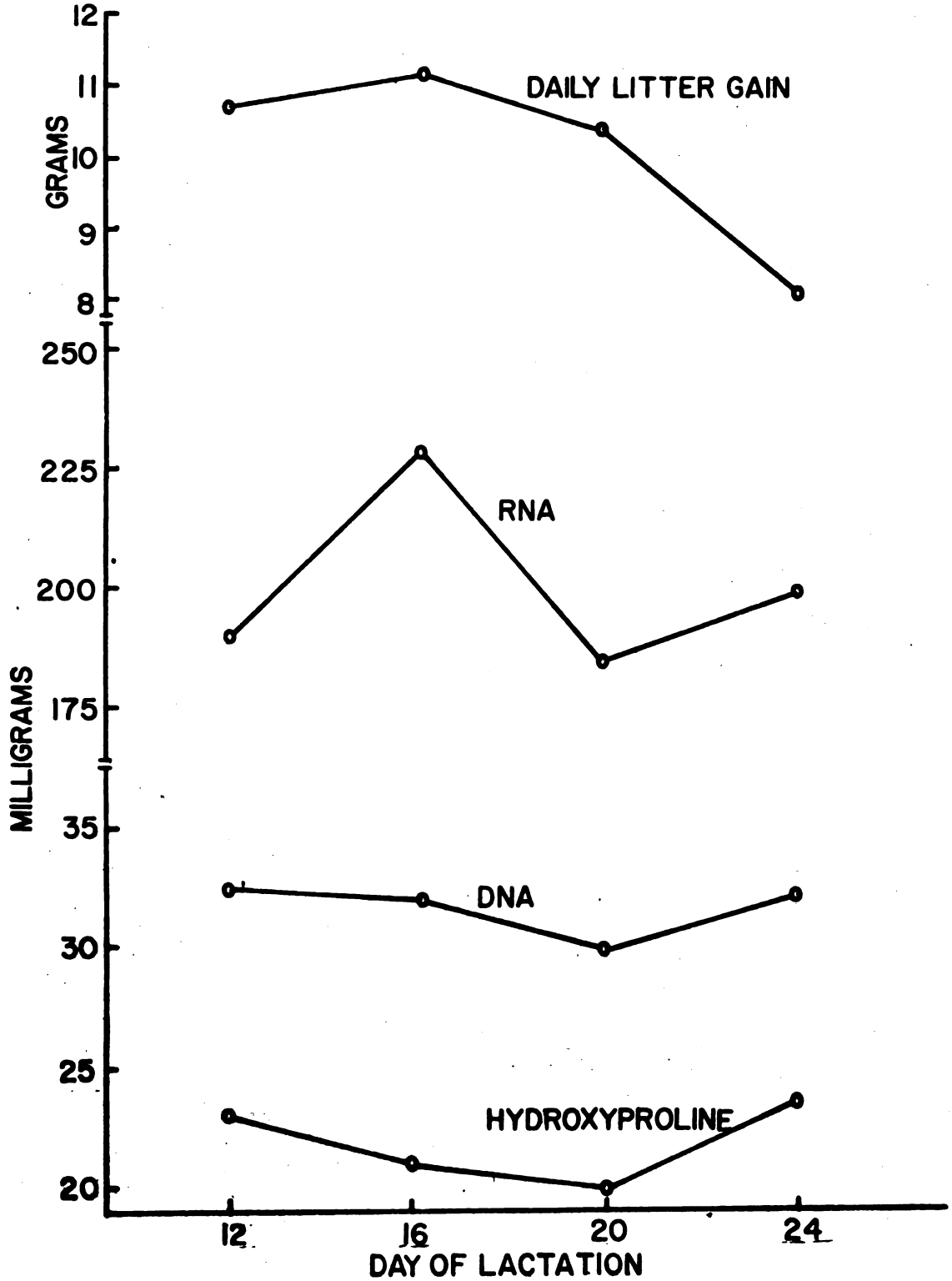
[b]Significant quadratic regression ($P < 0.05$) among days of lactation.

[c]Significant cubic regression ($P < 0.07$) among days of lactation.

of lactation. This is not in agreement with the results of Harkness and Harkness (1956). They reported that mammary gland hydroxyproline remained constant throughout pregnancy, lactation, and involution. However, the recent work of Traurig (1967) suggests that collagen is synthesized during lactation. Using the incorporation of labeled thymidine as an index of mitotic activity he reported a proliferation of fibroblasts during mammary epithelial proliferation. Since collagen is synthesized by the fibroblast (Asboe-Hansen, 1958; and Green et al., 1966), it is possible that the proliferating fibroblasts observed by Traurig are synthesizing hydroxyproline and account for the changes throughout lactation observed in this experiment.

The cubic response curve for hydroxyproline in the LNP animals parallels the cubic response curves for DNA and RNA (Table 5). It is important to note that although mammary hydroxyproline, DNA and RNA in LNP rats increased 14.8, 7.9 and 7.8 per cent, respectively, from the 20th to the 24th day of lactation, daily litter weight gain decreased 20.9 per cent during this period (Figure 4). From this, it is possible to speculate that the 7.8 per cent increase in DNA may indicate a proliferation of fibroblasts which could be actively synthesizing collagen, accounting for the increase in RNA and hydroxyproline, and that fewer parenchymal cells are available for milk synthesis, resulting in the 20.9 per cent decrease in daily litter weight gain.

Figure 4.--Relation between DNA, RNA, hydroxyproline of the mammary gland and daily litter weight gain for lactating, non-pregnant animals during latter stages of lactation.


12
11
10
9
8
GRAMS
DAILY LITTER GAIN
250
225
200
175
MILLIGRAMS
RNA
35
30
25
20
DNA
HYDROXYPROLINE
12
16
20
24
DAY OF LACTATION

Total hydroxyproline of the mammary gland for the LP animals was relatively constant from the 8th to the 20th day, but decreased 32.8 per cent from the 20th to the 24th day. On the 24th day, hydroxyproline for LP animals was 32.3 per cent less than corresponding LNP controls. Since collagen has a long half life (White et al., 1964), these results on the 24th day may indicate that advancing pregnancy or parturition caused an actual breakdown of collagen. How much of the hydroxyproline measured on the 24th day represented newly synthesized collagen or old collagen is unknown.

Mammary gland hydroxyproline synthesized per unit of RNA (Table 10) was consistently higher for those animals lactating and pregnant. This was especially evident on the 24th day of lactation. Since these animals delivered their second litters on the 24th day of lactation, these results were interpreted as indicating a preparation by the mammary gland stroma for future parenchymal cell proliferation.

The influence of concurrent pregnancy and lactation on hydroxyproline of the extraparenchymal fat pad is shown in Table 11. No significant trends could be detected for fat pad hydroxyproline from the 8th to the 24th day of lactation for either group. Also, LP animals (0.75 mg) did not differ ($P \approx 0.50$) from LNP controls (0.66 mg). It was originally hypothesized that the regressing mammary gland might leave its collagen in the fat pad. These data fail to support such an hypothesis.

TABLE 10.--Influence of pregnancy on hydroxyproline:RNA ratio of the mammary gland of lactating rats.

Day of lactation	Hydroxyproline:RNA ratio[a]	
	Lactating, pregnant	Lactating, non-pregnant
8	0.16	0.14
12	0.13	0.12
16	0.10	0.09
20	0.15	0.11
24	0.26	0.12
Overall mean	0.16	0.10

[a]Mean.

TABLE 11.--Influence of pregnancy on hydroxyproline content of the extraparenchymal fat pad of lactating rats.

Day of lactation	Total hydroxyproline[a] (mg)	
	Lactating, pregnant	Lactating, non-pregnant
8	0.67±0.07	0.67±0.08
12	0.81±0.11	0.60±0.06
16	0.76±0.14	0.64±0.09
20	0.82±0.17	0.73±0.08
24	0.67±0.05	0.67±0.06
Overall mean	0.75	0.66

[a]Mean and standard error of mean.

Hexosamine Content of the Mammary Gland

The influence of concurrent pregnancy and lactation on total hexosamine of the mammary gland relative to controls is found in Table 12. Analysis of variance revealed no difference ($P \simeq 0.70$) in hexosamine between LP (5.79 mg) and LNP (5.75 mg) animals. The interaction between day of lactation and pregnancy state, however, was significant ($P < 0.01$). Total hexosamine of the mammary gland for LP and LNP animals increased 19.7 and 31.0 per cent, respectively, from the 8th to the 16th day and then decreased 42.6 and 17.7 per cent, respectively, from the 16th to the 24th day. These quadratic ($P < 0.01$) regression

TABLE 12.--Influence of pregnancy on hexosamine content of the mammary gland of lactating rats.

Day of lactation	Total hexosamine[a] (mg)	
	Lactating, pregnant[b]	Lactating, non-pregnant[b]
8	5.56±0.44	4.83±0.26
12	7.04±0.37	6.13±0.39
16	6.92±0.50	7.00±0.65
20	5.44±0.32	5.98±0.53
24	4.00±0.50	5.76±0.47
Overall mean	5.79	5.75

[a]Mean and standard error of mean.

[b]Significant quadratic regression ($P < 0.01$) among days of lactation.

curves indicate that hexosamine, as a measure of the ground substance, is actively synthesized by the mammary gland during the peak of lactation.

Total hexosamine of the mammary gland paralleled the changes in RNA for both groups of animals. It is recognized that the ground substance bathes the cells and serves as a pathway for exchange between the blood stream and cells (Gersh and Catchpole, 1960). Thus, heightened metabolic activity of the mammary gland could be associated with increases in the amount of ground substance or hexosamine. Also, it has been shown that several of the hormones (thyrotrophic hormone and growth hormone) associated with increased mammary gland activity, can also cause increases in the amount of ground substance (Asboe-Hansen, 1958).

Weight of the Adrenal Gland

Knowledge of the weight of the adrenal glands has been used to estimate the functional status of these glands (Jones, 1957). Reports in the literature concerning changes in adrenal weight with advancing lactation are contradictory. Increases have been reported by Anderson and Kennedy (1933), and Anderson and Turner (1962) and decreases have been reported by Bearn et al. (1960).

The influence of concurrent pregnancy and lactation on weight of the adrenal gland relative to LNP controls is shown in Table 13. Adrenal gland weights for LP animals were

heavier ($P < 0.07$) than LNP controls. Mizuno (1960b) observed that adrenal gland weights for LP mice were 6.7 per cent higher than LNP controls on the 20th day of lactation. This agrees very well with the 7.7 per cent increase in adrenal weights for the LP rats, observed on the 20th day of lactation relative to LNP controls in this experiment. Linear increases in weight of the adrenal gland were observed from the 8th to the 24th day of lactation for both LP ($P < 0.07$) and LNP ($P < 0.01$) rats. These results agree with the results reported by Anderson

TABLE 13.--Influence of pregnancy on weight of the adrenal gland of lactating rats.

Day of lactation	Adrenal gland weight[a] (mg)	
	Lactating, pregnant[c]	Lactating, non-pregnant[d]
8	52.5±3.1	47.7±1.9
12	49.5±1.9	49.4±1.8
16	54.8±1.1	50.0±1.7
20	59.5±2.2	54.9±2.0
24	53.8±2.5	55.0±2.5
Overall mean	54.0[b]	51.4

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.07$) than lactating, non-pregnant controls.

[c]Significant linear increase ($P < 0.07$) among days of lactation.

[d]Significant linear increase ($P < 0.01$) among days of lactation.

and Kennedy (1933), and Anderson and Turner (1962) and confirm the observations of Poulton and Reece (1957) that ACTH and adrenal cortical activity were greater as lactation progressed.

Influence of Pregnancy During Various Lengths of the Dry Period

Body Weight

The influence of pregnancy on body weight during various length dry periods (periods of non-lactation between lactations) is summarized in Table 14. During the dry periods, body weights of pregnant rats (257 g) were heavier ($P < 0.01$) than non-pregnant controls (240 g). The interaction between days dry and pregnancy state was also significant ($P < 0.01$). Relative to the day of weaning at the 8th, 12th, 16th, and 20th day of lactation (Table 1) body weights of pregnant animals during the first four days of the dry period were depressed 5.6, increased 4.9, 2.1 and depressed 0.5 per cent, respectively. Body weights of non-pregnant animals during this interval decreased 6.3, 8.5, 6.9 and 4.2 per cent, respectively. This suggests that lactation is a stimulus for body growth in the non-pregnant animal, and an inhibitor to body growth in the pregnant animal. Others (Tucker and Reece, 1963d; and Griffith and Turner, 1961) have also reported decreases in body weights following weaning of lactating, non-pregnant rats. After weaning, pregnancy did not exert much of an effect in retarding weight loss before the 12th day of pregnancy

TABLE 14.--Influence of pregnancy on body weight during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Body weights (g)	
		Pregnant[c]	Non-pregnant
8	4	238±4	233±6
	8	252±7	236±6
	12	264±6	239±7
	16 (parturition)	266±7	240±6
12	4	258±6	236±4
	8	271±9	239±7
	12 (parturition)	257±7	243±6
16	4	251±4	238±5
	8 (parturition)	263±7	260±7
20	4 (parturition)	249±8	239±6
	Overall mean	257[b]	240

[a]Mean and standard error of mean.

[b]Significantly heavier ($P < 0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

(4th day after weaning on 8th day of lactation). After 12 days of gestation, however, body weights of pregnant rats were always heavier than non-pregnant controls (Table 14).

Weight of the Mammary Gland and Extraparenchymal Fat Pad

Mammary gland weights of pregnant (6.88 g) animals were heavier ($P < 0.01$) than non-pregnant (4.34 g) controls (Table 15). The interaction between day of the dry period and pregnancy state was also significant ($P < 0.01$). Mammary gland weights of pregnant animals were depressed 43.4, 17.2, 34.7, and 32.8 per cent during the first 4-day interval relative to weaning at the 8th, 12th, 16th and 20th day of lactation (Table 2). During this first 4-day dry period interval mammary gland weights of non-pregnant animals decreased 46.4, 58.0, 56.3, and 54.7 per cent, respectively. Thus, mammary glands of pregnant rats during the first 4 days of the 16-day dry period involuted to the same extent as mammary glands of non-pregnant animals. This failure of pregnancy to retard involution of the mammary gland during the early part of the 16-day dry period could be a limiting factor in the subsequent lactation. When weaned on the 8th day of lactation the mammary weights of pregnant rats increased progressively a total of 23.6 per cent, from the 4th to the 16th day of the dry period. Removal of the litter on the 12th day of lactation followed by 4-day interval measurements during the subsequent dry period

TABLE 15.--Influence of pregnancy on weight of the mammary gland during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Mammary gland weight[a] (g)	
		Pregnant[c]	Non-pregnant
8	4	5.51±0.37	5.03±0.26
	8	5.59±0.61	3.51±0.21
	12	6.21±0.28	3.30±0.18
	16 (parturition)	7.21±0.41	3.44±0.13
12	4	9.64±0.48	5.22±0.21
	8	6.02±0.23	4.06±0.24
	12 (parturition)	7.21±0.41	3.65±0.21
16	4	7.81±0.61	5.65±0.37
	8 (parturition)	7.04±0.38	4.07±0.17
20	4 (parturition)	6.60±0.48	5.43±0.32
	Overall mean	6.88[b]	4.34

[a]Mean and standard error of mean.

[b]Significantly greater ($P<0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

revealed that mammary weights declined for 8 days despite increasing stage of pregnancy, before increasing 16.5 per cent by the 12th day of the dry period (parturition). Weaning on the 16th day of lactation produced declines of 9.9 per cent between the 4th and 8th day of that dry period interval. Mammary weights in the non-pregnant groups were depressed an additional 31.6, 30.1, and 28.0 per cent between the first 4 days and the end of the dry period. Since mammary glands of pregnant animals involuted less than non-pregnant controls 4 days after the start of the dry period and grew during the dry period, it would appear that the hormones after 12 days of pregnancy are preventing appreciable involution and are causing mammary gland growth during the dry period.

The influence of pregnancy on weight of the extra-parenchymal fat pad during various length dry periods is shown in Table 16. During the dry periods, weights of the fat pad of pregnant rats (1.28 g) were not different ($P \simeq 0{,}50$) from non-pregnant controls (1.23 g). Between the day of weaning on the 8th, 12th, 16th and 20th day (Table 3) and the 4th day of the dry period, weights of the fat pad of pregnant animals increased 10.5, 25.0, decreased 0.1, and increased 8.7 per cent, respectively. Fat pad weights of non-pregnant animals increased 33.3, 26.6, 18.6 and 19.8 per cent, respectively. Unlike mammary glands which decreased in weight 4 days following weaning, fat pads of both

TABLE 16.--Influence of pregnancy on weight of the extra-parenchymal fat pad during dry periods of 4, 8, 12, and 16 days.

Day of lactation when weaned	Days dry	Fat pad weight[a] (g)	
		Pregnant[b]	Non-pregnant
8	4	0.95±0.11	1.56±0.12
	8	1.45±0.22	1.06±0.17
	12	1.37±0.16	1.22±0.16
	16 (parturition)	1.28±0.10	1.41±0.12
12	4	1.36±0.14	1.09±0.10
	8	1.76±0.22	1.05±0.15
	12 (parturition)	1.59±0.14	1.31±0.15
16	4	0.90±0.15	1.02±0.28
	8 (parturition)	1.25±0.11	1.42±0.20
20	4 (parturition)	0.92±0.16	1.16±0.11
	Overall mean	1.28	1.23

[a]Mean and standard error of mean.

[b]Day 1 of pregnancy = day 1 of lactation.

groups generally increased in weight. Weight of the fat pad of pregnant rats increased 25.8, 14.5 and 28.0 per cent, respectively, for the 4-16, 4-12, and 4-8 day dry period intervals, respectively. Weight of the fat pad of non-pregnant animals during these same periods decreased 9.6, increased 16.8 and 28.2 per cent, respectively. These changes in weight of the fat pad for both pregnant and non-pregnant animals were opposite the changes that occurred for weight of the mammary gland (Figures 5 and 6). From these changes it appears that the mammary gland grows into the fat pad during pregnancy and regresses out of it during involution as suggested by Linzell (1959).

DNA Content of the Mammary Gland and Extraparenchymal Fat Pad

The influence of pregnancy on total DNA of the mammary gland during the various length dry periods is presented in Table 17. Total DNA of the mammary gland of pregnant animals (19.5 mg) during the dry periods was greater ($P < 0.01$) than that of non-pregnant controls (10.6 mg). The interaction between day of the dry period and pregnancy state was also significant ($P < 0.01$). Weaning on the 8th, 12th, 16th, or 20th day of lactation (Table 5) caused losses totaling 49.1, 24.9, 27.9, and 32.8 per cent, respectively, during the first 4 days of the dry period despite concurrent pregnancy. Total DNA of non-pregnant animals during this first 4-day interval also decreased 59.8, 58.6, 57.3, and 52.4 per cent, respectively. On the 4th day of the dry period for rats weaned on the 8th day of lactation, pregnant animals showed approximately the same per cent decrease (49.1 per cent) as

Figure 5.--Relation between weight of the extraparenchymal fat pad and mammary gland of pregnant animals during various stages of lactation and involution.

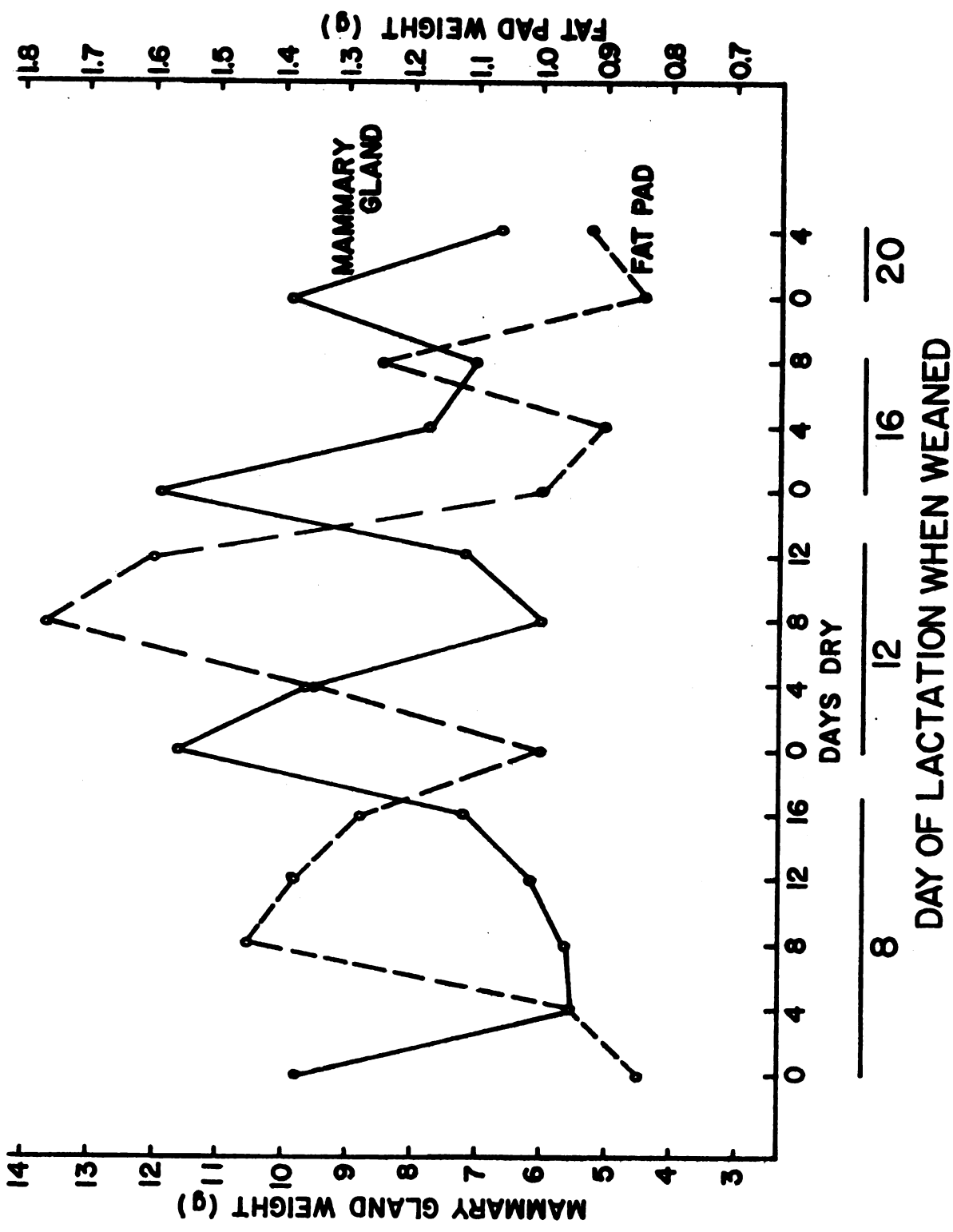

MAMMARY GLAND WEIGHT (g)
FAT PAD WEIGHT (g)
MAMMARY GLAND
FAT PAD
DAYS DRY
DAY OF LACTATION WHEN WEANED
8
12
16
20

Figure 6.--Relation between weight of the extraparenchymal fat pad and mammary gland of non-pregnant animals during various stages of lactation and involution.

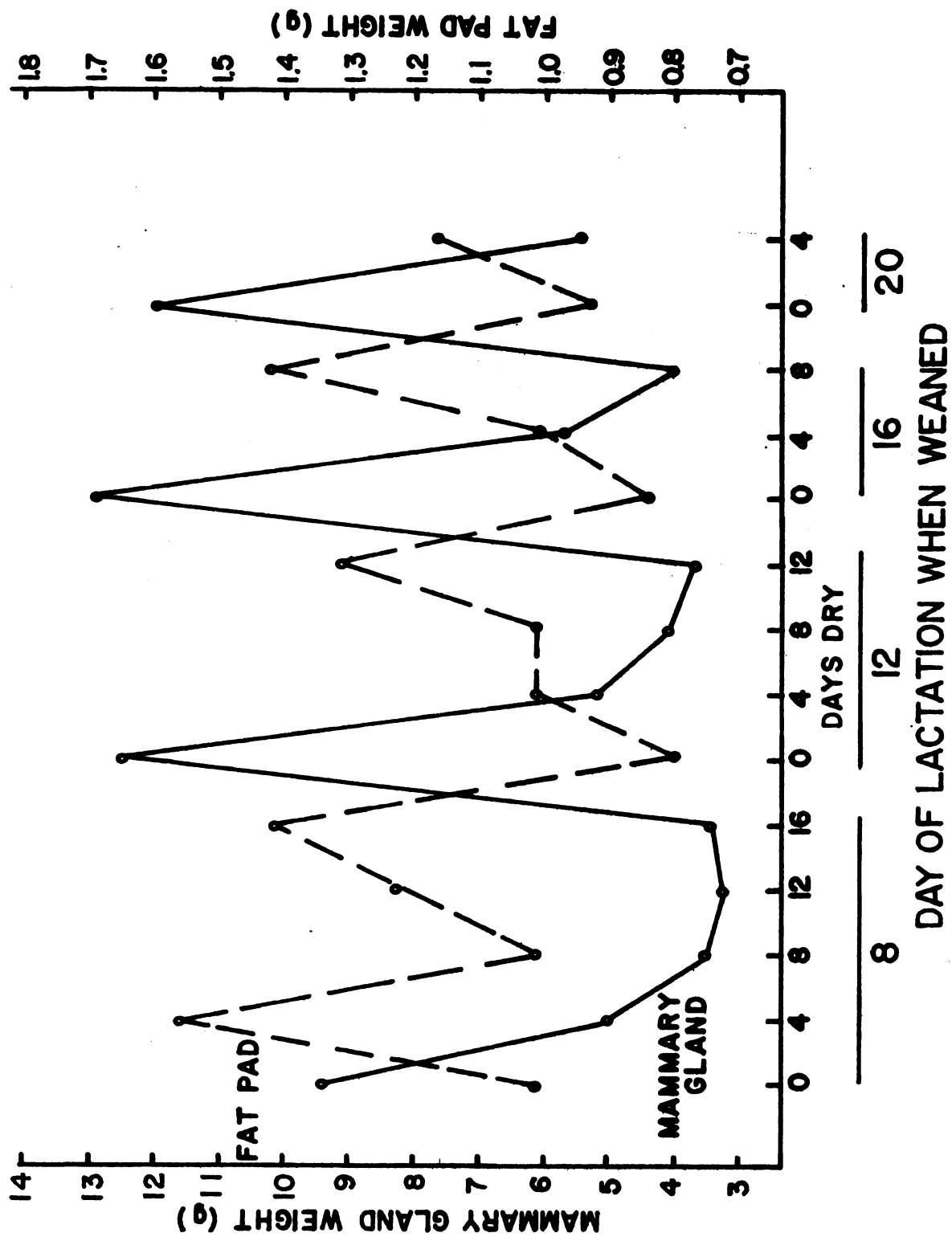

MAMMARY GLAND WEIGHT (g)
FAT PAD WEIGHT (g)
FAT PAD
MAMMARY GLAND
DAYS DRY
DAY OF LACTATION WHEN WEANED
8
12
16
20

TABLE 17.--Influence of pregnancy on DNA content of the mammary gland during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Total DNA (mg)	
		Pregnant[c]	Non-pregnant
8	4	15.4±0.7	12.1±0.8
	8	15.6±1.7	9.0±0.8
	12	17.9±0.8	7.8±0.5
	16 (parturition)	19.9±0.9	5.6±0.4
12	4	23.0±1.3	13.5±0.8
	8	18.4±1.3	10.0±0.7
	12 (parturition)	19.5±0.7	9.2±0.4
16	4	23.5±1.1	13.9±1.4
	8 (parturition)	22.5±1.1	10.4±0.6
20	4 (parturition)	19.7±1.3	14.2±0.9
	Overall mean	19.5[b]	10.6

[a]Mean and standard error of mean.

[b]Significantly greater ($P<0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

non-pregnant animals on the 4th day of the dry period following any of the weaning times. This suggests that the hormones before the 12th day of pregnancy were not being secreted in quantities large enough to retard the fall in DNA. This relatively constant per cent decrease in total mammary gland DNA 4 days after weaning lactating, non-pregnant animals on the 8th, 12th, 16th and 20th day, make it apparent that approximately the same per cent decline in total cells will occur regardless of what stage of lactation the non-pregnant animals are in when weaned. Also, Tucker and Reece (1963d) reported that total DNA decreased 70 per cent 12 days after weaning non-pregnant rats on the 20th day of lactation. We observed that total DNA decreased 74 and 72 per cent 12 days after weaning non-pregnant rats on the 8th and 12th days, respectively, of lactation. Our results on the 4th day after weaning LNP animals on the 20th day of lactation also agree with the results of Griffith and Turner (1961b) and Anderson and Turner (1963). They reported 42.0 and 53.4 per cent decreases, respectively, in total DNA 5 days after weaning on the 20th day of lactation.

Total DNA of the mammary gland of pregnant animals increased progressively a total of 21.7 per cent during the 16-day dry period after weaning on day 8 of lactation. However, when weaned on day 12, pregnancy failed to stimulate increases in cell number until between 8 days of involution and the end of the dry period when a 5.4 per cent increase

in DNA was observed. Similarly, in the 16th day weaning group a decline of 4.1 per cent in mammary DNA was observed between the 4th and 8th day of the dry period. Total DNA of non-pregnant animals from the 4th day of the dry period to the end of the dry period, decreased 53.4, 31.4 and 25.7 per cent, for the 8-, 12- and 16-day weaning groups, respectively. These results indicate that the hormonal condition after the 12th day of concurrent pregnancy was not only sufficient to retard mammary involution during the dry period but to stimulate net mammary cell mitosis during the 16- and 12-day dry periods.

The influence of pregnancy on total DNA of the extra-parenchymal fat pad during various length dry periods is summarized in Table 18. Total DNA of the fat pad of pregnant animals (0.76 mg) was not different ($P \simeq 0.75$) from non-pregnant controls (0.79 mg). On the 4th day of the dry period, following weaning at the 8th, 12th, 16th and 20th day total DNA of the fat pad of pregnant animals decreased 10.6, increased 10.2, 0.3, and decreased 13.7 per cent, respectively. Total DNA of the fat pad of non-pregnant animals during these same times increased 37.8, 32.0, 25.9 and 21.3 per cent, respectively. In general, the changes in fat pad DNA for pregnant and non-pregnant animals paralleled the changes in fat pad weight (Table 16). This same relationship also existed between mammary weight and DNA. Since cell growth in the mammary glands of pregnant

TABLE 18.--Influence of pregnancy on DNA content of the extraparenchymal fat pad during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Total DNA[a] (mg)	
		Pregnant[b]	Non-pregnant
8	4	0.55±0.04	0.89±0.05
	8	0.81±0.08	0.65±0.07
	12	0.78±0.06	0.63±0.10
	16 (parturition)	0.76±0.06	1.14±0.21
12	4	0.71±0.07	0.65±0.07
	8	1.27±0.39	0.82±0.09
	12 (parturition)	0.74±0.08	0.70±0.08
16	4	0.59±0.06	0.73±0.09
	8 (parturition)	0.75±0.09	0.91±0.10
20	4 (parturition)	0.58±0.06	0.74±0.05
	Overall mean	0.76	0.79

[a]Mean and standard error of mean.

[b]Day 1 of pregnancy = day 1 of lactation.

animals would be expected to be primarily epithelial cell growth and connective tissue cell growth (fibroblasts) in the fat pad, it appears that the factors regulating growth are the same for both types of cells.

RNA and RNA/DNA Ratio of the Mammary Gland

Total RNA of the mammary gland of pregnant animals averaged 49.1 mg during the various dry periods, and was greater ($P < 0.01$) than the 18.7 mg average found in non-pregnant controls (Table 19). The interaction between day of the dry period and pregnancy state was also significant ($P < 0.01$). On the 4th day of the dry period of rats weaned at the 8th, 12th, 16th and 20th day of lactation, total RNA of pregnant animals decreased 76.5, 60.2, 71.2 and 66.7 per cent, respectively, from the 0-day (Table 5) levels. Total RNA for non-pregnant animals decreased 82.9, 87.1, 87.0 and 85.3 per cent, respectively. This striking decrease in total RNA reflects the decreased protein synthesis associated with involution. Like DNA, the relatively constant per cent decreases in total RNA 4 days after weaning the non-pregnant animals at all stages of lactation suggests that the same per cent loss in total metabolic activity of the mammary gland will occur regardless of when the non-pregnant animals were weaned. Also, Tucker and Reece (1963d) reported that mammary RNA decreased 94.4 per cent 12 days after weaning non-pregnant rats on the 20th day of lactation. We observed

TABLE 19.--Influence of pregnancy on RNA content of the mammary gland during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Body weights (g)	
		Pregnant[c]	Non-pregnant
8	4	31.5±2.4	22.0±2.2
	8	28.0±4.8	13.3±1.2
	12	35.5±2.1	10.8±0.6
	16 (parturition)	61.1±4.2	12.2±2.2
12	4	69.1±5.3	24.4±1.4
	8	37.7±2.6	15.0±2.2
	12 (parturition)	53.0±4.0	15.3±1.4
16	4	61.4±4.5	29.8±3.4
	8 (parturition)	61.0±4.4	16.5±1.1
20	4 (parturition)	53.0±4.0	27.2±2.6
	Overall mean	49.1[b]	18.7

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

that total RNA decreased 91.6 and 92.0 per cent 12 days after weaning non-pregnant rats on the 8th and 12th days, respectively, of lactation.

Total RNA of the mammary gland of pregnant animals weaned on the 8th day of lactation increased 54.1 per cent from the 4th to the 16th day. Those pregnant animals weaned on the 12th day, however, did not reach minimal RNA values until the 8th day of the dry period (37.7 mg) and then increased 29.0 per cent to the 12th day dry. Most of the increases in RNA of those rats weaned at the 8th or 12th day were interpreted as probably being associated with initiation of the second lactation rather than being caused by advancing pregnancy. Rats weaned on the 16th day declined only 0.7 per cent between the 4th and 8th day of the dry period. Total RNA of non-pregnant animals between the 4th and last day of the dry period decreased 44.3, 37.2, and 44.7 per cent, respectively.

The influence of pregnancy on RNA/DNA ratios of the mammary gland during various length dry periods is shown in Table 20. The overall RNA/DNA ratio of pregnant animals (2.5) during the dry period was higher ($P < 0.01$) than non-pregnant controls (1.7). The interaction between day of the dry period and pregnancy state was also significant ($P < 0.01$). Between the day of weaning on the 8th, 12th, 16th and 20th day of lactation and the 4th day of the dry period, RNA/DNA ratios of pregnant animals decreased 53.4, 47.2, 60.3 and

TABLE 20.--Influence of pregnancy on RNA/DNA ratio of the mammary gland during the dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	RNA/DNA[a]	
		Pregnant[c]	Non-pregnant
8	4	2.0±0.1	1.8±0.1
	8	1.7±0.1	1.5±0.1
	12	2.0±0.1	1.4±0.1
	16 (parturition)	3.1±0.2	2.1±0.3
12	4	3.0±0.2	1.8±0.0
	8	2.1±0.1	1.4±0.1
	12 (parturition)	2.7±0.1	1.6±0.1
16	4	2.6±0.1	2.1±0.2
	8 (parturition)	2.7±0.1	1.6±0.1
20	4 (parturition)	2.7±0.1	1.9±0.1
	Overall mean	2.5[b]	1.7

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

49.2 per cent, respectively; and 57.8, 68.7, 69.5 and 69.5 per cent, respectively, for non-pregnant animals. Thus, the ratios for the pregnant and non-pregnant animals decreased to about 1/2 and 2/3, respectively, of what they were during lactation (Table 5). RNA/DNA ratios of the mammary gland increased slowly throughout pregnancy, becoming slightly greater in the later stages of pregnancy. These observations correspond quite well with the histological observations of Hammond (1927), Jeffers (1936) and Speert (1948) that the initiation of milk secretion is a gradual process.

Between the 4th and last day of the dry period the ratios increased 31.0, 12.7 and decreased 25.2 per cent for non-pregnant animals weaned on the 8th, 12th, and 16th day, respectively. The increases in the RNA/DNA ratios for the non-pregnant animal were not expected especially in view of the results of Tucker and Reece (1963d). Those authors weaned rats on the 21st day of lactation and reported that ratios on day 12 and 21 of involution were less than at other stages of involution. However, it has been shown that rats will start their estrous cycles 3 to 5 days after weaning (Toyoda, 1964) and that RNA/DNA of the mammary gland are higher during the estrous and metestrous stages of the cycle (Sinha and Tucker, 1967). It is possible that the increases we observed were due to abnormally large numbers of rats being killed by chance during the estrous and metestrous stages of the cycle.

Ethanol, Chloroform:Methanol, Ether (ECME) Extract of the Mammary Gland and Extraparenchymal Fat Pad

The data showing the influence of pregnancy on ECME extract of the mammary gland during various length dry periods is in Table 21. Total ECME extract of the mammary gland during the dry period in pregnant animals (4.11 g) was greater ($P < 0.01$) than in non-pregnant controls (3.23 g). The interaction between day of the dry period and pregnancy state was also significant ($P < 0.01$). For the first 4 days of the dry period, commencing at the 8th 12th, 16th and 20th day of lactation, total ECME extract of pregnant animals decreased 14.7, 4.6, 2.8, and increased 14.4 per cent, respectively. Total ECME extract for non-pregnant controls during this time decreased 5.0, 27.8, 22.8, and 7.1 per cent, respectively. This loss of lipid 4 days after weaning lactating, non-pregnant animals does not agree with the results of Harkness and Harkness (1956). They found that the amount of lipid present in the mammary gland did not change throughout lactation or 21 days of involution. Whether the loss we observed was due to the loss of intra- or extra-cellular bound lipid (Melcher, 1967) or fat cells *per se* is not known.

From the 4th to the last day of the dry period, for rats weaned on the 8th, 12th, and 16th day of lactation, total ECME extract of the mammary gland of pregnant animals increased 24.3, 10.7, and 4.6 per cent, respectively; for

TABLE 21.--Influence of pregnancy on ECME extract of the mammary gland during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Total ECME extract[a] (g)	
		Pregnant[c]	Non-pregnant
8	4	3.48±0.19	3.60±0.18
	8	3.93±0.33	3.06±0.20
	12	4.34±0.26	2.99±0.23
	16 (parturition)	4.60±0.26	3.35±0.22
12	4	4.16±0.25	3.04±0.11
	8	4.18±0.32	3.43±0.21
	12 (parturition)	4.66±0.22	3.18±0.24
16	4	3.75±0.23	3.08±0.21
	8 (parturition)	3.93±0.31	3.15±0.15
20	4 (parturition)	4.03±0.43	3.38±0.18
	Overall mean	4.11[b]	3.23

[a]Mean and standard error of mean.

[b]Significanlty greater ($P < 0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

non-pregnant rats decreased 6.9, increased 4.4, and 2.2 per cent, respectively. From these results, it may be inferred that during the dry period, a reservoir of mammary gland lipid was being deposited in response to pregnancy which would probably be used as an energy source during the second lactation.

Total ECME extract of the extraparenchymal fat pad (Table 22) of pregnant animals (0.94 g) was not different ($P \simeq 0.75$) from non-pregnant controls (0.92 g). Four days after weaning on the 8th, 12th, 16th, and 20th day of lactation (Table 8) total ECME extract of the fat pad increased 1.9, 15.8, decreased 1.3, and increased 14.4 per cent, respectively. Total ECME extract for non-pregnant animals increased 14.6, 1.7, 19.9 and 35.3 per cent, respectively. From the 4th to the 16th, 4th to the 12th, and 4th to the 8th day of the dry period of rats weaned on the 8th, 12th or 16th day, total ECME extract of the fat pad for pregnant animals increased 35.7, 17.5 and 22.5 per cent, respectively. ECME extract of the fat pad for non-pregnant animals during these same periods increased 39.4, 25.7, and 27.7 per cent, respectively. These changes in ECME extract 4 days after weaning and during the dry periods follow the changes in fat pad weights during these same periods (Table 16). This suggests that lipid mobilization may be responsible for the weight changes observed in the fat pads during these times.

TABLE 22.--Influence of pregnancy on ECME extract of the extraparenchymal fat pad during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Total ECME extract[a] (g)	
		Pregnant[b]	Non-pregnant
8	4	0.68±0.07	0.70±0.11
	8	0.98±0.12	0.76±0.11
	12	1.04±0.10	0.90±0.16
	16 (parturition)	1.05±0.06	1.16±0.11
12	4	0.94±0.11	0.78±0.07
	8	1.25±0.09	0.93±0.12
	12 (parturition)	1.14±0.10	1.05±0.13
16	4	0.69±0.08	0.83±0.09
	8 (parturition)	0.89±0.11	1.15±0.17
20	4 (parturition)	0.79±0.11	0.94±0.06
	Overall mean	0.94	0.92

[a]Mean and standard error of mean.

[b]Day 1 of pregnancy = day 1 of lactation.

Hydroxyproline Content of the Mammary Gland and Extraparenchymal Fat Pad

The effects of pregnancy on total hydroxyproline of the mammary gland during the various dry periods are summarized in Table 23. The overall mean total hydroxyproline content of the mammary gland of pregnant animals (19.14 mg) was greater ($P < 0.01$) than non-pregnant controls (17.11 mg). By the 4th day of the dry period, for rats weaned at 8, 12, 16 and 20 days, total hydroxyproline of pregnant animals decreased 8.0, 14.7, increased 3.8, and decreased 18.4 per cent, respectively, from the 0-day (Table 9) levels. Total hydroxyproline of the mammary gland of non-pregnant animals during this same interval decreased 18.2, 18.7, 0.1 and 1.4 per cent, respectively. Unlike DNA, which showed large 30 and 50 per cent decreases 4 days after weaning lactating, pregnant and lactating, non-pregnant animals, respectively, hydroxyproline showed lower per cent decreases. It is suggested that hydroxyproline within the mammary parenchyma may be more resistant to the process of involution than is cell loss.

Total hydroxyproline of the mammary gland of pregnant animals increased 1.7 per cent from the 8th to the 16th day of the 8-day weaned group, 1.9 per cent from the 8th to the 12th day of the 12-day weaned group, and 1.4 per cent from the 4th to the 8th day of the 16-day weaned group. Total hydroxyproline of non-pregnant animals during these same

TABLE 23.--Influence of pregnancy on hydroxyproline content of the mammary gland during dry periods of 4, 8, 12, and 16 days.

Day of lactation when weaned	Days dry	Total hydroxyproline[a] (mg)	
		Pregnant[c]	Non-pregnant
8	4	20.20±1.15	14.93±0.86
	8	17.56±1.23	15.95±1.09
	12	17.68±1.23	15.10±1.05
	16 (parturition)	17.86±1.43	13.79±1.48
12	4	19.96±1.66	18.93±1.28
	8	17.79±1.46	19.11±1.97
	12 (parturition)	18.13±1.01	16.82±1.34
16	4	21.25±1.39	19.77±1.98
	8 (parturition)	21.55±1.83	16.80±1.48
20	4 (parturition)	19.44±1.05	19.89±0.81
	Overall mean	19.14[b]	17.11

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

periods decreased 13.5, 11.9, and 15.0 per cent, respectively. Unlike total DNA of the mammary gland, whose loss was retarded by the 12th day of pregnancy and then increased after the 12th day of pregnancy, hydroxyproline losses were not effectively retarded until the 16th day of pregnancy. Since estrogen stimulates the fibroblast to synthesize collagen (Asboe-Hansen, 1958) it is possible that the increase in connective tissue collagen was caused by estrogen, which is thought to be secreted in greater amounts during late pregnancy. The losses in hydroxyproline from the mammary gland following weaning do not agree with the results of Harkness and Harkness (1956). These authors reported that the hydroxyproline content of the mammary gland did not change from lactation through 21 days of involution.

Total hydroxyproline of the fat pad (Table 24) of pregnant animals (0.83 mg) was not different ($P \simeq 0.60$) from non-pregnant controls (0.85 mg). Relative to the day of weaning at the 8th, 12th, 16th and 20th day of lactation (Table 11) on the 4th day of the dry period hydroxyproline of the fat pad of pregnant animals increased 5.6, decreased 9.9, 10.5, and increased 3.5 per cent, respectively. Hydroxyproline of the fat pad of non-pregnant animals increased 23.0, 18.9, 14.7, and 23.2 per cent, respectively. These changes in hydroxyproline for pregnant and non-pregnant animals parallel the changes in DNA (Figures 7 and 8). However, increases in total hydroxyproline of the fat pad after

TABLE 24.--Influence of pregnancy on hydroxyproline content of the extraparenchymal fat pad during dry periods of 4, 8, 12 and 16 days.

Days of lactation when weaned	Days dry	Total hydroxyproline[a] (mg)	
		Pregnant[b]	Non-pregnant
8	4	0.71±0.05	0.87±0.10
	8	0.72±0.07	0.60±0.09
	12	0.97±0.13	0.81±0.10
	16 (parturition)	0.89±0.09	1.24±0.15
12	4	0.73±0.12	0.74±0.08
	8	0.85±0.09	0.93±0.11
	12 (parturition)	1.14±0.12	0.76±0.08
16	4	0.68±0.07	0.75±0.10
	8 (parturition	0.80±0.07	0.88±0.14
20	4 (parturition)	0.85±0.12	0.95±0.09
	Overall mean	0.83	0.85

[a]Mean and standard error of mean.

[b]Day 1 of pregnancy = day 1 of lactation.

Figure 7.--Relation between DNA and hydroxyproline of the fat pad for pregnant animals during various stages of lactation and involution.

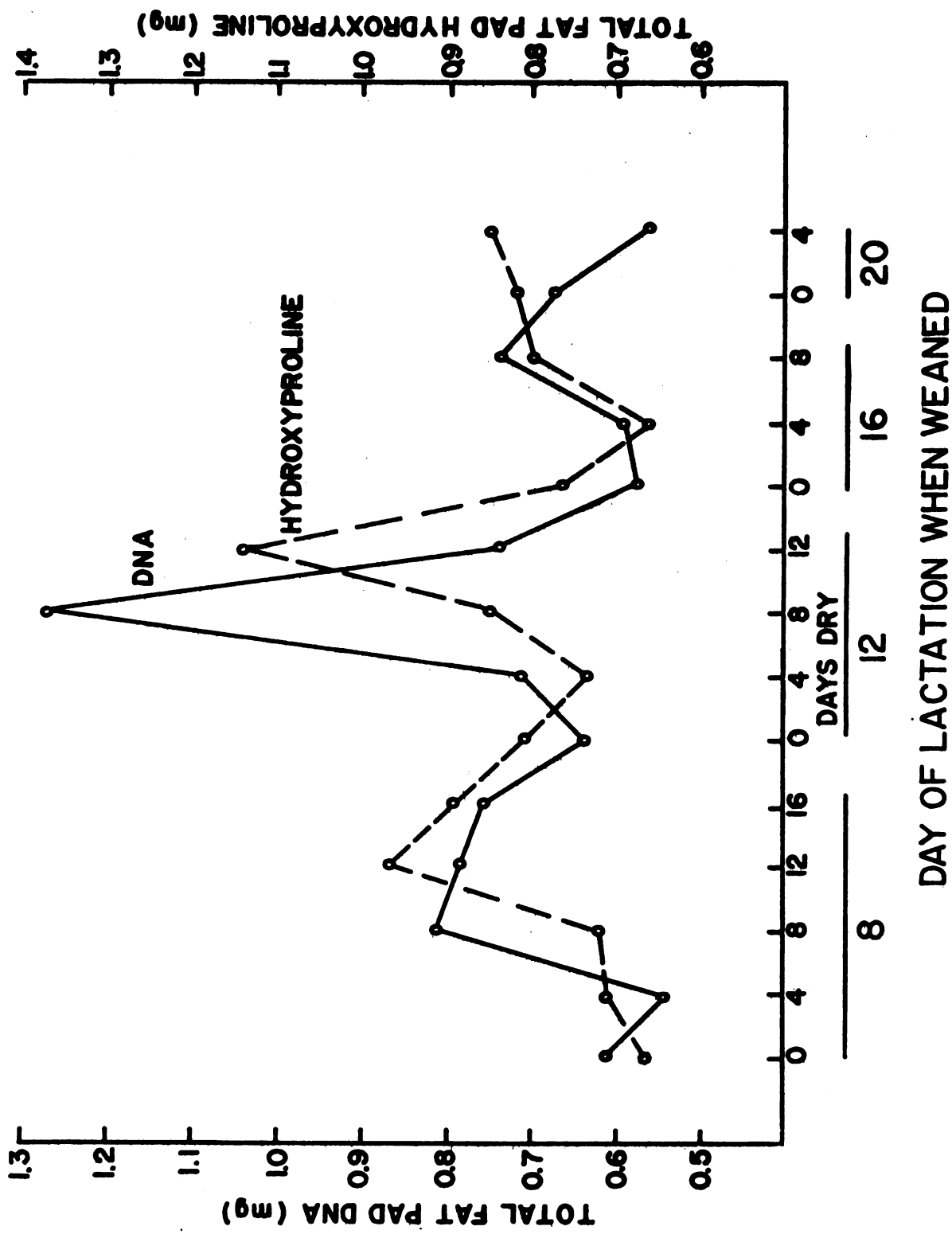
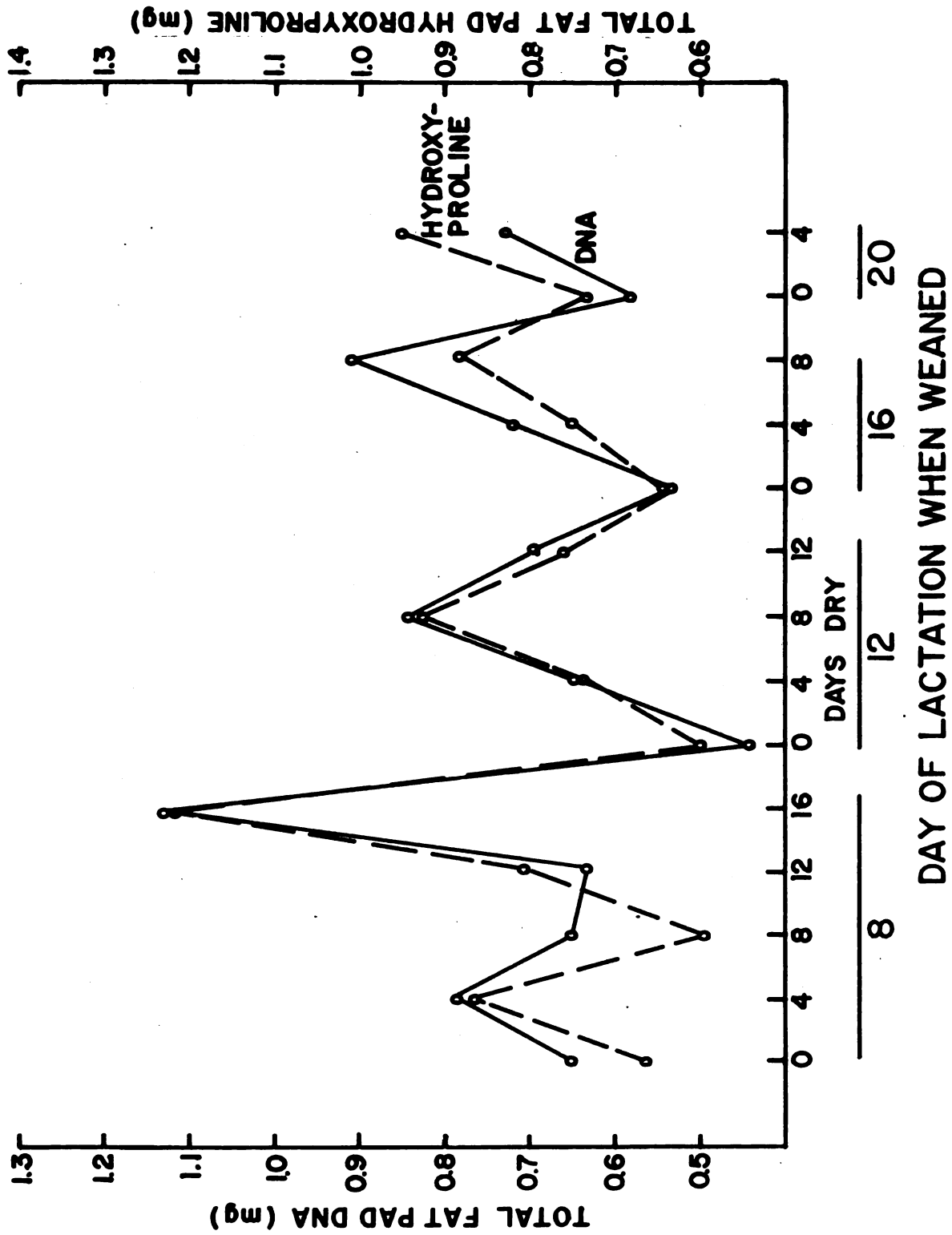

TOTAL FAT PAD DNA (mg)
TOTAL FAT PAD HYDROXYPROLINE (mg)
DNA
HYDROXYPROLINE
DAYS DRY
8
12
16
20
DAY OF LACTATION WHEN WEANED

Figure 8.--Relation between DNA and hydroxyproline of the fat pad for non-pregnant animals during various stages of lactation and involution.


TOTAL FAT PAD DNA (mg)
TOTAL FAT PAD HYDROXYPROLINE (mg)
HYDROXY-PROLINE
DNA
DAYS DRY
DAY OF LACTATION WHEN WEANED

weaning lactating, pregnant animals showed a 4-day lag period in relation to DNA. This suggests that the hormones secreted during late pregnancy may stimulate the fibroblasts to synthesize collagen 4 to 8 days following weaning, whereas the hormones secreted during the estrous cycle of the non-pregnant animals stimulate the fibroblasts to synthesize collagen 4 days after weaning. Thus, it appears that growth of the mammary gland produced by pregnancy during a dry period consists of two stages. First, a proliferation of epithelial and connective tissue cells occurs after the 12th day of pregnancy and second, a stimulation of the connective tissue fibroblasts by estrogen to secrete collagen occurs after the 16th day of pregnancy. Since the increases in hydroxyproline of the fat pad for both lactating, pregnant and lactating, non-pregnant animals has been attributed to recent synthesis by the fibroblasts, indicates that the regressing mammary gland did not leave extra collagen behind in the fat pad.

Hexosamine Content of the Mammary Gland

The hexosamine content of the mammary gland of pregnant animals totaled an average of 4.47 mg, which was greater ($P < 0.01$) than the average of 2.71 mg for non-pregnant controls (Table 25). On the 4th day of the dry period, after weaning on the 8th, 12th, 16th, and 20th day of lactation (Table 12), total hexosamine of the mammary

TABLE 25.--Influence of pregnancy on hexosamine content of the mammary gland during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Total hexosamine[a] (mg)	
		Pregnant[c]	Non-pregnant
8	4	4.20±0.58	3.35±0.29
	8	3.10±0.66	2.07±0.18
	12	2.88±0.28	1.82±0.18
	16 (parturition)	4.02±0.21	1.74±0.20
12	4	7.39±0.64	3.74±0.35
	8	3.24±0.23	2.60±0.31
	12 (parturition)	4.43±0.39	1.88±0.17
16	4	6.31±0.75	4.43±0.32
	8 (parturition)	4.63±0.29	2.13±0.14
20	4 (parturition)	4.54±0.45	3.33±0.33
	Overall mean	4.47[b]	2.71

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.01$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

gland for pregnant animals decreased 24.5, increased 4.7, decreased 8.8 and 16.5 per cent, respectively. Total hexosamine for non-pregnant animals decreased 30.5, 39.0, 36.7 and 44.3 per cent, respectively. Total hexosamine during each of the weaning groups for both pregnant and non-pregnant animals, continued to decrease until 4 days before the end of each dry period. At this time, hexosamine of the mammary gland for the pregnant animals showed a tendency to increase but for the non-pregnant animals it continued to decline. Pregnancy effectively reduced the loss of hexosamine after the 12th day of pregnancy but did not promote net increases until the 20th day of pregnancy.

Weight of the Adrenal Gland

Weight of the adrenal gland, as listed in Table 26, of pregnant animals (55.7 mg) was heavier ($P < 0.05$) than non-pregnant controls (52.5 mg). Four days after weaning on the 8th, 12th, 16th and 20th day of lactation the adrenal weight for pregnant animals decreased 10.4, increased 2.0, 5.6 and 2.0 per cent, respectively, and increased 3.4, 1.2, 8.4 and decreased 6.6 per cent, respectively, for non-pregnant animals. Pregnancy did not influence weight of the adrenal gland during the first 4 days following weaning. Towards the end of the dry period, however, weights of the adrenal of pregnant animals tended

TABLE 26.--Influence of pregnancy on weight of the adrenal gland during dry periods of 4, 8, 12 and 16 days.

Day of lactation when weaned	Days dry	Adrenal gland weight[a] (mg)	
		Pregnant[c]	Non-pregnant
8	4	47.0±1.6	49.4±1.3
	8	54.5±2.1	53.1±2.2
	12	57.2±3.1	51.5±1.5
	16 (parturition)	53.6±2.3	51.5±1.5
12	4	53.8±2.3	50.0±2.1
	8	55.5±1.7	54.1±2.3
	12 (parturition)	58.0±3.1	54.7±1.8
16	4	57.9±2.3	54.6±2.0
	8 (parturition)	59.2±2.0	55.1±2.2
20	4 (parturition)	60.7±2.5	51.3±1.9
	Overall mean	55.7[b]	52.5

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.05$) than non-pregnant controls.

[c]Day 1 of pregnancy = day 1 of lactation.

to increase. This may indicate that the adrenal may be responding to the stress of parturition or to the initiation of milk secretion at the start of the second lactation.

Influence of Length of the Dry Period on Subsequent Lactation

Body Weight

Initial body weights on the 3rd day of the first lactation were not different ($P < 0.50$) among the various treatment groups. Thus, at the start of this experiment, bias attributable to body weight difference was largely eliminated. Body weights on the first day (254 g) of the second lactation (Table 27) were less ($P < 0.01$) than body weights on the 8th (265 g) or 16th (277 g) days. Average body weight on the 8th day was also less ($P < 0.025$) than average body weight on the 16th day. On the 1st day of the second lactation, a linear increase ($P < 0.05$) in body weight with increasing dry period length was observed. On the average, rats which received the 16-day dry period weighed 11.7 per cent more on the 1st day than rats which received no dry period during their first lactation. On the 8th and 16 day of lactation length of the dry period did not influence body weight ($P < 0.05$). Results suggest that the role of nutrition, as affected by the dry period may be important on the 1st day of the second lactation but that this role of nutrition diminishes as lactation progresses. This is in contrast to results obtained in the

cow (Swanson, 1965). He reported that cows milked continuously throughout the first lactation remained 20 to 40 pounds heavier throughout the second lactation than cows which received 60-day dry periods.

TABLE 27.--Relation between length of the dry period and body weight during the second lactation.

Preceding dry period (days)	Body weight[a] (g)		
	Second lactation		
	1st day[d]	8th day	16th day
16	266±7	266±6	282 ±9
12	257±7	268±8	284 ±8
8	263±7	259±7	278±11
4	249±6	272±8	281±10
0	235±5	259±5	261 ±7
Overall mean	254[b]	265[c]	277

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significantly less ($P < 0.025$) than 16th day.

[d]Significant linear increase ($P < 0.05$) among dry periods.

Weight of the Mammary Gland and Extraparenchymal Fat Pad

The relation between length of the dry period and total weight of the mammary gland during the following lactation is shown in Table 28. Mammary weight on the 1st day (6.81 g) was less ($P < 0.01$) than the weight on the 8th (11.38 g) or 16th (13.43 g) day. Weights of the mammary gland on the 8th and 16th days of the second lactation were 17.5 and 3.6 per cent heavier, respectively, than weights of lactating, non-pregnant animals at the 8th (9.39 g) and 16th day (12.94 g) of the first lactation, respectively, and 14.5 and 11.1 per cent heavier, than lactating, pregnant animals at the 8th (9.73 g) and 16th day (11.96 g) of the first lactation, respectively. Thus, like the cow (Smith, 1959), the mammary gland of the rat increases in size with recurring lactations. Length of the dry period produced no effects ($P > 0.05$) in mammary weights on the 1st, 8th, or 16th day of the second lactation. The data suggest that length of the previous dry period plays a minor role in subsequent weight changes of the mammary gland.

The relation between length of the dry period and total weight of the extraparenchymal fat pad is shown in Table 29. Weight of the extraparenchymal fat pad was heavier ($P < 0.05$) on the 1st day (1.18 g) than on the 8th (1.06 g) or 16th (1.03 g) day of the second lactation.

These fat pad weights on the 8th and 16th days of the second lactation were 0.2 and 19.6 per cent heavier, respectively, than fat pad weights from lactating, non-pregnant animals at the 8th (1.04 g) and 16th day (0.83 g) of the 1st lactation, respectively, and 19.8 and 3.9 per cent heavier, than lactating, pregnant animals at the 8th (0.85 g) and 16th day (0.83 g) of the 1st lactation, respectively. The interaction between length of the dry period and day of lactation was also significant ($P < 0.05$). On the 1st day

TABLE 28.--Relation between length of the dry period and weight of the mammary gland during the following lactation.

Preceding dry period (days)	Mammary gland weight[a] (g)		
	Second lactation		
	1st day	8th day	16th day
16	7.21±0.41	11.34±0.47	13.79±1.33
12	7.21±0.41	11.35±0.85	13.89±0.60
8	7.04±0.38	11.37±0.37	13.64±0.82
4	6.60±0.48	11.57±0.65	13.78±0.79
0	6.01±0.36	11.28±0.63	12.07±0.63
Overall mean	6.81[b]	11.38[c]	13.43

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significantly less ($P < 0.01$) than 16th day.

there was a linear increase ($P < 0.01$) in fat pad weight with increasing length of the dry period. The significantly lower weight of the fat pad on the 1st day for the 0- and 4-day dry periods did not inhibit the mammary gland from growing (Table 28) at the subsequent 8th and 16th day of lactation. No trends ($P > 0.05$) in fat pad weight due to dry periods could be detected on the 8th or 16th day. This suggests that the effect of dry period length on weight of the fat pad is lost by the 8th day of the second lactation.

TABLE 29.--Relation between length of the dry period and weight of the extraparenchymal fat pad during the second lactation.

Preceding dry period (days)	Fat pad weight[a] (g)		
	Second lactation		
	1st day[c]	8th day	16th day
16	1.28±0.10	1.16±0.13	1.16±0.11
12	1.59±0.14	0.97±0.12	1.06±0.15
8	1.25±0.11	1.01±0.09	0.99±0.10
4	0.92±0.16	1.12±0.13	0.97±0.11
0	0.86±0.13	1.04±0.11	0.99±0.09
Overall mean	1.18[b]	1.06	1.03

[a]Mean and standard error of mean.

[b]Significantly greater ($P < 0.05$) than 8th and 16th day.

[c]Significant linear increase ($P < 0.01$) with increasing length of the dry period.

Total Litter Weight Gain

The relation between length of the dry period and total litter weight gain (milk production) is shown in Table 30. The total gain of the litter from the 8th to the 16th day of the first lactation for lactating, non-pregnant (81.3 g) and lactating, pregnant (84.6 g) animals was 10.4 and 10.3 per cent less, respectively, than litter weight gain from the 8th to the 16th day of the second lactation. Thus, milk production in the rat

TABLE 30.--Total litter weight gain from the 3rd to the 8th and 8th to the 16th day of the second lactation after various length dry periods.

Preceding dry period (days)	Total litter weight gain[a] (g)	
	Second lactation	
	3rd-8th day	8th-16th day[c]
16	46.7±3.8	97.1±4.7
12	49.8±2.9	94.9±3.3
8	52.2±1.9	93.8±4.4
4	44.0±4.2	104.7±6.4
0	43.9±3.2	81.7±5.3
Overall mean	47.3[b]	94.4

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th to 16th day weight.

[c]Significant cubic regression ($P < 0.01$) among dry periods.

continues to increase like it does for the cow (Lush and Shrode, 1950; and Smith, 1959) during subsequent lactations. It has also been reported (Wrenn, et al., 1966) that rats stimulated by two pseudo-pregnancies showed a 15 per cent increase in litter weight during the subsequent lactation.

Length of the dry period had no effect on litter weight gain between the 3rd and 8th day of the second lactation ($P > 0.05$). Between the 8th and 16th day, however, those animals given a 4-day dry period produced approximately 22.0 and 9.3 per cent heavier litters than animals given a 0-day or longer (8-, 12-, 16-day) dry periods, respectively. These data indicate that a 4-day dry period is optimum for maximal litter weight gains for the rat, and in general agree with results obtained in the cow (Johansson, 1962). That is, the lactation yield increased with increasing length of the dry period up to a certain point, but a further increase in length of the dry period had no beneficial effect on the yield of milk in the following lactation. This beneficial effect of a 4-day dry period was not anticipated because dry periods had no effect on weight of the mammary gland on the 1st, 8th, or 16th day of lactation. This could mean that mammary glands from rats which received a 4-day dry period contained more secretory tissue than mammary glands from rats which received the 0-, 8-, 12, or 16-day dry periods.

Nucleic Acid Content of the Mammary Gland and Extra-parenchymal Fat Pad

The relation between length of the dry period and total DNA of the mammary gland during the following lactation is summarized in Table 31. Total DNA on the 1st day (20.8 mg) was less ($P < 0.01$) than total DNA on the 8th (35.4 mg) and 16th day (37.1 mg). There was no difference ($P \simeq 0.80$) between the 8th and 16th day. The interaction between dry period and day of lactation was also significant ($P < 0.05$). DNA of the mammary gland during the 8th and 16th day of the second lactation was 15.1 and 12.1 per cent higher, respectively, than DNA of lactating, non-pregnant animals on the 8th (30.0 mg) and 16th day (32.6 mg), respectively; and 14.7 and 12.2 per cent higher, than lactating, pregnant animals on the 8th (30.2 mg) and 16th day (32.6 mg), respectively, of the first lactation. These results, although not strictly applicable, agree with observations by Wada and Turner (1959), that recurring pregnancies without nursing periods will increase DNA in the mammary gland.

On the 1st day of the second lactation, those animals given an 8- or 0-day dry period contained approximately 13.3 per cent more DNA ($P < 0.05$) than rats given 16-, 12-, or 4-day dry periods. Greater levels of DNA were expected for rats receiving no dry period because these rats were continually nursing a litter, and suckling could be

maintaining the DNA of the mammary gland (Tucker and Reece, 1963). On the 8th and 16th day of the second lactation, significant quadratic regression curves ($P < 0.01$) in response to the various dry periods were obtained (Table 31). On the 8th day, mammary glands of rats which received 0- or 16-day dry periods contained 14.8 and 13.2 per cent less DNA respectively, than rats which

TABLE 31.--Relation between length of the dry period and DNA content of the mammary gland during the second lactation.

Preceding dry period (days)	Total DNA[a] (mg)		
	1st day[c]	8th day[d]	16th day[e]
16	19.9±0.9	33.4±1.6	35.5±2.1
12	19.5±0.7	36.0±1.2	37.7±1.9
8	22.5±1.1	38.5±0.7	38.3±1.6
4	19.7±1.3	36.4±1.6	40.3±2.4
0	22.5±1.2	32.8±1.3	33.5±1.4
Overall mean	20.8[b]	35.4	37.1

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significant cubic regression ($P < 0.05$) among dry periods.

[d]Significant quadratic regression ($P < 0.01$) among dry periods.

[e]Significant quadratic regression ($P < 0.01$) among dry periods.

received an 8-day dry period. By the 16th day of lactation mammary glands of rats which received 0- or 16-day dry periods contained 16.8 and 11.8 per cent less DNA, respectively, than rats which received a 4-day dry period. Although length of the dry period had no effect on weight of the mammary gland within a particular day of lactation (Table 28), it did have an effect on the number of cells in the gland. This suggests that factors other than cell number are accounting for this equality in weight of the mammary gland. The reason for the low number of mammary cells on the 8th and 16th day of the second lactation for rats which received no dry period could be attributed to the involution of the old cells of the first lactation. These cells were prevented from involuting during the first lactation because of the continuous suckling stimulus, and when these cells were carried into the second lactation, they may have been lost during the first 8 days and not replaced later in lactation by new cells. This confirms the observations by Altman (1947) that a dry period was necessary to allow rapid regeneration of the secretory epithelium before the next lactation. For those rats which received 16-day dry periods (weaned on the 8th day of pregnancy) the opposite situation existed. Since pregnancy was not effective in retarding cell loss until the 12th day of pregnancy (Table 18), the mammary glands which received the 16-day dry period involuted more than mammary glands

which received 4-, 8-, or 12-day dry periods. Therefore, these animals had to grow more cells during the dry period and went into the second lactation with proportionally more new cells than the other dry period groups. It is possible that this additional new cell growth during the 16-day dry period expended some of the energy and co-factors required for normal cell growth that should have occurred during the second lactation.

If we consider the 4- to 8-day dry period as optimum for the rat, then our results on the 1st day of the second lactation agree with results published for the cow (Pardue and Swanson, 1963). That is, on the 1st day of the second lactation, total DNA of the mammary gland for cows given a 2-month dry period in their first lactation, did not differ from total DNA of cows given very short dry periods. If the cow also behaves like the rat during the latter stages of lactation differences in DNA between the two groups of cows would have been noted at the later stages of lactation. Thus, the stage of lactation chosen for this type of comparison is important and the relation one observes on the 1st day of a subsequent lactation will not necessarily be true later in lactation.

The relation between length of the dry period and total DNA of the extraparenchymal fat pad during the following lactation is shown in Table 32. Total DNA of the fat pad on the 1st day (0.69 mg) was not different ($P \simeq 0.90$)

from the 8th (0.70 mg) and the 16th day (0.65 mg). Total DNA of the fat pad during the 8th and 16th day of the second lactation was 21.4 and 16.9 per cent higher, respectively, than fat pad DNA of lactating, non-pregnant animals on the 8th (0.55 mg) and 16th day (0.54 mg), respectively, and 12.9 and 12.3 per cent higher than lactating, pregnant animals on the 8th (0.61 mg) and 16th day (0.57 mg), respectively, of the first lactation. No response curves ($P < 0.05$) were obtained due to length of the dry period on the 1st, 8th or 16th day of the second lactation. The data suggest that factors which regulate mammary epithelial cell numbers during a second lactation may not be regulating fat pad connective

TABLE 32.--Relation between length of the dry period and DNA content of the extraparenchymal fat pad during the second lactation.

Preceding dry period (days)	Fat pad weight[a] (g)		
	Second lactation		
	1st day	8th day	16th day
0	0.62±0.04	0.82±0.09	0.63±0.03
4	0.59±0.06	0.69±0.05	0.64±0.05
8	0.75±0.07	0.69±0.05	0.64±0.06
12	0.75±0.08	0.65±0.06	0.67±0.09
16	0.76±0.06	0.67±0.06	0.67±0.06
Overall mean	0.69	0.70	0.65

[a]Mean and standard error of mean.

tissue cell numbers. The constant number of cells on the 1st day of lactation among the various dry periods, does not follow the linear increase in weight of the fat pad with increasing length of the dry period shown to exist at this time (Table 29). Possibly, during the short dry periods, some of the lipid was mobilized out of the fat cells located in the fat pad, leaving behind intact fat cells with low amounts of lipid.

The relation between length of the dry period and total RNA of the mammary gland during the second lactation is shown in Table 33. Total RNA on the 1st day (58.9 mg) was less ($P < 0.01$) than RNA on the 8th (172.6 mg) and 16th day (247.0 mg), and RNA on the 8th day was less ($P < 0.01$) than RNA on the 16th day of lactation. RNA during the 8th and 16th day of the second lactation was 25.4 and 7.0 per cent higher, respectively, than RNA of lactating, non-pregnant animals on the the 8th (128.8 mg) and 16th day (229.8 mg), of the first lactation, respectively; and 22.2 and 13.8 per cent higher than lactating, pregnant animals on the 8th (134.3 mg) and 16th day (213.0 mg) of the first lactation, respectively. Thus, total protein synthetic activity of the mammary gland increased with each recurring lactation.

The various dry periods had no affect on total mammary gland RNA during the 1st day of the second lactation ($P > 0.05$). However, quadratic response curves for total RNA

were obtained on the 8th ($P < 0.05$) and 16th day ($P < 0.01$) of the second lactation among the various dry period groups. On the 8th day, mammary glands of rats given an 8-day dry period contained approximately 16 per cent more RNA than mammary glands which received 16- and 0-day dry periods. On the 16th day, mammary glands of rats given a 4-day dry period contained 14.3 and 22.1 per cent more RNA than

TABLE 33.--Relation between length of the dry period and RNA content of the mammary gland during the second lactation.

Preceding dry period (days)	Total RNA[a] (mg)		
	Second lactation		
	1st day	8th day[d]	16th day[e]
16	61.1±4.2	159.2±11.1	231.1±20.1
12	53.0±4.0	177.8± 8.7	262.4±16.0
8	61.0±4.4	188.8± 6.0	260.5±11.5
4	53.0±4.0	178.1±12.1	270.5±25.4
0	62.1±6.2	159.1± 9.1	210.7±13.8
Overall mean	58.0[b]	172.6[c]	247.0

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significantly less ($P < 0.01$) than 16th day.

[d]Significant quadratic regression ($P < 0.05$) among dry periods.

[e]Significant quadratic regression ($P < 0.01$) among dry periods.

mammary glands which received 16- and 0-day dry periods, respectively. In view of the DNA results, the trends established within a particular stage of lactation among dry period groups were expected. Since fewer mammary cells were formed in animals which received no dry period or very long dry periods, it is reasonable to assume that mammary RNA would also be reduced in these treatment groups. This was confirmed by the RNA/DNA ratios (Table 34). On the 1st, 8th and 16th day of lactation, length of the dry period

TABLE 34.--Relation between length of the dry period and RNA/DNA ratio of the mammary gland during the second lactation.

Preceding dry period (days)	RNA/DNA ratio[a]		
	Second lactation		
	1st day	8th day	16th day
16	3.1±0.2	4.7±0.2	6.5±0.3
12	2.7±0.1	4.9±0.2	6.9±0.2
8	2.7±0.1	4.9±0.2	6.8±0.2
4	2.7±0.1	4.9±0.2	6.6±0.3
0	2.8±0.2	4.9±0.3	6.3±0.3
Overall mean	2.8[b]	4.9[c]	6.6

[a]Mean and standard error or mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significantly less ($P < 0.01$) than the 16th day.

had no effect ($P > 0.05$) on the RNA/DNA ratios. Thus the various dry periods are not causing reduced synthesis per cell because cells from mammary glands of rats which received the 16- or 0-day dry periods were just as capable of synthesizing protein as were cells which received the 4- or 8-day dry period. These data indicate that the dry period is having its effect on subsequent lactation by causing loss of cells or preventing mitosis during the second lactation rather than loss of synthesis per cell.

RNA/DNA ratios of mammary glands during the 8th (4.9) and 16th (6.6) day of the second lactation were 12.0 per cent more and 5.6 per cent less, respectively, than ratios for non-pregnant animals on the 8th (4.3) and 16th day (7.0), respectively; and 10.0 and 1.4 per cent more, respectively, than ratios for pregnant animals on the 8th (4.4) and 16th (6.5) day, respectively, of the 1st lactation. Thus, recurring lactation did not appreciably alter the synthesizing capability of each cell.

Ethanol, Chloroform: Methanol, Ether (ECME) Extract of the Mammary Gland and Extraparenchymal Fat Pad

The relation between length of the dry period and total ECME extract of the mammary gland is shown in Table 35. Total ECME extract of the mammary gland on the 1st day (4.10 g) was not different ($P \simeq 0.50$) from the ECME extract on the 8th (4.38 g) or 16th day (4.23 g). Total ECME extract of the mammary gland during the 8th and 16th day of

the second lactation was 13.5 and 5.7 per cent higher, respectively, than lactating, non-pregnant animals on the 8th (3.79 g) and 16th day (3.99 g), respectively, of the first lactaiton and 6.8 and 8.7 per cent higher than lactating, pregnant animals on the 8th (4.08 g) and 16th day (3.86 g), respectively, of the first lactation.

Linear increases in ECME extract of the mammary gland with increasing length of the dry period were

TABLE 35.--Relation between length of the dry period and ECME extract of the mammary gland during the second lactation.

Preceding dry period (days)	Total ECME extract[a] (g)		
	Second lactation		
	1st day[b]	8th day	16th day[c]
16	4.60±0.26	4.40±0.39	4.93±0.73
12	4.66±0.22	4.52±0.45	4.08±0.19
8	3.93±0.31	4.05±0.21	4.63±0.32
4	4.03±0.43	4.43±0.26	4.11±0.30
0	3.26±0.26	4.49±0.41	3.38±0.32
Overall mean	4.10	4.38	4.23

[a]Mean and standard error of mean.

[b]Significant linear increase ($P < 0.01$) with increasing length of the dry period.

[c]Significant linear increase ($P < 0.05$) with increasing length of the dry period.

observed on the 1st ($P < 0.01$) and 16th day ($P < 0.05$) of the second lactation. Thus, at the beginning and towards the end of the second lactation, the shorter dry periods caused mobilization of lipid out of the mammary gland, probably to be used as energy sources in response to the initiation and maintenance of lactation.

The relation between length of the dry period and total ECME extract of the extraparenchymal fat pad is shown in Table 36. Total ECME extract on the 1st day (0.90 g)

TABLE 36.--Relation between length of the dry period and ECME extract of the extraparenchymal fat pad during the second lactation.

Preceding dry period (days)	Total ECME extract[a] (g)		
	Second lactation		
	1st day[b]	8th day	16th day
16	1.05±0.06	0.97±0.10	0.83±0.05
12	1.13±0.10	0.73±0.11	0.85±0.03
8	0.89±0.11	0.81±0.08	0.82±0.12
4	0.79±0.11	0.80±0.11	0.83±0.03
0	0.64±0.08	0.93±0.11	0.64±0.05
Overall mean	0.90	0.85	0.79

[a]Mean and standard error of mean.

[b]Significant linear increase ($P < 0.01$) with increasing length of the dry period.

was not different (P ≈ 0.25) from the 8th (0.85 g) and 16th (0.79 g) day. A linear increase (P < 0.01) with increasing length of the dry period was observed on the 1st day of the second lactation. This paralleled the linear increase in weight of the fat pad with increasing length of the dry period (Table 29). However, DNA of the fat pad was the same regardless of dry period length (Table 32). This suggests either a mobilization of lipid from the fat cell, leaving behind a fat cell devoid of lipid, or a mobilization of extracellular bound lipid, without altering the fat cell numbers.

Hydroxyproline Content of the Mammary Gland and Extraparenchymal Fat Pad

The relation between length of the dry period and total hydroxyproline content of the mammary gland during the following lactation is listed in Table 37. Total hydroxyproline on the 1st day (18.60 mg) was less (P < 0.01) than total hydroxyproline on the 8th (22.37 mg) and 16th day (27.37 mg). Total hydroxyproline on the 8th day was less (P < 0.01) than on the 16th day of the second lactation. During the 8th and 16th day of the second lactation total hydroxyproline was 18.4 and 20.6 per cent higher than lactating, non-pregnant animals on the 8th (18.26 mg) and 16th day (21.74 mg) of the first lactation, respectively, and 1.8 and 25.3 per cent higher, than lactating, pregnant animals on the 8th (21.96 mg) and 16th day (20.44 mg) of the first lactation, respectively. Since mammary glands also weighed more in the second lactation, it is reasonable to

expect that these glands should contain more collagen, possibly due to a larger connective tissue framework needed in these larger glands.

On the first day of the second lactation a quadratic regression ($P < 0.05$) among dry periods was obtained.

TABLE 37.--Relation between length of the dry period and hydroxyproline content of the mammary gland during the second lactation.

Preceding dry period (days)	Total Hydroxyproline[a] (mg)		
	Second lactation		
	1st day[d]	8th day[e]	16th day[f]
16	17.86±1.43	23.14±1.34	28.56±3.16
12	18.13±1.01	26.12±1.26	28.11±2.03
8	21.55±1.83	23.63±1.35	32.20±2.08
4	19.44±1.05	20.37±0.78	25.14±1.46
0	16.00±0.92	18.59±1.35	22.84±1.09
Overall mean	18.60[b]	22.37[c]	27.37

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significantly less ($P < 0.01$) than 16th day.

[d]Significant quadratic regression ($P < 0.05$) among dry periods.

[e]Significant linear increase ($P < 0.01$) with increasing length of dry period.

[f]Significant quadratic regression ($P < 0.01$) among dry periods.

Mammary glands of animals which received the 16- and 0-day dry periods contained 17.1 and 25.8 per cent less hydroxyproline, respectively, than animals which received the 8-day dry period. Since collagen is necessary for future mammary development (Lasfarques, 1957), these lower amounts of hydroxyproline produced on the 1st day by those glands which received the 16- and 0-day dry periods, could be one of the reasons why these same glands contained reduced numbers of mammary cells in later lactation (Table 31). The amount of hydroxyproline synthesized per unit of RNA (Table 38) was

TABLE 38.--Relation between length of the dry period and hydroxyproline/RNA ratio of the mammary gland during the second latation.

Preceding dry period (days)	Hydroxyproline/RNA ratio[a]		
	Second lactation		
	1st day	8th day	16th day
16	0.31	0.15	0.12
12	1.33	0.15	0.12
8	0.35	0.13	0.12
4	0.36	0.12	0.10
0	0.26	0.12	0.11
Overall mean	0.32	0.13	0.11

[a]Mean.

highest on the 1st day of the second lactation. Also, on the 1st day the ratios were lowest for those animals which received 16- and 0-day dry periods. Thus it appears that the mammary gland was synthesizing more hydroxyproline per unit of RNA on the 1st day in those rats given the optimal dry periods, in possible preparation for future mammary parenchymal cell growth. The low hydroxyproline/RNA ratio for those animals which received 0-day dry periods, suggests that a greater portion of the RNA was being used to code for milk protein rather than structural protein, since these animals were continually nursing a litter.

Total hydroxyproline content of the extraparenchymal fat pad (Table 39) on the 1st day (0.87 mg) of the second lactation was not different ($P \simeq 0.40$) from the 8th (0.89 mg) and 16th day (0.81 mg). The interaction between length of the dry period and day of lactation was significant ($P < 0.05$). Total hydroxyproline during the 8th and 16th day of the second lactation was 24.7 and 21.0 per cent higher than lactating, non-pregnant animals on the 8th (0.67 mg) and 16th day (0.64 mg), respectively; and 24.7 and 6.2 per cent higher than lactating, pregnant animals on the 8th (0.67 mg) and 16th day (0.76 mg) of the first lactation, respectively. Possibly, the extra hydroxyproline may account for the larger fat pads observed during the second lactation.

On the 1st and 16th day of the second lactation, linear increases ($P < 0.01$) of hydroxyproline content of the fat pad with increasing length of the dry period were obtained. Since these longer dry periods also resulted in heavier fat pads, collagen together with ECME extract is probably contributing to the heavier fat pad weight.

TABLE 39.--Relation between length of the dry period and hydroxyproline content of the extraparenchymal fat pad during the second lactation.

Preceding dry period (days)	Total hydroxyproline[a] (mg)		
	Second lactation		
	1st day[b]	8th day	16th day[c]
16	0.89±0.09	0.86±0.11	0.97±0.10
12	1.14±0.12	0.80±0.09	0.96±0.17
8	0.80±0.07	0.84±0.06	0.77±0.06
4	0.85±0.12	0.89±0.09	0.74±0.08
0	0.67±0.09	1.07±0.16	0.60±0.05
Overall mean	0.87	0.89	0.81

[a]Mean and standard error of mean.

[b]Significant linear increase ($P < 0.01$) with increasing length of the dry period.

[c]Significant linear increase ($P < 0.01$) with increasing length of the dry period.

Hexosamine Content of the Mammary Gland

The relation between length of the dry period and total hexosamine content of the mammary gland during the following lactation is shown in Table 40. Total hexosamine was less ($P < 0.01$) on the 1st day (4.32 mg) than on the 8th (6.39 mg) and 16th day (7.08 mg). Total hexosamine was significantly less ($P < 0.06$) on the 8th

TABLE 40.--Relation between length of the dry period and hexosamine content of the mammary gland during the second lactation.

Preceding dry period (days)	Total hexosamine[a] (mg)		
	Second lactation		
	1st day	8th day	16th day[d]
16	4.02±0.21	6.32±0.55	7.77±1.00
12	4.43±0.39	6.19±0.47	6.98±0.36
8	4.63±0.29	6.92±0.50	7.39±0.42
4	4.54±0.45	6.10±0.37	6.73±0.36
0	4.00±0.50	6.42±0.47	6.54±0.37
Overall mean	4.32[b]	6.39[c]	7.08

[a]Mean and standard error of mean.

[b]Significantly less ($P < 0.01$) than 8th and 16th day.

[c]Significantly less ($P < 0.05$) than 16th day.

[d]Significant linear increase ($P < 0.05$) with increasing length of the dry period.

day than on the 16th day of lactation. Total hexosamine content of the mammary gland during the 8th and 16th day of the second lactation was 24.4 and 1.1 per cent higher, respectively, than lactating, non-pregnant animals on the 1st (12.80 mg) and 16th (7.00 mg) day of the first lactation; and 12.8 and 2.2 per cent higher, than lactating, pregnant animals on the 8th (5.57 mg) and 16th (6.92 mg) day of the first lactation, respectively. During the second lactation, total RNA was also higher than the first lactation, notably so on the 8th day of the second lactation. Since the ground substance surrounds each cell and serves as the pathway between the cell and bloodstream, an increase in the metabolic activity of the cell (RNA) could presumably increase the amount of ground substance (hexosamine).

On the 16th day of the second lactation there was a linear increase ($P < 0.05$) in hexosamine with increasing length of the dry period (Table 40). The reason for this linear increase could be related to the increase in adrenal weight (Table 41) at this time. Both estrogen and mineralocorticoids which are secreted by the adrenal glands have been shown to increase hexosamine levels of a tissue (Dorfman and Schiller, 1961). It is possible that these larger adrenals are producing more hormones, thus increasing total mammary gland hexosamine.

Weight of the Adrenal Gland

The relation between length of the dry period and adrenal gland weight during the following lactation is shown in Table 41. There was no difference ($P \simeq 0.20$) in adrenal gland weight on the 1st, 8th and 16th day of the second lactation. Adrenal glands on the 8th (55.1 mg) and 16th (54.9 mg) day of the second lactation were 13.4 and 8.9 per cent and 4.7 and 0.2 per cent heavier than adrenal glands from lactating, non-pregnant or lactating, pregnant animals on the 8th and 16th day (50.0 mg) of the first lactation, respectively.

Quadratic response curves in adrenal gland weight in response to length of the dry period were obtained on the 1st ($P < 0.01$) and 8th ($P < 0.05$) day of lactation. This adrenal weight change, in response to length of the dry period parallels the DNA and RNA data reported for this experiment. Thus, it appears that size of the adrenal glands may be associated with mammary gland growth and synthetic activity. On the 16th day of lactation, an increase in adrenal weight for those animals which received a 16-day dry period, paralleled the increase in total hexosamine content of the mammary gland. This suggests that hormones elaborated by the adrenal at this time could be responsible for this increase in hexosamine.

TABLE 41.--Relation between length of the dry period and weight of the adrenal gland during the second lactation.

Preceding dry period (days)	Adrenal gland weight[a] (g)		
	Second lactation		
	1st day[b]	8th day[b]	16th day
16	53.6±2.3	54.1±2.2	57.2±1.8
12	58.0±3.1	55.1±1.5	53.2±3.4
8	59.2±2.0	57.1±2.0	56.3±2.1
4	60.7±2.5	58.2±2.2	55.1±2.2
0	53.8±2.5	51.0±1.5	52.7±1.6
Overall mean	57.1	55.1	54.9

[a]Mean and standard error of mean.

[b]Significant quadratic regression ($P < 0.05$) among dry periods.

SUMMARY AND CONCLUSIONS

The influence of pregnancy on lactation and mammary gland development was studied during various stages of lactation and also at various stages following weaning.

Lactating, pregnant (LP) animals weighed less ($P < 0.05$) than corresponding non-pregnant controls only on the 24th day of lactation (246 g versus 254 g). This depression in body weight was attributed to water loss associated with parturition and/or to advancing pregnancy. Following weaning at the 8th, 12th, 16th and 20th day of lactation, body weights of pregnant animals tended to increase whereas those of non-pregnant animals decreased.

Weight of the mammary gland of LP animals (9.85 g) was less ($P < 0.01$) than lactating, non-pregnant (LNP) controls (11.65 g). Weights of the mammary gland for LP and LNP animals decreased 49.0 and 11.0 per cent, respectively, from the 16th to the 24th day of lactation. During the first 4-day interval following weaning on the 8th, 12th, 16th and 20th day of lactation, weights of the mammary gland of LP animals were depressed 43.4, 17.2, 34.7, and 32.8 per cent, respectively. Weights of the gland of LNP animals during these times decreased 46.4-58.0, 56.3, and 54.7 per cent, respectively. During

the dry period, pregnancy did not alter weight of the mammary gland before the 12th day of pregnancy.

Weight of the extraparenchymal fat pad for either LP (0.91 g) or LNP (0.90 g) animals was the same and did not change ($P \simeq 0.75$) between the 8th and 24th day of lactation. Following weaning, fat pads of both pregnant and non-pregnant animals increased in weight. A plot of the relation between weight of the mammary gland and fat pad during various stages of lactation and involution, revealed that the changes for the fat pad were opposite the changes for the mammary gland. This suggests that the mammary gland grew into the fat pad during pregnancy and regressed out of it during involution.

Pregnancy reduced ($P < 0.01$) daily litter weight gain from the 8th to the 24th day of lactation (6.9 g versus 10.2 g). By the 24th day of lactation litters from LP animals were losing 1.7 g a day, whereas litters from LNP animals were gaining 8.4 g a day.

DNA and RNA of the mammary gland for both LP and LNP animals were the same from the 8th to the 20th day of lactation. However, from the 20th to the 24th day, DNA for LP animals decreased 23.3 per cent, but increased 7.9 per cent for LNP controls, whereas RNA decreased 61.6 per cent for LP animals and also increased 7.9 per cent for LNP controls. These response curves were quadratic ($P < 0.01$) and cubic ($P < 0.01$) for LP and LNP groups, respectively.

RNA/DNA ratios were the same for both LP and LNP animals up to the 16th day of lactation, but then decreased 52.5 and 12.4 per cent, respectively, from the 16th to the 24th day.

From these results, it was concluded that pregnancy may be exerting its inhibitory effect on lactation in two ways: (1) between the 20th and 24th day of lactation it may be reducing the amount of synthesis per cell, and (2) on the 24th day it may be causing a further decrease in milk synthesis by reducing the total number of mammary cells present.

During the period of involution (dry period) following weaning on the 8th, 12th, 16th and 20th day of lactation, DNA, RNA, and RNA/DNA ratios of the mammary gland of pregnant animals were greater ($P < 0.01$) than non-pregnant controls. During the dry periods pregnancy retarded the fall in DNA after the 12th day of gestation. On the 8th day of the dry period, following weaning on the 8th and 12th day of lactation, DNA, RNA and RNA/DNA ratios of pregnant animals gradually increased up to the time of parturition.

DNA of the extraparenchymal fat pad of LP animals (0.62 g) was greater ($P < 0.05$) than LNP (0.54 g) controls. From the 8th to the 24th day of lactation, for both LP and LNP animals, a relatively constant percentage of DNA in the fat pad (1.3 to 2.6 per cent) was observed relative to

total mammary DNA (mammary gland DNA plus extraparenchymal fat pad DNA). Following weaning, DNA of the fat pad of pregnant animals tended to decrease whereas DNA of the fat pad of non-pregnant animals tended to increase. These changes in DNA of the fat pad paralleled changes in weight of the fat pad.

Hydroxyproline of the mammary gland for LNP animals increased 21.5 per cent from the 8th to the 12th day, decreased 13.3 per cent from the 12th to the 20th day, and increased 14.8 per cent from the 20th to the 24th day of lactation. Hydroxyproline for LP animals was relatively constant from the 8th to the 20th day of lactation, but decreased 32.8 per cent from the 20th to the 24th day. On the 24th day, hydroxyproline for LP animals was 32.3 per cent less than corresponding LNP controls. Although hydroxyproline, DNA, and RNA of the mammary gland for LNP animals increased 14.8, 7.9, and 7.8 per cent, respectively, from the 20th to the 24th day of lactation, daily litter gain decreased 20.9 per cent. From this, it is possible to speculate that the 7.9 per cent increase in DNA may indicate a proliferation of fibroblasts which could be actively synthesizing collagen, accounting for the increases in RNA and hydroxyproline, but that fewer parenchymal cells were available for milk synthesis, causing the 20.9 per cent decrease in daily litter gain.

From the 8th, 12th, 16th, and 20th day of lactation to the 4th day after weaning, hydroxyproline of the mammary gland for both pregnant and non-pregnant animals showed changes ranging from a 3.8 per cent increase to an 18.7 per cent decrease. This is in contrast to the 30 to 50 per cent decreases in DNA 4 days following weaning for both LP and LNP animals. Thus, hydroxyproline appeared to be more resistant to the process of involution than DNA. Also, unlike DNA whose loss was retarded after the 12th day of pregnancy, hydroxyproline losses were not retarded until the 16th day of pregnancy. This late response of hydroxyproline was attributed to estrogen which, when secreted in greater amounts during late pregnancy, probably stimulated the fibroblasts to synthesize collagen.

Following weaning, hydroxyproline of the extraparenchymal fat pad for both pregnant and non-pregnant animals increased. However, the increases in hydroxyproline after weaning lactating, pregnant animals showed a 4-day lag period in relation to the increases in fat pad DNA.

Hexosamine of the mammary gland for LP and LNP animals increased 19.7 and 31.0 per cent, respectively, from the 8th to the 16th day and decreased 42.6 and 17.7 per cent, respectively, from the 16th to the 24th day. These quadratic curves ($P < 0.01$) indicate that hexosamine, as a measure of the ground substance, was actively synthesized

by the mammary gland at the peak of lactation. Following weaning, hexosamine for both pregnant and non-pregnant animals decreased until 4 days before the end of each dry period but net synthesis of hexosamine occurred by the time of parturition.

From these results it appears that mammary growth following weaning of LP animals consists of the following steps: (1) a proliferation of epithelial and connective tissue cells starting on the 12th day of pregnancy; (2) a stimulation of the connective tissue cells on the 16th day of pregnancy by estrogen to synthesize collagen (hydroxyproline); (3) an increase in cell protein synthetic activity (RNA) starting on the 20th day of pregnancy, in preparation for the initiation of lactation on the 24th day (parturition); (4) an increase in the ground substance (hexosamine) surrounding each cell in response to the increased passage of products between cell and blood supply.

Weight of the adrenal gland (54.0 mg) for LP animals was heavier ($P < 0.07$) than LNP controls (51.4 mg). Linear increases in weight of the adrenal gland were observed from the 8th to the 24th day of lactation for both LP ($P < 0.07$) and LNP ($P < 0.01$) rats. Four days following weaning, no trends could be established for weight of the adrenal gland for either pregnant or non-pregnant animals. Toward the end of the dry periods however, weight of the adrenal

gland for pregnant animals tended to increase. This may indicate that the adrenal gland is responding to the stress of parturition or to the initiation of milk secretion at the start of the second lactation.

In order to determine the influence of various lengths of a dry period on the carryover of mammary gland secretory and connective tissue components into a second lactation, some LP rats were allowed to deliver their second litter and various biochemical parameters of the mammary gland were measured on the 1st, 8th, and 16th day of the second lactation.

From the 3rd to the 8th day of the second lactation, dry period length did not influence total litter weight gain. Between the 8th and 16th day, however, animals given a 4-day dry period produced 22.0 and 9.3 per cent heavier litters than animals given 0-day or longer (8-, 12-, 16-day) dry periods, respectively.

On the 1st day of the second lactation, animals given an 8- or 0-day dry period contained approximately 13.3 per cent more DNA than rats given 16-, 12-, or 4-day dry periods. On the 8th day of the second lactation, mammary glands of rats which received 0- or 16-day dry periods contained 14.8 and 13.2 per cent less DNA, respectively, than rats which received an 8-day dry period. By the 16th day, 0- or 16-day dry period groups contained 16.8 and 11.8 per cent less DNA, respectively, than rats

which received a 4-day dry peirod. From these results, it is evident that the stage of lactation chosen to study the influence of length of the dry period on DNA of the mammary gland is important, and the relationship one observes on the 1st day of lactation will not necessarily be true later in lactation.

The various dry periods had no effect on total mammary gland RNA during the 1st day of the second lactation ($P > 0.05$). However, on the 8th day, mammary glands of rats given an 8-day dry period contained on the average, 15.8 per cent more RNA than glands which received 16- and 0-day dry periods. By the 16th day, 16- and 0-day dry period groups contained 14.3 and 22.1 per cent less RNA than rats given the 4-day dry period. Length of the dry period had no effect ($P > 0.05$) on the RNA/DNA ratios on the 1st, 8th, or 16th day of the second lactation. Thus, the short or long dry periods were not causing reduced synthesis per cell because cells from mammary glands of rats which received the 16- or 0-day dry periods were just as capable of synthesizing protein as cells which received the 4- or 8-day dry period.

Linear increases in ECME extract (crude lipid) of the mammary gland with increasing length of the dry period were observed on the 1st ($P < 0.01$) and 16th day ($P < 0.05$) of the second lactation.

On the 1st, 8th, and 16th day of the second lactation, mammary glands of rats which received the 0- or 16-day dry period contained less hydroxyproline, than rats which received the 4-, 8-, or 12-day dry periods. Since collagen is necessary for future mammary development (Lasfarques, 1957), these lower amounts of hydroxyproline produced on the 1st day, by those glands which received the 16- and 0-day dry periods, could be one of the reasons why these same glands contained reduced numbers of mammary cells in later lactation.

Length of the dry period did not effect hexosamine content of the mammary gland on the 1st or 8th day of the second lactation ($P > 0.05$). However, by the 16th day, a linear increase ($P < 0.05$) with increasing length of the dry period was observed. This linear increase in hexosamine seemed to be related to the linear increase in weight of the adrenal gland. Since both estrogen and mineralcorticoids increase the amounts of hexosamine in a tissue (Dorfman and Schiller, 1961), it is possible that these larger adrenals are producing more hormone, thus increasing mammary hexosamine.

LIST OF REFERENCES

Ackerman, R. A., Thomas, R. O., and Butcher, D. F. 1967. Effects of length of dry period on production. J. Dairy Sci., 50:976.

Altman, A. D. 1945. Izmeneniya v vimeni korov v prosesse razdoya (Udder changes in lactating cows). Vest. Zhivot., 1:85. [cited by Dairy Sci. Abstr., 9: 287. 1947-1948]

Anderson, D. H. and Kennedy, H. S. 1933. Studies on the physiology of reproduction. V. The adrenal cortex in pregnancy and lactation. J. Physiol., Lond. 77:159.

Anderson, R. R., and Turner, C. W. 1963. Involution of the mammary gland duct and lobule-alveolar systems in the mouse. Proc. Soc. Exptl. Biol. Med., 113:333.

Anderson, R. R. and Turner, C. W. 1962. Adrenal ascorbic acid levels during pregnancy and lactation in rats and mice. Endocrinology, 70:796.

Asboe-Hansen, G. 1958. Hormonal effects on connective tissue. Physiol. Rev., 38:446.

Baldwin, R. L. and Milligan, L P. 1966. Enzymatic changes associated with the initiation and maintenance of lactation in the rat. J. Biol. Chem., 241:2058.

Bearn, J. G., Gould, R. P., and Jones, H. E. H. 1960. The response of the adrenal gland of the rat to pregnancy and lactation. Acta Anat., 41:273.

Blandau, R. J. and Soderwall, A. L. 1941. Post-parturitional heat and the time of ovulation in the albino rat. Anat. Rec., 81:4.

Boas, N. F. 1953. Method for the determination of hexosamines in tissues. J. Biol. Chem., 204:553.

Brookreson, A. D. and Turner, C. W. 1959. Normal growth of mammary gland in pregnant and lactating mice. Proc. Soc. Exptl. Biol. Med., 102:744.

Bruce, H. M. 1961. Observations on the suckling stimulus and lactation in the rat. J. Reprod. Fert., 2:17.

Cole, H. A. 1933. The mammary gland of the mouse, during the oestrous cycle, pregnancy and lactation. Proc. Roy. Soc. B., 114:136.

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

Cross, B. A. and Silver, I. A. 1956. Milk ejection and mammary engorgement. Proc. Roy. Soc. Med., 49:978.

Dickerson, G. E. and Chapman, A. B. 1939. The effect of age and dry period on production at differing levels of producing ability. Proc. Am. Soc. Animal Prod., p. 73.

Dix, Arnold, P. T. and Becker, R. B. 1936. Influence of preceding dry period and of mineral supplementation on lactation. J. Dairy Sci., 19:257.

Dorfman, A. and Schiller, S. 1958. Effects of hormones on the metabolism of acid mucopolysaccharides of connective tissue. Recent Progr. Hormone Res., 14:427.

Emmel, V. E., Weatherford, H. L. and Streicher, M. H. 1946. Leucocytes and lactation. Am. J. Anat., 38:1.

Enzmann, E. V., Saphir, N. R. and Pincus, G. 1932. Delayed pregnancy in mice. Anat. Rec., 54:325.

Fekete, E. 1938. A comparative morphological study of the mammary gland in a high and a low tumor strain of mice. Am. J. Pathol., 14:557.

Gaines, W. L. 1926. Rate of milk secretion as affected by advance in lactation and gestation. Ill. Agr. Exp. Sta. Bull. No. 272.

Gersh, I. and Catchpole, H. R. 1960. The nature of ground substance of connective tissue. Perspectives in Biol. and Med., 3:282.

Gibson, L. M. 1930. A comparative study of the life history of the female mammary gland in two strains of albino mice. J. Cancer Res., 14:570.

Gorman, G. M. and Swanson, E. W. 1967. Effect of oxytocin injected during the dry period on the succeeding lactation. J. Dairy Sci., 50:620.

Green, H., Ephrussi, B., Yoshida, M., and Hamerman, D. 1966. Synthesis of collagen and hyaluronic acid by fibroblast hybrids. Proc. Nat. Acad. Sci., 55:41.

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

Griffith, D. R. and Turner, C. W. 1959. Normal growth of rat mammary glands during pregnancy and lactation. Proc. Soc. Exptl. Biol. Med., 102:619.

Griffith, D. R. and Turner, C. W. 1961a. Normal growth of rat mammary glands during pregnancy and early lactation. Proc. Soc. Exptl. Biol. Med., 106:448.

Griffith, D. R. and Turner, C. W. 1961b. Normal and experimental involution of rat mammary glands. Proc. Soc. Exptl. Biol. Med., 107:668.

Hammond, J. 1927. The Physiology of Reproduction in the Cow. Cambridge University Press, London.

Harkness, M. L. R. and Harkness, R. D. 1956. The effect of pregnancy and lactation on the collagen content of the mammary gland of the rat. J. Physiol., 132:476.

Hasselberg, C. and Loeb, L. 1937. The structure of the secreting and retrogressing mammary gland in the guinea pig. Anat. Rec., 68:103.

Hooker, C. W. and Williams, W. L. 1940. Retardation of mammary involution in the mouse by irritation of the nipples. Yale J. Biol. Med., 12:559.

Jeffers, K. R. 1935. Cytology of the mammary gland of the albino rat. I. Pregnancy, lactation, and involution. Am. J. Anat., 56:257.

Johansson, I. 1962. Genetic Aspects of Dairy Cattle Breeding. Oliver & Body Ltd., pp. 144-146.

Jones, I. C. 1957. The Adrenal Cortex. 1st ed., The University Press, Cambridge.

Kirkham, W. R. and Turner, C. W. 1953. Nucleic acids of the mammary glands of rats. Proc. Soc. Exptl. Biol. Med., 83:123.

Klein, J. W. and Woodward, T. E. 1943. Influence of length of dry period upon the quantity of milk produced in the subsequent lactation. J. Dairy Sci., 26:705.

Kuramitsu, C. and Loeb, L. 1921. The effect of suckling and castration on the lactating mammary gland in rat and guinea pig. Am. J. Physiol., 56:40.

Kuretani, K. 1957. Studies on biochemical components in rat mammary glands. Kagaku-Kenkyu-Jo Hokoku, 27:7.

Lasfarques, E. Y. 1957. Cultivation and behavior in vitro of the normal mammary epithelium of the adult mouse. Anat. Rec., 127:117.

Lataste, F. 1891. Des variations de duree de la gestation chez les mammiferes et des circonstances qui determinent ces variations. Mem. Soc. Biol., 43:21.

Lewin, I. 1957. Discussion of mammary tumors. Xanthine oxidase activity in normal and abnormal growth. Proc. R. Soc. Med., 50:563.

Linzell, J. L. 1959. Physiology of the mammary glands. Physiol. Rev. 39:534.

Lush, J. L., and Shrode, R. 1950. Changes in milk production with age and milking frequency. J. Dairy Sci., 33:338.

Maeder, L. M. A. 1922. Changes in the mammary gland of the albino rat (Mus norvegicus albinus) during lactation and involution. Am. J. Anat., 31:1.

Mantalenakis, S. J. and Ketchel, M. M. 1966. Frequency and extent of delayed implantation in lactating rats and mice. J. Reprod. Fert., 12:391.

Mayer, G. 1956. Interferences entre lactation et gestation. Ann. Endocr., Paris, 17:557.

Mayer, G., and Klein, M. 1961. Histology and cytology of the mammary gland. In: Milk: The Mammary Gland and Its Secretion, Vol. I, Ed. by S. K. Kon and A. T. Cowie. Academic Press, New York, Chap. II, pp. 72-116.

McLean, P. 1958. Carbohydrate and metabolism of mammary tissue. II. Levels of oxidized and reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide in the rat mammary gland. Biochim. Biophys. Acta., 30:316.

Meites, J. 1966. Control of mammary growth and lactation. In: Neuroendocrinology. Academic Press, New York, 1:669.

Meites, J. and Turner, C. W. 1948. Studies concerning the induction and maintenance of lactation. I. The mechanism controlling the initiation of lactation at parturition. Univ. Mo. Agric. Expt. Station Res. Bull. No. 415.

Meites, J. and Turner, C. W. 1950. Lactogenic Hormone. In: Hormone Assay, Academic Press Inc., p. 237.

Melcher, A. H. 1967. Extracellular bound lipid in developing connective tissue. British J. of Exptl. Path., 48:188.

Mizuno, Hideo. 1960a. The effects of administration of prolactin, progesterone or concurrent pregnancy on the mammary gland involution and respiratory activity in the mouse. Endocrinol. Japon., 7:121.

Mizuno, Hideo. 1960b. The effect of concurrent pregnancy on lactation in the mouse. Endocrinol. Japon., 7:322.

Mizuno, Hideo. 1961. The changes of nucleic acids content in the mouse mammary glands in the course of involution and the effects of pregnancy, prolactin or progesterone on them. Endocrinol. Japon., 8:27.

Moon, R. C. 1962. Mammary gland cell content during various phases of lactation. Am. J. Physiol., 203:939.

Munford, R. E. 1963. Changes in the mammary glands of rats and mice during pregnancy, lactation and involution. 2. Levels of deoxyribonucleic acid, and alkaline and acid phosphatases. J. Endocrinol., 28:17.

Myers, F. J. and Myers, J. A. 1921. Studies on the mammary gland. VIII. Gross changes in the mammary gland in the female albino rat during the period of involution. Anat. Rec., 21:74.

Nicoll, C. S. and Tucker, H. A. 1965. Estimates of parenchymal, stromal, and lymph node deoxyribonucleic acid in mammary glands of C3H/Crg 1/2 mice. Life Sci., 4:993.

Okada, M. 1956. Histology of the mammary gland. II. Effects of stagnant milk on the colostrum bodies in the mammary glands of rats. Tohoku J. Agr. Res., 7:115.

Oshima, M. and Goto, T. 1955. A study of involution of the mammary gland after weaning in the albino rat. Bull. Nat. Inst. Agr. Sci., Japan (Series G, No. 11), 30:81.

Ostle, B. 1964. Statistics in Research. Iowa State Univ. Press, Ames, Iowa.

Ota, Katuaki. 1964. Mammary involution and engorgement after arrest of suckling in lactating rats indicated by the contents of nucleic acids and milk protein of the gland. Endocrinol. Japon., 11:146.

Pardue, R. E., and Swanson, E. W. 1963. Mammary gland desoxyribonucleic acid (DNA) as affected by dry periods. J. Dairy Sci., 46:625.

Poulton, B. R. and Reece, R. P. 1957. The activity of the pituitary-adrenal cortex axis during pregnancy and lactation. Endocrinology, 61:217.

Prockop, D. J. and Udenfriend, S. 1960. A specific method for the analysis of hydroxyproline in tissues and urine. Anal. Biochem., 1:228.

Ragsdale, A. C., Turner, C. W., and Brody, S. 1924. The effect of gestation upon lactation in the dairy cow. J. Dairy Sci., 7:24.

Reece, R. P. 1958. Mammary gland development and function. In: The Endocrinology of Reproduction. J. T. Velardo, Ed., VII, Oxford University Press, New York.

Reece, R. P. and Warbritton, V. 1953. The rate of cell division in the mammary glands of rats. J. Dairy Sci., 36:589.

Sanders, H. G. 1928. The variation in milk yields caused by season of the year, service, age, and dry period, and their elimination. IV. Dry period standardization of yields. J. Agr. Sci., 18:209.

Schmidt, G. and Thannhauser, S. J. 1945. A method for the determination of desoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem., 161:83.

Shimizu, H. 1957. Biochemical studies on the mammary gland functions. I. The determination of nucleic acids in the mammary glands of rats. Tohoku J. Agr. Res., 7:339.

Shimizu, H. and Ugami, S. 1959. Biochemical studies in the mammary gland functions. IV. Changes in the catalase activity of the rat mammary gland. Tohoku J. Agr. Res., 10:307.

Silver, I. A. 1956. Vascular changes in the mammary gland during engorgement with milk. J. Physiol., 133:65p.

Sinha, Y. N. and Tucker, H. A. 1967. Mammary nucleic acids and pituitary prolactin content during the estrous cycle. J. Dairy Sci., 50:971.

Slater, T. F. 1961. Ribonuclease activity in the rat mammary gland during pregnancy, lactation, and mammary involution. Biochem. J., 78:500.

Slater, T. F. 1962. Studies on mammary involution. I. Chemical changes. Arch. Intern. Physiol. et Biochim., 70:167.

Smith, V. R. 1959. Physiology of Lactation. Iowa State Univ. Press., Ames, Iowa, 192.

Smith, A., Wheelock, J. V. and Dodd, F. H. 1966. Effect of milking throughout pregnancy on milk yield in the succeeding lactation. Dairy Sci. Res., 49:895.

Smith, T. C. and Richterich, B. 1958. Patterns of succinoxidase, cytochrome oxidase, nucleic acids and other constituents in mammary glands of pregnant and lactating rats. Arch. Biochem. Biophys., 74:398.

Speert, H. 1948. The normal and experimental development of the mammary gland of the rhesus monkey, with some pathological correlations. Carnegie Inst. Wash. Pub. 575, Contrib. Embryol., 32:9.

Stanley, D. E. and Reece, R. P. 1967. Effect of dietary protein on mammary gland nucleic acid content of lactating pregnant rats. J. Dairy Sci., 50:972.

Swanson, E. W. 1962. Lactation depression due to no dry period. J. Dairy Sci., 45:696.

Swanson, E. W. 1965. Comparing continuous milking with sixty-day dry periods in successive lactations. J. Dairy Sci., 48:1205.

Toyoda, Yutaka. 1964. The effect of suckling on ovarian function. Gunma Symposia on Endocrinol., 1:215.

Traurig, H. H. 1967. Cell proliferation in the mammary gland during late pregnancy and lactation. Anat. Rec., 157:489.

Tucker, H. A. 1964. Influence of number of suckling young on nucleic acid content of lactating rat mammary gland. Proc. Soc. Exptl. Biol. Med., 116:218.

Tucker, H. A. and Reece, R. P. 1963a. Nucleic acid content of mammary glands of pregnant rats. Proc. Soc. Exptl. Biol. Med., 112:370.

Tucker, H. A. and Reece, R. P. 1963b. Nucleic acid content of mammary glands of lactating rats. Proc. Soc. Exptl. Biol. Med., 112:409.

Tucker, H. A. and Reece, R. P. 1963c. Nucleic acid content of mammary glands of rats lactating 41 and 61 days. Proc. Soc. Exptl. Biol. Med., 112:688.

Tucker, H. A. and Reece, R. P. 1963d. Nucleic acid content of rat mammary glands during post-lactational involution. Proc. Soc. Exptl. Biol. Med., 112:1002.

Tucker, H. A. and Reece, R. P. 1964. Nucleic acid content of mammary glands of lactating rats simultaneously pregnant. Proc. Soc. Exptl. Biol. Med., 115:885.

Turner, C. W. and Gomez, E. T. 1933a. The normal development of the mammary gland of the male and female albino mouse. I. Intrauterine. II. Extrauterine. Mo. Agr. Exp. Sta. Res. Bul. No. 182.

Turner, C. W. and Gomez, E. T. 1933b. The normal development of the mammary gland of the male and female guinea pig. Mo. Agr. Exp. Sta. Res. Bul. No 194.

Turner, C. W. and Reineke, E. P. 1936. A study of the involution of the mammary gland of the goat. Mo. Agr. Exp. Sta. Res. Bul. No. 235.

Wada, H. and Turner, C. W. 1959. Effect of recurring pregnancy on mammary gland growth in mice. J. Dairy Sci., 42:1198.

White, A., Handler, P. and Smith, E. L. 1964. Principles of Biochemistry. McGraw-Hill Book Company.

Williams, W. L. 1942. Normal and experimental mammary involution in the mouse as related to the inception and cessation of lactation. Am. J. Anat., 71:1.

Wrenn, T. R., DeLauder, W. R., and Bitman, J. 1965. Rat mammary gland composition during pregnancy and lactation. J. Dairy Sci., 48:1517.

Wrenn, T. R., DeLauder, W. R., Wood, J. R., and Bitman, J. 1966. Influence of prior pseudo-pregnancy on lactation and reproduction of rats. J. Dairy Sci., 49:1001.

Yoshinaga, K. and Hosi, T. 1958. On the delayed implantation in the lactating pregnant rat. Part I. The effect of estrogen. Jap. J. Animal Repro., 3:93. [Cited by Dairy Sci. Abstr., 23:222. 1961.]

APPENDICES

APPENDIX I

COMPOSITION OF RAT FEED

The animal ration was composed of the following ingredients:

Gound shelled corn (1/8 inch screen)	607.0 lb
Soybean oil meal, 50% protein	280.0 lb
Alfalfa meal, 17% protein	20.0 lb
Fishmeal, 65% protein	25.0 lb
Dried whey	25.0 lb
Pro-strep 20, 0.54% penicillin and 2.72% streptomycin	4.0 oz
Pro-Gen, 20% arsanilic acid	0.5 lb
Vitamin A, 10,000 units/g	364.0 g
Irradiated yeast, 9.000 units/g	38.0 g
Choline chloride	318.0 g
D, Ca Pantothenate	2.5 g
Riboflavin	1.5 g
Nicotinic acid (Niacin)	15.0 g
Vitamin B-12 (0.1% mannitol trituration)	3.0 g
DL alpha tocopherol acetate, 250 IU vitamin E/g	8.8 g
Menadione (vitamin K)	1.0 g
DL methionine	227.0 g
Limestone	16.0 lb
Dicalcium phosphate	17.5 lb
Iodized salt	5.0 lb

Manganous sulphate ($MnSo_4.H_2O$)	
32.5% manganous	168.9 g
Ferrous sulfate ($Fe\ SO_4.7H_2O$)	
20.9% iron	215.2 g
Calcium carbonate ($CaCO_3$)	
40.4% calcium	83.8 g
Zinc carbonate, basic ($ZnCO_3$)	
56.0% zinc	40.2 g
Cupric sulfate ($CuSO_4.5H_2O$)	
25.45% copper	12.9 g
Cobalt chloride ($CoCl_2.6H_2O$)	
24.77% cobalt	4.7 g
Potassium iodide (KI)	
76.45% iodine	2.2 g

This ration gave the following analysis:

protein	21.2 %
fat	3.9 %
crude fiber	2.5 %
productive net energy	902 C/lb

APPENDIX II

SOLUTIONS USED IN THE ANALYTICAL PROCEDURE FOR NUCLEIC ACID

Orcinol reagent used in the RNA procedure.

1. A stock solution of 1.6% ferric chloride ($FeCl_2.6H_20$) was prepared by adding 1.6 g of ferric chloride to 1 liter of concentrated hydrochloric acid.
2. A 1% solution of orcinol in 1.6% ferric chloride solution, was prepared 1 hour before use.

APPENDIX III

SOLUTIONS USED IN THE ANALYTICAL PROCEDURE FOR HYDROXYPROLINE

Resin-charcoal preparation.

1. Twenty g of analytical grade cation-exchange resin (Ag 1-X8, 200-400 mesh, chloride form) was mixed with 10 g Norit A.
2. The mixture was washed several times with 6N HCl in a coarse sintered-glass funnel.
3. Mixture was washed with ethanol and ether and then dried to a fine powder.

Potassium Borate Buffer.

1. 61.84 g of boric acid and 225.00 g of potassium chloride were added to 800 ml distilled water.
2. the pH was adjusted to 8.7 with 10 N KOH and the final volume adjusted to 1000 ml.

Alanine Solution.

1. Ten g DL alpha alanine was added to 90 ml of distilled water.
2. The pH was adjusted with 10 N KOH and the final volume adjusted to 100 ml.

Sulfuric acid Ehrlich's Reagent.

1. 27.4 ml of concentrated sulfuric acid was slowly added to 200 ml of absolute ethyl alcohol.
2. 120 g of p-dimethylamino-benzaldehyde was added to 200 ml of absolute ethyl alcohol in a separate large beaker.
3. The acid alcohol was slowly added with stirring to the p-dimethylamino-benzaldehyde.

APPENDIX IV

SOLUTIONS USED IN THE ANALYTICAL PROCEDURE FOR HEXOSAMINE

Dowex-50, 250-500 mesh, cationic exchange resin.

1. The resin was washed several times on a Büchner funnel with 2N sodium hydroxide, 2N hydrochloric acid and distilled water in that order, and freed of excess moisture by suction.
2. A 1:1 (weight per volume) suspension of the resin in water was prepared and 5 ml was pipetted into a 16 ml plastic centrifuge tube, centrifuged at 17,000 rpm for 5 minutes, and the water decanted off.

Hydrochloric acid Ehrlich's reagent.

1. A 2.67% solution (weight per volume) of p-dimethylaminobenzaldehyde, in a 1:1 mixture of absolute ethyl alcohol and concentrated hydrochloric acid was prepared.