THE LACTATIONAL STRATEGY OF *THRICHOMYS APEREOIDES*

by

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(ABSTRACT)

The lactational strategy of Thrichomys apereoides, a tropical hystricomorph rodent was examined. Milk composition and yield, and mammary gland composition was determined.

Milk samples were collected at days 2, 7, 14, 21, 28, and 35 post-partum. Milk yield peaked at day 14 and ceased by day 35. Milk fat content was high ranging from 30.2 ± 0.8% at day 2 to 21.0 ± 1.0% on day 21. Protein increased from 11.9 ± 0.6% at day 14 to 17.4 ± 0.9% at day 28. Lactose content decreased from 4.62 ± 0.1% at day 7 to 2.65 ± 0.1% at day 21.

Chemical parameters of the mammary gland examined were nucleic acid concentration (DNA and RNA), percent fat and percent protein. Mammary DNA concentration (ug. DNA/mg.mammary tissue) did not change over the lactation period. RNA concentration (ug.RNA/mg. mammary tissue) peaked by day 14 post-partum and started to decline by day 21. Mammary gland fat and protein content did not change over the lactation period. Mammary gland histology supported the results of the chemical test and milk yields

as to the activity of the glands. Percent lumina and stroma were inversely related, with lumina peaking at day 14. Involution occurred by day 28 in the inguinal glands and day 35 in the laterals glands.

The unusual milk composition and extended lactation period is theorized to be one of several adaptations associated with reproduction in an unpredictable, xeric environment. Milk high in fat and low in lactose allows a lactating dam to decrease the demand on maternal water reserves; while providing the neonates with a high fat diet which results in the production of the greatest amount of metabolic water. The extended lactation period also insures that the neonates are provided with a reliable source of food (energy) in an unpredictable environment.

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I would also like to acknowledge the memory of my grandfather . Whose walks through the woods taught me to appreciate the beauty and wonders of nature, and inspired me to wonder why?

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INTRODUCTION

Thrichomys apereoides is a tropical hystricomorph rodent from the Caatinga of Brazil and the Chaco region of Paraguay. While most other echimyids inhabit tropical riverine forests, T. apereoides inhabit a xeric rocky scrubland environment, where rainfall is an unpredictable event within and between years (Streilein, 1982a).

While similar to other echimyids in body mass, litter size, neonatal growth rate and neonatal mass, selective pressures from this harsh unpredictable environment have caused a divergence in some reproductive characteristics. The gestation period is 50% longer, allowing a 50% slower prenatal growth rate. Conception to weaning time is 40% longer (Roberts et al., 1988). These divergent characteristics appear to be adaptations for minimizing maternal energy expenditures while maximizing reproductive output.

While various aspects of the gestation period have been examined (Roberts et al., 1988; Nicoll and Thompson, 1987), little is known about the lactation period. Lactation represents a major portion of maternal energetic investment. In some mammals (ie. ungulates) lactation may constitute 70-80% of maternal energetic effort (Oftedal, 1984). Since selective pressures from this unpredictable, harsh environment have substantially altered typical gestational reproductive characteristics, it is expected

that the lactational strategy has been altered as well.

In order to study the lactational strategy, it is necessary to study both the mammary glands and milk produced. The results of these analyses in comparison to data available from that of other rodents will support the contention that selective pressures have caused the evolution of an atypical lactational strategy.

Literature Review

All mammals share in common both the maternal energetic investment of the gestation and lactation of neonates. Due to variability in biotic and abiotic factors such as habitat, and nutritional requirements, different species employ different reproductive strategies. The objective of this research was to examine several parameters of lactation in a South American rodent, Thrichomys apereoides, (punare), which is distributed in the hot, xeric Caatinga of Brazil and Chaco region of Paraguay. Parameters evaluated included milk composition, mammary gland composition and histology of mammary tissue.

Milk composition

Milk is essential for the growth and development of young mammals during the maternal dependency period. The concentrations and proportions of the major constituents of milk (fat, lactose and protein), vary greatly among mammalian species. Some of these constituents are synthesized by the mammary gland itself, while others are withdrawn from those circulating in the maternal bloodstream. Constituents synthesized in the gland itself include:milk fat, lactose, caseins and a & b lactalbumins. Milk composition for over 200 of the 4000 species of mammals has been analyzed (Jenness, 1986; Oftedal et al., 1987). Some of these studies have encompassed entire lactation period while others were determined from a single

sample from a single organism (Oftedal, 1984).

During the course of lactation milk composition changes in a characteristic fashion for each species (Oftedal, 1984). Macropod marsupials, exhibit the greatest amount of change in milk composition among mammals (Jenness, 1986). Not only does the composition vary, but different glands within the same female can produce markedly different milks at the same time as has been reported for the Red Kangaroo (Macropus rufus) and the Tammar wallaby (Macropus eugenii) (Jenness, 1986). Interspecific differences in milk composition reflects differences in rates at which the constituents, including water, are secreted. Rates of secretion are hormonally controlled. Relations of mechanisms of synthesis and secretion and the resulting milk composition are summarized by Holt (1983).

Milk fat is produced in the cytosol of the secretory cells appearing as well defined droplets (Hollman, 1974). Milk fat is secreted from the apical side of the cells into the alveolar lumen. The relative rates of secretion of the lipid droplets and aqueous phase (water and ions) determines the fat concentration in the milk. High percentages of fat are typical for arctic mammals such as the blue whale, grey seal, and polar bear (42%, 53%, and 33%, respectively) (Oftedal, 1984). Aquatic and arctic species secrete milk high in fat content, thus allowing

young to rapidly lay down an insulating layer of subcutaneous fat, and to obtain high calorific energy from its oxidation (Oftedal et al., 1987; Brody, 1945; Peaker and Good, 1978). A high fat content also allows higher energy transfer with minimal secretion rates. Rodents from different habitats produce milk with various fat content values, 10.3% for Rattus norvegicus, and 13.1% for Mus musculus, temperate species, and 5.7 % for Cavea porcellus, a tropical inhabitant (Oftedal, 1984, Mepham and Beck, 1973). Species which have long intervals (ie. tree shrews-2 days,) between suckling bouts are also reported to have somewhat increased milk fat (Jenness, 1986; Martin, 1984).

Lactose is another major constituent of milk, and like fat, its chief function is to supply energy as the principal carbohydrate source to suckling young. Lactose production and transport involves both transcellular and paracellular mechanisms (Jenness, 1986). It is first synthesized into Golgi vesicles derived from the endoplasmic reticulum (Jenness, 1986; Palmiter, 1969). These Golgi vesicles then move to a cell's apical membrane and discharge lactose into the alveolar lumen by exocytosis. Lactose content differences over the lactation period are compensated for by diffusible ions, which maintain a constant osmolarity. There is a reciprocal relationship between osmotically effective salts and the

amount of lactose, in some species of seals, where little or no lactose is found; their osmolar range however, is similar to other mammals. Peaker (1977) has suggested that a decrease in milk lactose content decreases stress on maternal water reserves due to the reduction in the amount of carbohydrate transferred in an aqueous media, between dam and juvenilies. Lactose is beneficial as a source of carbohydrate energy because it is a disaccharide; and as such carries twice the caloric content of a monosaccharide per osmotic increment.

Milk proteins are the third major constituent found in milk. Like lactose, they are synthesized in a vacuole of the Golgi apparatus, and are released by exopinocytosis. Some are seen as protein granules, and are the source of amino acids required by the the young for the synthesis of proteins. Protein contents range from 1% in man to 20% in the rabbit (Jenness, 1986; Cowie, 1969). It supplies about 50% of the caloric output of some rodents, ie. guinea pigs (Cavia aperea); while it furnishes only 10% of the calories in both low energy (ie.human) and high energy milk (ie. whales) (Jenness, 1986). Several protein types are found in milk including: caseins, lactalbumins and immunoglobulins. Casein proteins are principally involved in supplying amino acids and in the transport of phosphate and calcium. The whey proteins differ greatly among species, and function in providing the rest of the

essential dietary amino acids. The proportion of casein to whey protein varies from 0.8:1 in some primates to 4:1 in some ungulates (Jenness, 1986).

Mammary Gland Anatomy

Mammary gland numbers, location, shapes and sizes vary among mammals. Their principal function is the secretion of milk for nourishment of the young. In all placental mammals (Eutherians), the glands are highly developed and have teats. In most species, the gland is composed of a teat, duct system, and lobes and lobules of the secretory tissue (Cross, 1977) . Within the Order Rodentia various anatomical differences in gland location, shape and numbers are found. The guinea pig has two mammary glands and teats located in the inguinal region, while the rat has six pairs-three thoracic, two inguinal and one abdominal. Flat, sheet-like, and two dimensional in appearance, though all are separate glands, they look undistinguishable to the eye.

Histology and Cytology

Milk is produced by a single layer of epithelial cells that line the lumen of the alveolus. The alveolar epithelium is columnar in shape when the lumen is empty. When the alveolar lumen is filled with secretion, the cells flatten. Myoepithelial cells are located between the basement membrane and epithelial cells. When stimulated by

oxytocin myoepithelial cells contract, causing milk to be ejected from the secretory epithelial cell into the lumen (Cross, 1977; Oftedal et al., 1987). Each alveolus is spherical. Milk secreted into the lumen is drained by intercalary ducts, these ducts join to form intralobular ducts which carry milk to the exterior of the lobule. Each alveolus is surrounded by capillaries, which carry milk precursors. Groups of alveoli encapsulated by connective tissue are called lobules. They are drained by a common duct. The lobules join and are drained by yet a larger duct. The lobules cluster to form a lobe, which in turn is surrounded by more connective tissue (Schmidt, 1971). Rats and mice have a single streak canal which leads to a cistern, which acts as a collecting vessel for all lobes in the gland (Hollman, 1974).

The mammary gland is made up primarily of parenchyma (secretory cells) and stroma (ie. connective tissue, blood vessels. Even though milk composition varies greatly mammary gland cells of different species are almost structurally identical. These cells modulate milk composition and yield by changing the number, volume and surface area of organelles when different synthetic activity is required (Hollmann, 1974). Densely packed epithelial cells result in a spongy appearance to a distended alveolus.

If secretion of galactopoietic hormones is impaired

mammary secretory cell organization is disrupted (Hollman, 1974). The alveoli lose the round outline of the lumina, narrow and they become engorged with lipid droplets. If the secretion of prolactin, and oxytocin are maintained, but suckling does not occur lactation can be reinstated, within a certain period by the resumption of a suckling stimuli. If all hormones necessary for lactation are maintained that nursing can be resumed even after a 5 day period of rat pup removal. As cellular organization deteriorates, cells shrink and disappear by lysis or expulsion from the alveolus. Dying cells are shed into the lumen (Hollmann, 1974). Regression and involution of the gland is characterized by the disappearance of a bulk of the parenchyma tissue. The secretory cells change their shape and become cubic or flat, the microvilli disappear so that the lumen has a smooth and irregular appearance, and the nuclei lose their round shape and become infolded (Hollmann, 1974). Involution is characterized by a decrease in the and total number of alveoli, as well as a decrease in the number of cells associated with each alveoli. There is either a proliferation, or more obvious appearance of connective tissue (Hollmann, 1974). Also found is the presence of a great number of lysosomes (Wellings and DeOme, 1963). Necrotic cell debris are moved into the connective tissue space where they are further degraded and

eventually disappear. There are two processes that cause the reduction in mammary gland parenchyma, (1) a decrease in the individual cell size and the organelles of the surviving cell population, and (2) the progressive degeneration of the other epithelial cells. There is some descrepancy as to the importance of macrophages in the involution of the mammary gland. Wellings and DeOme (1963) found them of little importance in involution since they are not abundant in the epithelial or stromal spaces during rat mammary gland involution; however Helminen and Ericsson (1968) reported that macrophages played an important role in milk protein uptake and in epithelial cell cytoplasm in the rat. Hollmann (1974) agrees with Wellings and DeOme (1963), and reported that macrophagelike cells are difficult to distinguish from degenerating epithelial cells, due to fine structure similarity and because they are both shed into the alveolar lumen.

Nucleic Acids

With the ability to determine tissue nucleic acid content, researchers have been able to study both glandular development and gland activity over the lactation period. Currently, the measurement of total deoxyribonucleic acid (DNA), is the most common method to determine the total cell numbers of the mammary gland. This is based on the finding that DNA content per mammary gland cell, (as per any somatic cell) is constant within a species. (Munford,

1964). Through the use of "Total DNA" studies it has been shown that while mammogenesis continues through the beginning of lactation in some species, ie. the guinea pig, mice, and rats (Anderson et al.,1982; Tucker and Reece, 1963a), it is complete at parturition in others, ie. the golden hamster,and sheep (Sinha et al., 1970; Anderson, 1975). Various research has focused on mammary gland growth in lactating mice (Brookreson and Turner,1959; Knight and Peaker, 1982). Mizuno (1961), reported that total DNA increased to a maximum at day 14 of lactation and decreased from there until day 19. Concurrent pregnancy prevented total decrement of the DNA content. DNA synthesis and the number of secretory alveolar cells increased during early, but not during established lactation; with the most rapid and prominent cell volume increase occurring with the initiation of lactation (Foster, 1977). Mammary gland DNA content in mice decreased 53% between day 20 (day of weaning) and day 25. (Schmidt, 1971). It was also reported that DNA content peaked in mice at day 5 (Knight and Peaker, 1982). Mammary DNA in rats continues to increase during early lactation (Tucker and Reece, 1963a), however the suckling stimuli is not enough to maintain DNA levels. Despite replacing 16 day old rat litters every 4 days with 12 day old litters, DNA content still decreased even though suckling stimulus levels were

high (Thatcher and Tucker, 1968).

The amount of ribonucleic acid (RNA) in various body tissues has been shown to be associated with the intensity of protein synthesis (Munford, 1964). Since there is a correlation (r=0.93) between mammary RNA content and litter weight gain, RNA has been used as a good estimator of synthetic activity and therefore a good estimator of lactational potential (Kirkham and Turner, 1953). Thatcher and Tucker (1968) showed that RNA content decreased after peak lactation. Frequent replacement of foster litters to maintain intensive nursing stimuli failed to prevent declines in DNA and RNA content between days 20 and 36. The reduction of RNA during extended lactation was much greater than that of DNA. Thus it appears that the factors controlling protein synthesis limited milk synthesis more than factors influencing total cell numbers.

The ratio of mammary RNA to mammary DNA (R/D) is an additional measure of glandular synthetic activity. In the rat, the R/D ratio is 1.9 shortly after parturition and by day 16, the ratio has increased to 4.2, with increasing glandular output, and reaches maximal R/D ratio on day 21 (Kirkham and Turner, 1953; Tucker and Reece, 1963), or between day 16 to day 20 (Tucker, 1966). In the guinea pig (Cavia aperea) mammary RNA and milk yield increased to a maximum near day 6, with a R/D=4.2 (Nelson, et al., 1962). Declining lactation is associated with reduced R/D in rats,

implying depressed cellular activity (Thatcher and Tucker, 1968).

Thrichomys apereoides

Thrichomys apereoides is a crepuscular, hystricomorph rodent, with a disjunct distribution in the Caatinga of northeastern Brazil, and the Chaco region of Paraguay (Streilein, 1982a). The region, although xeric in nature, is subjected to extremes of precipatation, experiencing both drought and heavy rainfall. Thrichomys apereoides is the only Echimyid species which inhabits a xeric environments (Streilein,1982). They are found solely in areas of rock outcroppings. Thrichomys, like other hystricomorph rodents have a long gestation period and produce precocial young (Weir, 1974). Though there is no absolute distinction between precocial and altricial mammalian young, as there is in birds, (being nidifugous or nidicolous), precocial mammalian young are said to be those which at birth are well furred, and whose eyes and ears are open (Weir, 1974). Varying degrees of precocial development are reported among hystricomorph rodents. While Chinchillids (Chinchillas laniger), and guinea pigs (Cavia aperea), display precocial characteristics, other hystricomorphs such as degus (Octodon degus), and tuco-tucos (Ctenomys knightii) are born with eyes closed and are less well furred (Weir, 1974). Thrichomys appear to

produce one of the most precocial offspring of rodent species. Gestation length averages 97 days, and litter sizes range from 1-8, with an average of 3.2 young (Roberts et al., 1988). Suckling appears to occur for at least 30 days some 40% longer than other Echimyids (Roberts et al., 1988). Kleiman (1974) suggested that this extended period of suckling in hystricomorph rodents is due to the need of social bonding, and that nutritional lactation is curtailed much earlier. Preliminary studies show that from 2 days post-partum Thrichomys apereoides young can feed independently and within a week possibly thermoregulate on their own when separated from the adults. In Myocastor coypus, another South American hystricomorph rodent precocial young lactate for 7.7 weeks in the wild, but in the lab are able to survive on solid food from 5 days of age (Gosling et al, 1984).

Thrichomys apereoides have many behavioral and physiological adaptations which enable them to survive in the xeric Caatinga environment. Thrichomys apereoides utilize micro-habitats within the environment which are high in humidity due to runoff percolation into the crevices. Such crevices allow for the pooling of water both from rain and runoff from higher elevations (Streilein, 1982c). Physiologically T. apereoides is able to withstand extreme water deprivation. In a drinking water deprivation experiment, where dried corn (% water

content unknown), was the only food source, 19 out of 20 specimens survived, losing on average 12 % of their original body mass after 18 days. When given access to water for 12 hrs., they regained all but 2.8% of their original mass. In a second deprivation experiment , where cacti pads were available, along with the dry food, the individuals lost only 2.5% of their starting mass, as compared to 10% during a similar time period within the first water deprivation study (Streilen, 1982d). Additional possible adaptations to a dry environment may be the consumption of the water containing feces and urine of the young (Maltz and Schkolnik, 1984). This could be in addition to preventing nest fouling and detection by predator. Preliminary studies show that copography is exhibited by adults at a diurnal rhythm, with two types of fecal pellets produced, one looking and feeling drier than the other. Besides the recycling of undigested nutrients, it may be possible that the Thrichomys may be using copography as another method to conserve water. It needs to be determined which pellets are consumed.

Water Restricted Animals

Desert adapted mammals have been reported to have significantly different milk compositions from species which inhabit temperate environments. The ibex (Capra ibex) and gazelle (Gazella dorcas), two arid adapted

species of desert ruminants are reported to have milk with twice the solids content (fat, protein, lactose, ash), and a higher fat percentage than non-desert ruminants (Maltz & Shkolnik, 1984). Kooyman (1963) analyzed milk composition in the Kangaroo rat (Dipodomys merriami) and reported that the milk from pooled samples from unknown stages of lactation, contained an average of 23.5% fat. Irving (1960) suggested that high fat content is advantageous as less water is required for its storage when compared to carbohydrate and protein. Fat has the greatest amount of calorific content per gram, and when it is metabolized, it produces the most metabolic water. On combustion each gram of fat yields 1.07g. of water, while protein yields just 0.40g, and carbohydrates only 0.56g (Brody, 1945). Milk composition analyses from several Australian desert rodents (Notomys alexis, N. cervinus, N. mitchelliand Pseudomys australis) reported that milk fat content was no greater than that observed in the laboratory rat, dog, or rabbit (Chalk and Barley, 1979; Baverstock, et al., 1976; Oftedal, 1984). This could be attributed to efficient recycling and conservation of the maternal excretory system. Notomys alexis is able to concentrate urine to 9370 mosmol/L, as compared to 3394 mosmol/L for the Thrichomys. Since Notomys are so efficient in water conservation (Baverstock et al., 1976) it may not be necessary for them to have altered their lactational

strategy as well. Since the environment in which the Thrichomys naturally occur is not as dry as that where the Notomys is, the selection pressure for water conservation is probably not as great. It is possible that the xeric environment is physiologically stressful only for pregnant and lactating females and for this reason, these aspects of Thrichomys apereoides life history strategy has been altered.

Chapter 1

Milk Yield and Composition in Thrichomys apereoides

Abstract

Milk composition of Thrichomys apereoides, a tropical hystricomorph rodent, was determined from milk samples collected by manual palpation. Milk samples were pooled across teats within a female and sampling period. Milk was collected at days 2, 7, 14, 21, 28, and 35 post-partum. Milk production peaked at day 14 and ceased by day 35. Percent fat composition of the milk was unusually high, ranging from 30.2 $\pm$ 0.8 % on day 2, to 21.0 $\pm$ 1.0 % on day 21. Percent protein changed significantly over time, ranging from 11.9 $\pm$ 0.6 % on day 14 to 17.4 $\pm$ 0.9 % on day 28. Lactose content was low, and decreased significantly over time, from 4.62 $\pm$ 0.1 % at day 7 to 2.65 $\pm$ 0.1 % at day 21. It is theorized that this unusual milk composition for a tropical rodent, is one of several adaptations associated with reproduction in a xeric environment. Milk high in fat and low in lactose, allows the dams to decrease demand on maternal water reserves, and for neonates to metabolically produce their own water from the metabolism of the high fat content milk.

Introduction

Thrichomys apereoides is a hystricomorph rodent in the family Echimydae which has life history characteristics

similar to other echimyids (body mass, litter size, neonatal growth rate and neonatal mass) but have diverged in some reproductive characteristics. Gestation length and conception to weaning time are longer, prenatal growth rate has decreased (Roberts et al.,1988) and a lower metabolic rate is reported (Nicoll and Thompson, 1987).

Unlike most other echimyids which inhabit tropical riverine forests, T. apereoides are distributed in the xeric rocky scrubland environments of Brazil and Paraguay. (Streilein, 1982a). Though the regions are classified as semi-arid (based on total annual rainfall), there is no seasonality to precipitation in the area. Rainfall is an unpredictable event from one year to the next, and within the same year (Streilein, 1982a). Selection does not promote seasonal reproductive patterns due to a lack of environmental seasonal predictability; thus, reproduction is a year round event (Streilein, 1982b).

Thrichomys apereoides' reduced basal metabolic rate may be an adaptation to reduce the cost of maintenance in an unpredictable environment (Nicoll and Thompson, 1987). A 50% slower prenatal growth rate, 50% longer gestation period and 40% greater conception to weaning period also appear to be adaptations for minimizing the rate of maternal energy expenditures for reproduction, while maximizing reproductive output.

As lactation represents a major portion of maternal reproductive effort (Oftedal, 1984; Oftedal et al., 1987), it is probable that selective pressures have also altered facets of the lactation period. Lactational performance of a species has evolved from interactions of factors including: food and water availability, environmental stress, and neonatal growth rates. Studies of compositional changes in milk over the course of lactation demonstrate that a high degree of variation in milk composition occurs interspecifically. For this reason milk from mammals within similar genera and samples from different times during the lactation period should not be assumed similar. Mammals with altricial young (ie. carnivores, rodents), generally produce a more concentrated milk while those which produce precocial young (ie. ungulates) have more dilute milks containing high sugar content (Martin, 1984). Two precocial species, the guinea pig (Cavia aperea) and the brown hare (Lepus capensis) produce milk more similar to ungulates with single or twin young, than to more closely related species but with larger litter sizes (Oftedal, 1984).

Thrichomys apereoides has a medium sized litter, with an average 3.2 individuals (Roberts et al., 1988), though litter sizes do range from 1-8 individuals. Neonates are extremely precocial; fully furred, with eyes open at birth. Preliminary studies showed that from 2 days post-

partum neonates could feed independently and within 10 days thermoregulate (at ambient temperature) when separated from adults. Suckling occurs for approximately 6 weeks (Roberts et al., 1988) well beyond a time of nutritional dependence. It has been suggested that the extended suckling is more important as a bonding process, than for nourishment, particularly, in older neonates (Kleiman, 1974; Walser, 1977). This extended suckling period is thought to maintain the mother-young bond when the young are still vulnerable to predation (Kleiman, 1974).

The objective of this study was to determine both the length of nutritional suckling as well as the composition of the milk produced. If Thrichomys apereoides follow a lactational strategy typical of tropical precocial rodent, one would expect to find a short lactation period with a dilute, low energy milk (Mepham and Beck,1973; Oftedal, 1984). Because Thrichomys are the only echimyids to inhabit a xeric environment, it is expected that the lactation process has been evolutionarily altered to improve survivorship of the neonates in this unpredictable environment.

Materials and Methods

The Thrichomys apereoides used in this study were derived from the Biology department colony at Virginia Polytechnic Institute and State University. The original

stock was derived from a colony at the National Zoological Park (Smithsonian Institution), in 1980. The animals were maintained on a photoperiod LD 16:8 at 20 $\pm$ 1°C, and were provided with lab chow (Agway 3000 Lab Blox) and water ad libitum. The animals were housed in plastic tubs (30cm x 42cm x 34 cm), with wood shavings for bedding and were cleaned weekly. Experimental litters varied in size from 3 to 6 neonates.

Milk samples were collected from 16 tranquilized females, which were separated from their young 6 hours prior to milk collection to permit accumulation of milk in the gland. Females were tranquilized with an intramuscular injection (IM) of Ketamine (Vetelar; 22 mg/kg). This was followed by an IM injection of 0.4 units of oxytocin. Milk was collected by massaging the mammary tissue and drawing the resulting droplets into a pipet tip, by oral suction. The small total milk volume obtained from each female per milking necessitated pooling all teat samples within a female and sampling period. Milk collected was measured after the gland was expressed as completely as possible.

Females were milked on days 2, 7, 14, 21, 28, and 35 post-partum. Milk samples were immediately analyzed for percent fat content, which was determined by centifugation of three replicate samples in 75 mm capillary tubes (Ganguli et al.; 1969: Schoknecht et al., 1985). The remaining milk was then centrifuged at 3700 x g for 30

minutes, and then frozen. Protein content was measured using a dye binding Bio-Rad protein determination assay (Bradford, 1976) and lactose content by the colorimetric picric acid procedure of Perry and Doan (1950) as modified by Erb et al., (1977) (Schokencht et al., 1985).

To test for the effect of exogenous oxytocin on milk composition, milk was collected at peak lactation from six females prior to oxytocin administration. Three samples were individually tested for fat content. The remaining three milk samples were pooled with the samples from the fat determination samples and the pooled sample was divided into three replicate samples and used for protein and lactose assays.

Individual milk components were analyzed using a two way analysis of variance, with female and time being the source of variation. Student T-tests were used to further evaluate data for which the ANOVA was significant (SAS, 1982).

Results

To determine if the exogenous oxytocin had any effect on milk components, milk was collected, at peak lactation (day 14) from 6 females, prior to oxytocin administration. Three of these samples were individually tested for fat content, and found to be comparable to the milk that was collected later (22%, 22.5% and 23%), under oxytocin

stimulation. The remaining 3 samples, as well as the remaining fat assay samples were pooled and assayed for protein and lactose content. The pooled subsamples were found to be within the expected range of values observed for that period of lactation (12.1% protein and 4.28% lactose).

Changes in estimated milk production occurred over the lactation period (fig 1.1). On day 2 post-partum, less than 0.2 ml. of milk was obtained from each female milked. At this time in lactation, the 2 inguinal glands produced half of the milk sample that was obtained. By day 7 post-partum, milk production had increased to 1.0 $\pm$ 0.1 ml, with the lateral and inguinal glands producing equally. Milk production continued to increase until day 14, when yields averaged 2.0 $\pm$ 0.2 ml per female, (range 1.5 -3.0 ml), with lateral glands producing twice the volume of inguinals. By day 21 milk yield decreased to 1.25 $\pm$ 0.2 ml., with decreases in the production of all the glands. Milk production ceased in the inguinal glands by day 28, with less than 0.5 ml collected from the lateral glands.

Milk composition changed markedly over the lactation period (fig 1.2). Fat, the major solid constituent of the Thrichomys milk decreased significantly from day 2 to day 14, but was unchanged thereafter. (fig. 1.2). At day 2 the milk averaged 30.2 $\pm$ 0.8 % fat and significantly decreased ($p < 0.0001$, $t=-6.0$) by day 7, to 24.5 $\pm$ 0.8 %.

Another significant drop occurred between day 7 and day 14 14 when fat decreased to 20.8 ± 2.0% ($p < 0.0001$, $t=-5.67$). Concentrations did not differ significantly between day 14, day 21, day 28, with percentages of 20.8 ± 2.0, 21.0 ± 1.0, and 22.6 ± 0.6 %, respectively ($p > 0.08$, $t=1.89$).

Total percent protein in the milk changed over the lactation period, decreasing significantly from 13.9 ± 0.6 % on day 7 to 11.9 ± 0.6 % on day 14 ($p < 0.003$, $t=-6.43$). No change occurred from day 14 to day 21 (11.9 ± 0.6 % & 12.3 ± 1.0 %, respectively; $p >0.51$, $t=0.69$) but protein increased significantly between day 21 and day 28 ($p < 0.008$, $t=6.42$) when protein levels reached 17.4 ± 0.9%.

Similar to fat and protein, lactose (major milk sugar) concentration changed over the lactation period. Lactose decreased as lactation progressed. At day 7 the percent lactose was 4.62 ± 0.09 % and it decreased to 4.31 ± 0.06 % ($p < 0.026$, $t=-2.73$) at day 14. Lactose concentration continued to decrease through day 21 where a significant drop to 2.65 ± 0.12 % ($p < 0.0001$, $t=-13.71$) occurred. Lactose concentration at day 28 (2.88 ± 0.14 %), did not differ significantly from day 21 ($p > 0.95$, $t=0.07$).

Discussion

In contrast to other hystricomorph rodents which

suckle for extended periods after the point of nutritional independence (Kleiman, 1974), Thrichomys apereoides exhibits a longer period of milk production, with milk produced through day 28 post-partum. Roberts et al. (1988) reported nutritional weaning to occur at 21 ± 6 days for T. aperoides. In this experiment all females continued to lactate through day 28, but had ceased by day 35. This discrepancy in duration may be due to the method of weaning determination. Roberts et al. (1988) determined that lactation ceased when 80 % of fostered juveniles were no longer sucessfully reared. Since the ages of the juveniles were not reported it cannot be argued whether milk had actually stopped being produced, or if possibly, there was milk produced, just not in quantities great enough to sustain the young. Milk production in this study was determined by the manual palpation of the mammary gland and the subsequent appearance or lack of appearance of product.

An extended period of milk production is highly unusual for such precocial rodents. Guinea pigs (Cavia aperea), which produce highly precocial young wean them before they reach 3 weeks of age. Guinea pig milk yield peaks from day 5-8, and steadily declines thereafter until day 18-21 (Mepham and Beck, 1973).

Not only does T. apereoides have an extended nursing period, but milk composition is also atypical. Milk composition was uncharacteristically high in fat and low in

lactose, peaking at day 2 with 30.2 $\pm$ 0.8% fat and thereafter declining to a fat content of 21.0 $\pm$ 1 % by the end of lactation. The initial decline in fat content may be due to the presence of colostrum in early lactation milk. While colostrum in primates, carnivores, and ungulates are typically high in proteins, in rodents, fat is usually elevated (Oftedal, 1984). Even when fat content reached its lowest percent of composition, it was still substantially higher than that of most other rodents, including both an altricial temperate species Rattus norvegicus with 8.8 %, and a precocial, tropical species- Cavia porcellus with 5.7% (Table 1.1)(Oftedal, 1984). As shown, Thrichomys apereoides milk is comparable to that of many marine mammals where high fat content allows the suckling young to quickly lay down an insulating layer of subcutaneous fat (Oftedal et al., 1987; Lavigne et al., 1982). Though this may be an important adaptive strategy for aquatic mammals, it is an improbable one for the tropical Thrichomys dam and neonates. It appears there must be another adaptive significance, or selective pressure for high fat content milk. Milks high in fat content have been found in species where there is a long interval between suckling bouts, such as the tree shrew, which has been reported to suckle every 2 days (Martin, 1968). Milk with high fat allows the greatest amount of caloric energy to be

transferred in the least amount of time (Oftedal, 1984). Since little is known about the suckling behavior of Thrichomys apereoides in the wild, it is hard to determine if this strategy applies. The lactose percent also low. Irving (1960) suggested that reduced lactose concentration, reduces the amount of water needed to transport the carbohydrate in the aqueous form. A milk high in fat and low in lactose is more characteristic of aquatic and arctic mammals.

While reproduction in a cold environment is not a problem for Thrichomys aperoides, as it is for the arctic mammals; neonatal survivorship in a xeric, unpredictable climate is a problem. In an environment where precipitation is not a seasonal event, it is possible that high fat content milk is an adaptation for year round reproduction. By increasing the fat portion of the milk the dam decreases the aqueous portion, thereby reducing stress on maternal water reserves. In the harp seal, percent fat almost doubles between the beginning and end of lactation (Table 1.1). One reason suggested, in addition to insulating the pup, is as an adaptation of the non-feeding dam to maintain her water balance (Lavigne, et al., 1982). The neonate benefits from this strategy because metabolizing fat, produces proportionally more metabolic water than would the metabolism of other milk components (Peaker, 1977). From each gram of fat oxidized, 1.04g. of water are produced,

while only 0.40g., and 0.56g are produced from each gram of carbohydrate and protein, respectively (Brody, 1945). Milk composition of various desert adapted rodent species has been determined. Kooyman (1963) analyzed milk composition in the kangaroo rat (Dipodomys merriami) and reported that the milk from a pooled sample, of unknown lactation stage, averaged 23.5% fat. In contrast, analysis of milk composition of several species of Australian desert rodents (Notomys alexis, N. cervinus, N. mitchelli) show fat content of milk is no greater than that observed in the lab rat, dog, or rabbit (Baverstock et al., 1976). This could be attributed to the efficient recycling and conservation of the maternal excretory system. While the N. alexis is able to concentrate urine to 9370 mosmol/L, the Thrichomys can only to 3394 mosmol/L. The ability to recycle water reserves in Notomys is so efficient that they may not need to increase fat content of milk. Whereas in the Thrichomys the selective pressure of lack of environmental water is not as great, and possibly is only a stress when lactating, so they may have evolved a different adaptive strategy.

Total solids (fat, protein, lactose) was also substantially higher in Thrichomys milk, again more similar in characteristic of the aquatic mammals, than of rodents (Table 1). If solids take up a greater proportion of the milk , water percentage is decreased. Some desert adapted

mammals are found to have significantly different milk composition from their temperate inhabiting relatives. The ibex and gazelle, two arid adapted species of ruminants are reported to have milk with twice the solids content, and a higher percentage of fat, than temperate inhabiting ruminants (Maltz and Shkolnik, 1984).

Besides being a source of water for the neonates, it is possible that this extended period of lactation may be a way in which the dam can be certain that no matter what the environmental conditions are at the time of parturition, her young will have a food source.

Table 1.1 MILK COMPOSITION OF VARIOUS AQUATIC AND RODENT SPECIES

(%)	FAT	PROTEIN	LACTOSE	TOTAL SOLIDS
Thrichomys apereoides (this study)				
early lactation	30.2	----	-----	----
mid lactation	20.8	11.9	4.3	37.0
late lactation	22.6	17.4	2.6	42.6
Cavia porcellus Guinea pig (Oftedal, 1984)				
mid lactation	5.7	6.3	4.8	16.8
Rattus norvegicus Brown rat (Oftedal, 1984)				
mid lactation	8.8	8.1	3.8	20.7
Arctocephalus gazellus Antarctic fur seal (Bonner, 1968)				
lactation period unknown	26.4	22.4	0.1	48.8
Phoca groenlandica Harp seal (Lavigne et al., 1982)				
early lactation	23.0	6.6	2.3	31.9
late lactation	40.0	6.6	2.3	48.9

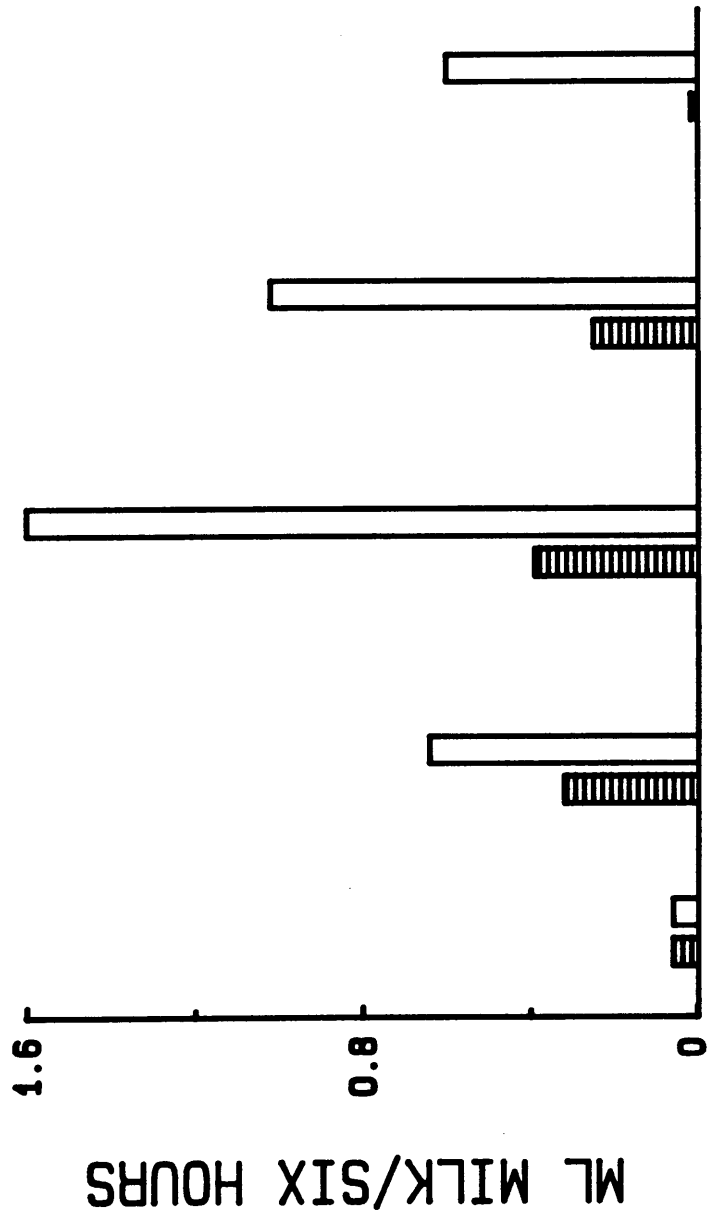


Fig. 1.1 Mean milk yield after 6 hours of accumulation for the inguinal (horizontal bars) and lateral (open bars) glands over the lactation period.

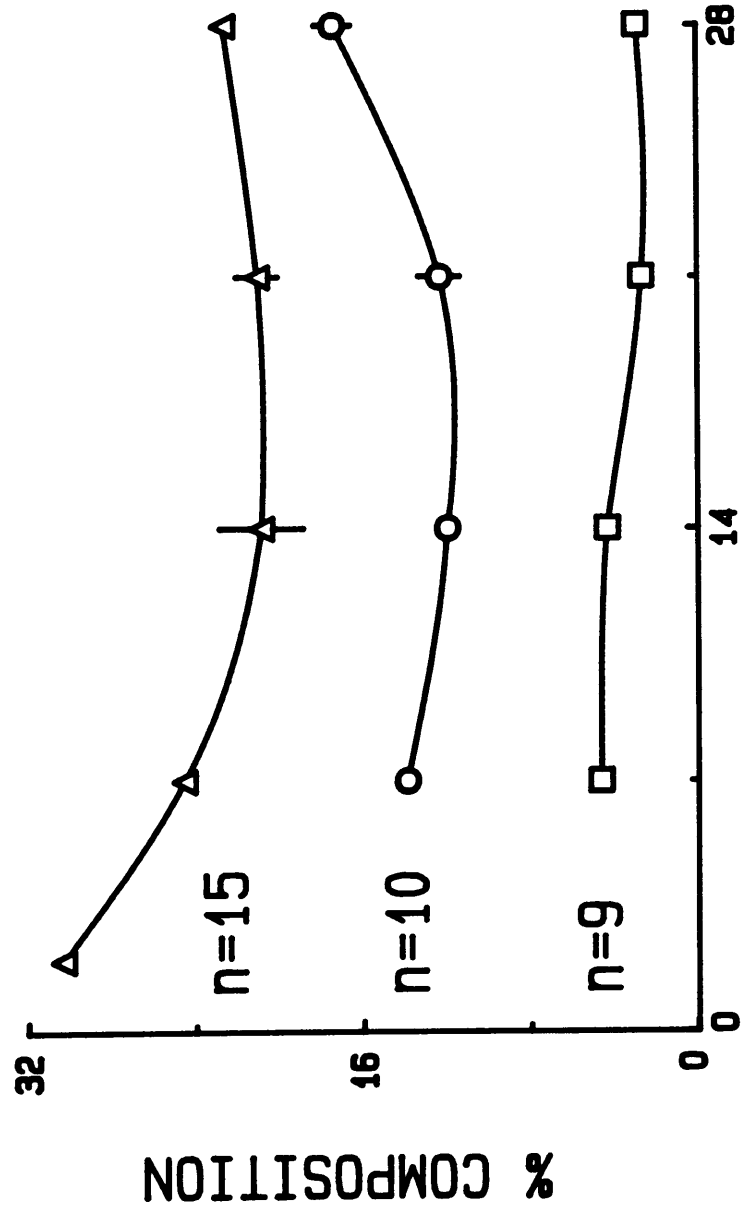


Fig. 1.2 Mean milk composition from day 2 through day 28 post-partum. % fat (triangles), % protein (circles), % lactose (squares). S.e.m. where not shown is less than the symbol height. n=minimum number of samples per sampling period.

Chapter 2

Mammary Gland Composition in Thrichomys apereoides

(Abstract)

Chemical and histological composition of mammary glands of Thrichomys apereoides were evaluated including: nucleic acid concentration (DNA and RNA), percent fat and percent protein. RNA concentration (ug./mg. mammary tissue) and the RNA/DNA ratio, two measures of synthetic activity of the gland, both peaked at day 14 of lactation, and started to decline by day 21. DNA concentration did not change over time. There was no significant difference in percent fat or protein of mammary tissue over time.

Mammary gland histology, supported the results of the chemical tests and milk yields as to the stage of development of the gland. Percent luminal space and stroma were inversely related, with epithelium being constant until the end of lactation. Luminal space peaked by day 14 at 52.5 $\pm$ 0.9 % , while percent stroma was at its lowest at 12.4 $\pm$ 1.5 %. Percent epithelium between sampling periods did not differ significantly until day 28, when it decreased from 35.3 $\pm$ 1.3 % at day 21 to 24.4 $\pm$ 1.1 % at day 28. Inguinal and lateral glands showed similar trends in mammogenesis, with the inguinals peaking (% lumina, % stroma), one week before the laterals. Involution of inguinal glands occurred by day 28, while laterals

involuted by day 35.

Introduction

Mammogenesis has been studied in various species: rats (Tucker and Reece, 1963a), mice (Brookreson and Turner, 1959; Mizuno, 1961), cows (Tucker, 1969), hamsters (Sinha et al., 1970) and sheep (Anderson, 1975). Macroscopic measures of mammary gland development have included: tissue weights, water displacement, and sonorays, the latter two most useful in udder-like glands. Histological evaluation of mammary tissue development has been reported for a variety of species (Chalkey, 1943; Akers et al., 1977).

The most common biochemical method to evaluate mammary development involves measurement of tissue nucleic acid concentrations (deoxyribonucleic acid-DNA, and ribonucleic acid- RNA). DNA content has been used as an index of total mammary cell number with the discovery that somatic cell DNA concentration is constant within a species (Kirkham and Turner, 1953). Because the amount of RNA in various body tissues is correlated with the rate of protein synthesis (Munford, 1964); and there is a strong correlation between mammary RNA content and litter weight gain (r=0.93), RNA concentration has been used as an estimator of glandular synthetic activity (Kirkham and Turner, 1953).

The lactation period of Thrichomys apereoides, a South

American hystricomorph rodent, is unusually long and milk composition is very differs from that of other studied rodents. Not only is time from conception to weaning time 40% longer (Roberts et al., 1988), but milk with high fat content is produced throughout lactation (ranges from 21.0-30.2% -Meyerson, this volume).

The objective of this study to quantify mammary gland development in T. apereoides chemically (nucleic acid conc., fat content, protein content) and histologically. Comparative consideration of these results may indicate the mechanism of this atypical lactational strategy in T. apereoides.

Materials and Methods

The Thrichomys apereoides used in this study were derived from the Biology Department colony at Virginia Polytechnic Institute and State University. The original stock was derived from a colony at the Smithsonian Institution's National Zoological Park in 1980. The animals were maintained on a photoperiod LD 16:8 at 20 $\pm$ 1 ^{o}C, and were provided lab chow (Ag Way 3000) and water ad libitum. Animals were housed in plastic tubs (30cm x 42 cm x 34 cm) with wood shaving bedding material and cages were cleaned weekly. Litters were randomly culled at birth to a litter size of three, and the sire remained with the dam and offspring.

Determination of gland composition involved histological and chemical measurements. Three females were killed with CO_2 at days-2, 7, 14, 21, 28, 35 post-partum. Female body and individual mammary gland mass were determined. Randomly selected mammary glands on one side of each female were used for the histological analyses, while the contra lateral glands were used for the chemical determinations.

Before sacrifice, each female was completely milked after injection of oxytocin (0.4 units). Mammary tissue samples (approx. 1 cm^3) were removed from each of the two lateral and one inguinal glands. Samples were transferred to a petri dish and immediately cut into 1 mm^3 blocks and placed for 6 hours in a fixative containing: 0.5 % formaldehyde and 1.3% glutaraldehyde in 0.1M phosphate. Samples were maintained in buffered phosphate until all samples were collected. Tissues were subsequently dehydrated in alcohol, washed in propylene oxide, and embedded in Epon 812-Araldite 502 Resin 50:50 (Akers et al., 1977; Geisleman and Burke, 1973). Thin-sections (approx. 1 micron) were cut on an ultramicrotome (Sorvall MT-1) and stained with Azure II for light microscopy.

Quantitative morphological analysis was used to determine the percent composition of the following mammary tissue and cell types: epithelial cells, alveolar lumena, and stroma (Akers et al., 1977; Chalkey, 1943).

Chemical analysis of the mammary tissue included: DNA, RNA, fat, and protein content. The DNA content of the gland was determined by using a modification of the Burton Method (Burton, 1956) for measurement of DNA in tissues, while the RNA was determined using an orcinol reaction to quantify ribose (Cerotti, 1955; Appendix-1). Mammary gland lipid content was extracted using chloroform/methanol and determined gravimetrically. Protein content was determined using a dye binding Bio-Rad Protein assay, with bovine serum albumin for the protein standard (Bradford, 1976).

Histological results were analyzed using an Anested analysis of variance procedure, to determine differences between the period of lactation and cell type percentage, between females and within subsections of the same mammary gland. Student T-tests were used to further evaluate data for which the ANOVA was significant. Chemical results were analyzed using the SAS GLM procedure (SAS, 1982). Student T-tests were used when appropriate.

Results

Relationships between mass specific mammary weight, RNA/DNA ratio and nucleic acid concentrations over the lactation period were were found (fig. 2.1). Significant changes in mass specific gland size occurred over time ($p < 0.04$, $F=3.49$). Mammary gland mass doubled between day 2 and day 7 of lactation with means of .01 and 0.02 g mammary

tissue/g body mass , respectively ($p < 0.05$, $T=2.16$). Thereafter, mass did not change significantly until a decrease on day 21 (0.013 g.) and remained low over the rest of lactation. Significant changes in mammary RNA concentration occurred over time ($p < 0.0004$, $F=5.73$). At day 2 post-partum RNA concentration was 7.75 ± 0.20 ug/mg. RNA conc. continued to increase through day 14 with 8.81 ± 0.46 ug/mg (fig. 1.1) A significant decrease occurred by day 21 ($p < 0.05$, $T=2.16$). Thereafter RNA content did not change. While mammary RNA concentration changed proportionate to body mass DNA content did not. DNA concentration did not differ significantly over time ranging from 1.43 ± 0.1 to 1.58 ± 1.6 ug/mg ($p > 0.84$, $F=0.40$). A second measure of synthetic activity RNA/DNA ratio was determined however since DNA concentration was relatively constant, the RNA/DNA was proportionate to the concentration of RNA (fig. 2.1).

Percent fat and percent protein of the mammary glands exhibited no significant changes in concentration over time (fig. 2.2 and fig. 2.3, respectively; $p > .07$, $F=1.84$; $p > 0.05$, $T=2.021$ respectively).

Thin sections of the mammary glands showed that glandular morphology changed markedly over the lactation period (fig. 2.4). At day 2 (fig. 2.4a) alveoli were apparent and epithelial secretory cells were cuboidal in appearance and uniformly lined the alveolar lumen. Luminal

spaces were small, although secretion is evident. Stromal tissue was abundant. By day 14 (peak lactation-fig. 2.4b) the luminal areas were larger, epithelial cells were typically flattened from secretion, and stromal tissue area reduced. By day 28 histological appearance of lateral and inguinal glands were markedly dissimilar (Fig. 2.5). Involution was beginning in the lateral glands (fig. 2.4c & 2.5a). Alveoli were smaller, epithelial cells appeared sloughed into the luminal spaces and stromal tissue was abundant. In contrast, where involution was only beginning in the lateral glands, it was almost complete in the inguinal (fig. 2.5b). By day 35 (fig.2.4d) both lateral and inguinal glands were involuted.

Quantitative analyses showed significant differences in percent epithelial, luminal, and stromal tissue types over the lactation period ($p < 0.003$, $F=9.15$; $p < 0.0001$, $F= 21.41$; $p < 0.0001$, $F=42.04$; respectively; fig. 2.6). At day 2 mammary tissue was comprised of 32.2 $\pm$ 0.2% epithelial cells , 36.1 $\pm$ 0.9% luminal space, and 31.4 $\pm$ 0.2% stromal tissue. By peak lactation, (day 14) there was a significant increase in luminal space (53.7 $\pm$ 2.7%) and decrease in stromal tissue (11.4 $\pm$ 2.9%) ($p < 0.05$, $T= 2.23$; $p < 0.05$, $T=2.23$; respectively). Mammary gland regression had begun by day 28, as evidenced by significant increases to 35.2 $\pm$ 1.1% stromal material and

decreases in luminal space to 40.9 $\pm$ 0.8% ($p < 0,05$, $T=2.23$). Percent epithelium did not change significantly until day 28, when it decreased to 24.0 $\pm$ 1.5% of the alveolar area ($p < 0.05$, $T=2.23$). Relative changes in histological development of inguinal and lateral glands were similar, although inguinal glands reached maximal development (ie. maximal luminal and epithelial area) 1 week before laterals (fig. 2.7).

Discussion

Measurements of total DNA content of mammary glands, have shown that mammogenesis continues through the beginning of lactation in most species studied, e.g. the guinea pig (Anderson et al., 1982), mice (Mizuno, 1961), and rats (Tucker and Reece, 1963b). In some species however, mammogenesis has been found completed by parturition (ie. the golden hamster, and sheep) in a few species. (Sinha et al., 1970; Anderson, 1975). Mammogenesis in Thrichomys apereoides was completed by day 2 post-partum; with no significant differences in either total glandular DNA or DNA concentration over the duration of lactation. Synthetic activity (RNA conc. and RNA/DNA ratio) peaked by day 14. This is coincident with observations of maximxal milk yield (Meyerson, this volume). Previous studies report that milk yield peaked at day 14 and started declined by day 21. Milk production ceased in the inguinal glands by day 28 and in the laterals

by day 35. Subsequent decreases in mammary RNA were also associated with periods of decreased milk output. Similar results are reported for the guinea pig (Cavea porcellus) where both mammary RNA and milk yield increased to a maximum by day 6 of lactation (Nelson et al., 1962). Changes in nucleic acid content and histological data changed in close correspondence. Peaks in the percent of the parenchyma occupied by luminal space occurred coincident with peaks in milk production (Meyerson, this volume). Increased luminal space was expected since additional storage space is required for the increased secretion (Schmidt, 1971). Since T. apereoides, like other echimyids, suckle for extended periods beyond the point of nutritional dependence the length of the lactation period is not obvious. Both 21 and 28 days are reported as the length of lactation (Roberts et al., 1988; Meyerson, M.S. Thesis, respectively). Roberts et al. (1988) determined lactation had ceased when 80% of cross fostered offspring were not successfully reared. It is possible that milk was produced but not at a level sufficient to support the fostered neonates. Meyerson (this volume) reported that lactation extended through day 28 but ceased by day 35 post-partum. Milk was collected by manual palpation after stimulation of the milk ejection reflex with exogenous oxytocin. Exogenous oxytocin has been

associated with maintaining mammary gland integrity (Oftedal, 1984; Benson and Folley, 1957). The effect of the oxytocin depending upon the amount and frequency of administration. Results from this study indicate that the lactation period extends beyond day 28, and is not an artifact resulting from exogenous oxytocin administration. Peaks in both alveolar luminal space and mammary synthetic activity of females which had no prior exposure to exogenous oxytocin corresponded to peaks in milk yield of females which had been exposed.

Physical stimulation provided by the milking process also has been shown to increased the length of lactation (Oftedal, 1984). Since Thrichomys suckle for 6 weeks (Roberts et al., 1988), mammary glands are stimulated by the suckling neonates beyond the point of nutritional weaning. Therefore it is unlikely that milking the dams weekly had any effect on mammary gland integrity.

In animals where mammogenesis is not complete at parturition, (eg. rat and mouse), changes in percent protein and fat of the mammary glands are reported (Wrenn, et al., 1965). With increasing gland size there is an increase in parenchyma tissue, therefore an increase in protein content of the gland. In rats percent fat in the gland declined from 67% in non-pregnant animals to 17% by the end of lactation. This decrease was due to the rapid increase in glandular tissue during gestation and lactation

(Wrenn et al., 1965). In T. apereoides no changes were found over the lactation period in percent fat, or protein of mammary tissue. With development completed by day 2 post-partum, significant changes were unlikely.

This study confirmed previous reports (Meyerson, this volume) that the period of milk production in Thrichomys apereoides exceeds 28 days but is less than 35 days in duration. Both histological and chemical evaluations show that the mammary gland tissue is still active by day 28 post-partum. Neither chemical or histological results differ from that observed in other studied rodents which produce milk substantially different in composition.

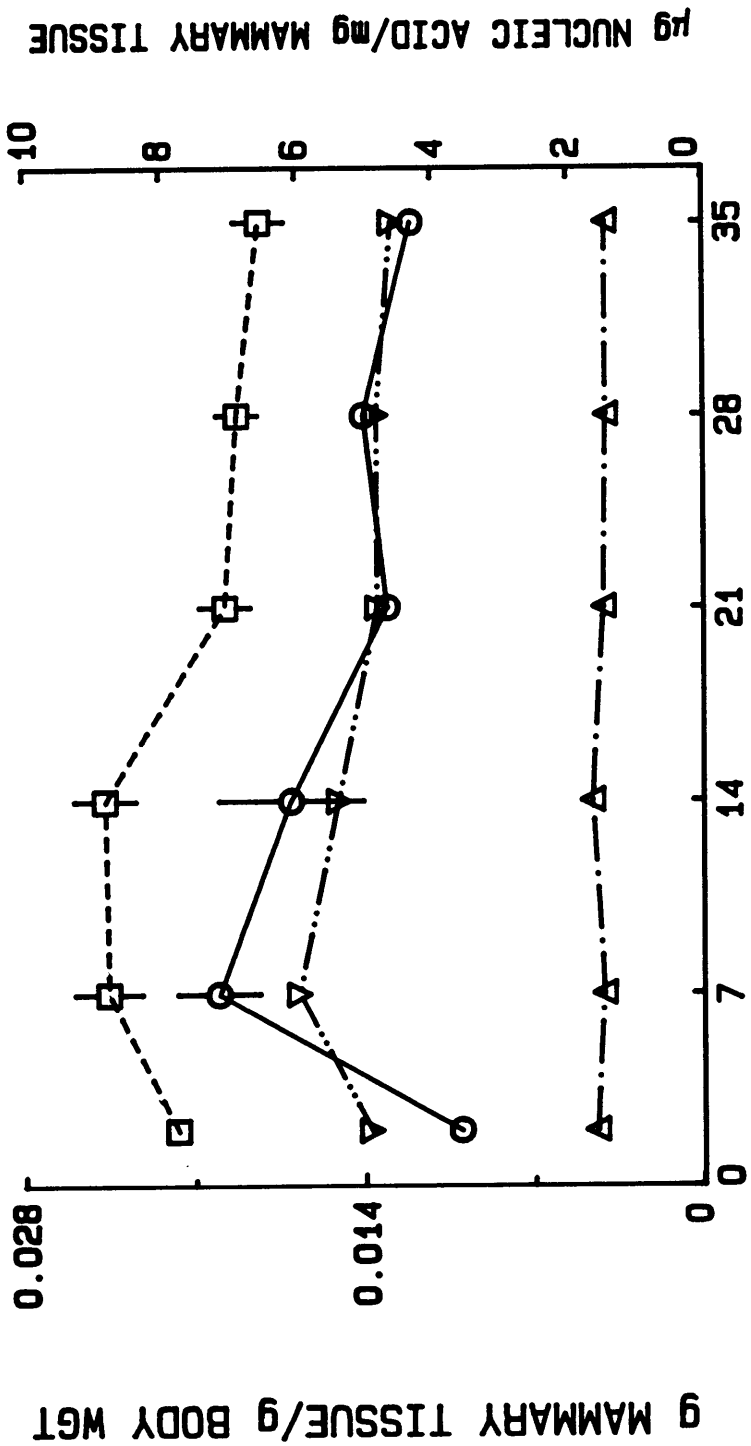


Fig. 2.1 Changes in mammary gland composition and mass over the 5 week period following parturition. ug RNA/mg mammary tissue (squares), ug DNA/mg mammary tissue (triangle-base down), g mammary tissue/g body (circles), ug DNA/ug RNA (triangles-base up). All glands were pooled for each sampling period. S.e.m. where not shown is less than the symbol height.

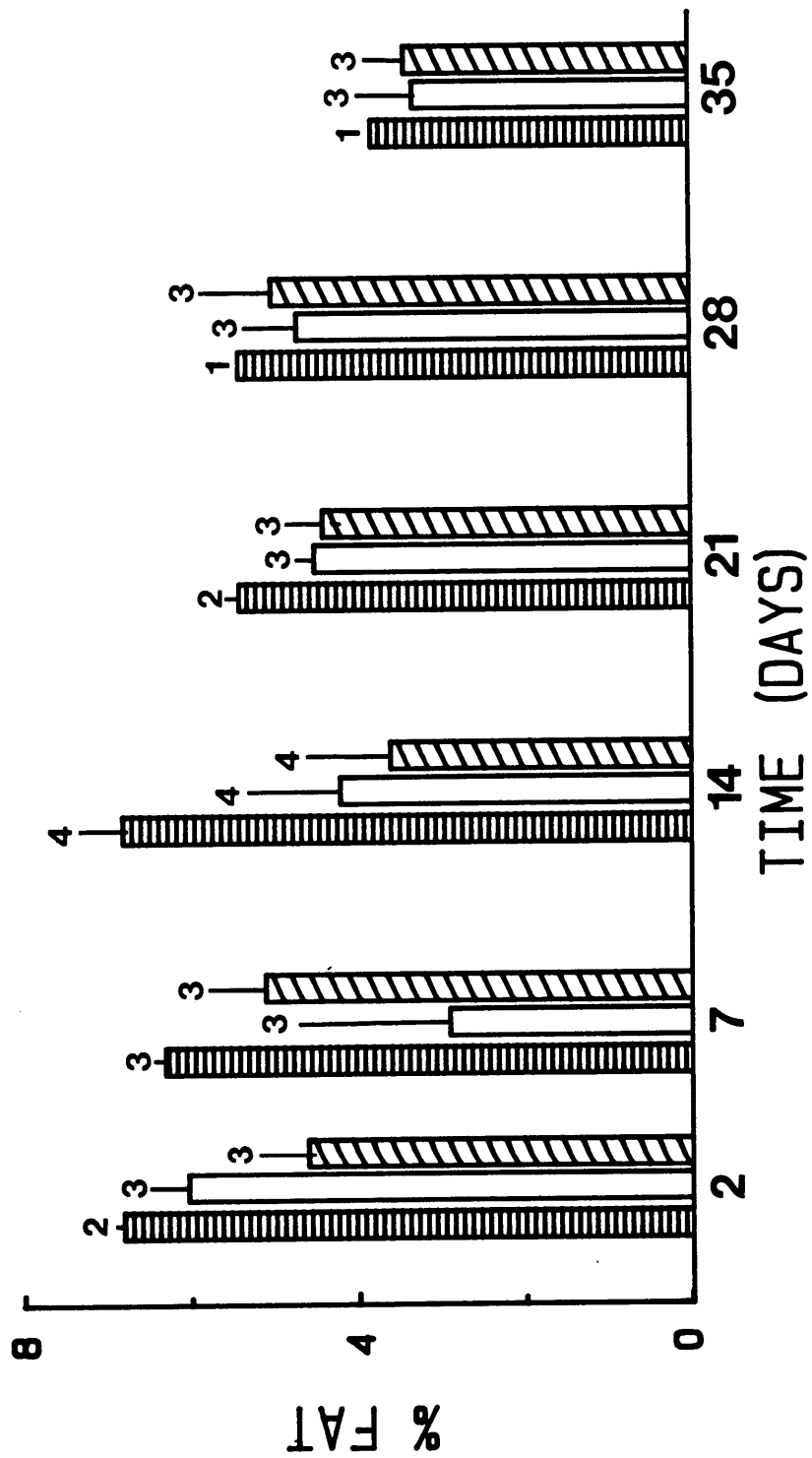


Fig. 2.2 Differences in percent fat of the mammary glands over the lactation period. Inguinal glands (horizontal bars), ant. lateral glands (open bars), post. lateral glands (slanted bars). Data expressed as mean $\pm$ s.e.m. Number over the error bars is the sample size (n).

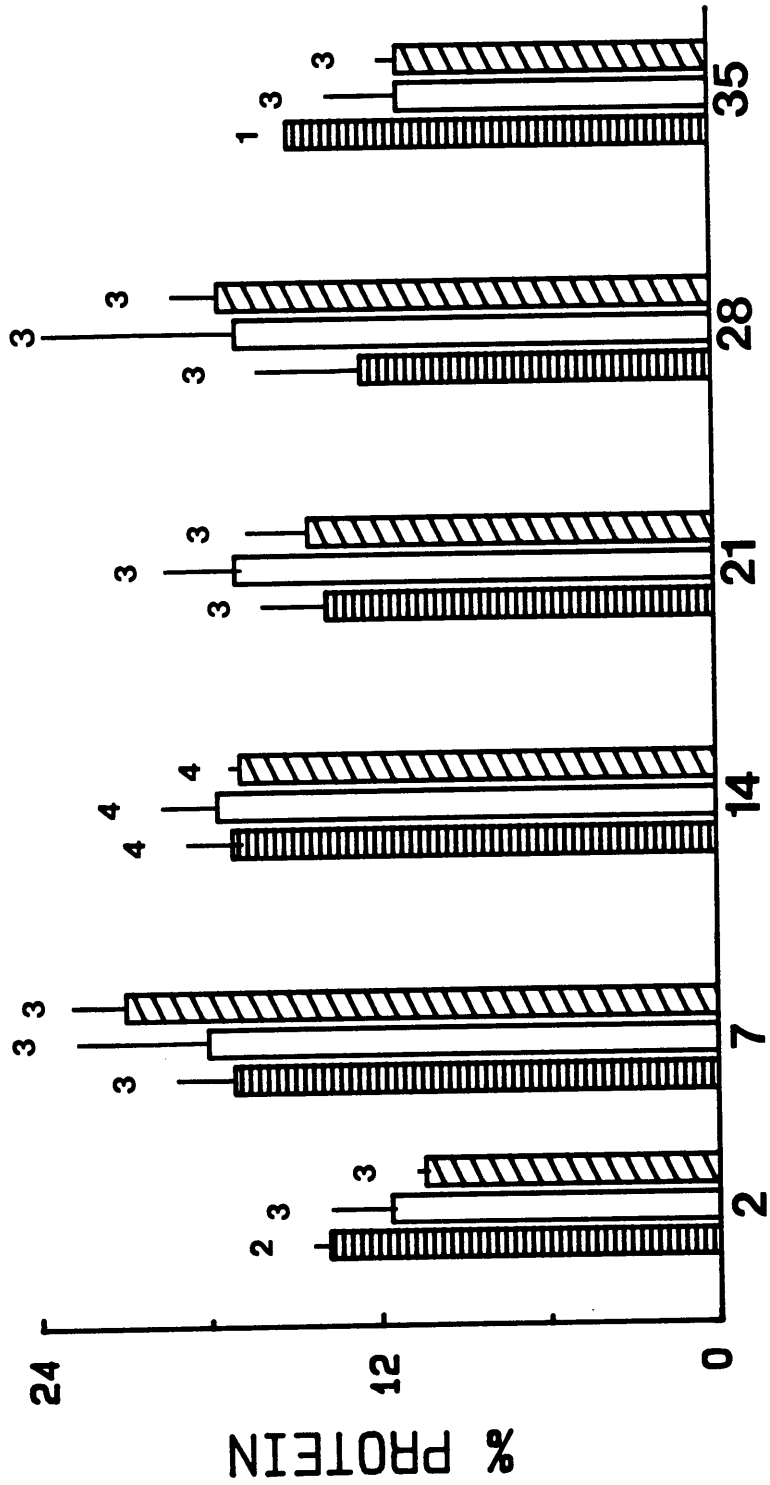


Fig. 2.3 Differences in percent protein of the mammary glands over lactation. Inguinal glands (horizontal bars), ant. lateral glands (open bars), post. lateral glands (slanted bars). Data expressed as mean ± s.e.m. Number over the error bars is the sample size (n).

Fig. 2.4 Lateral mammary gland anatomy in a lactating female from day 2 through day 35 of lactation.
(a) mammary development at day 2 post-partum.
(b) mammary development at peak lactation, day 14.
(c) mammary development at early involution, day 28.
(d) involuted mammary gland, day 35.
S=stromal tissue (all tissue excluding luminal space and epithelial cells), E=epithelial cells (a) lining lumen, (c) sloughed into lumen, M=milk. Magnification=25X

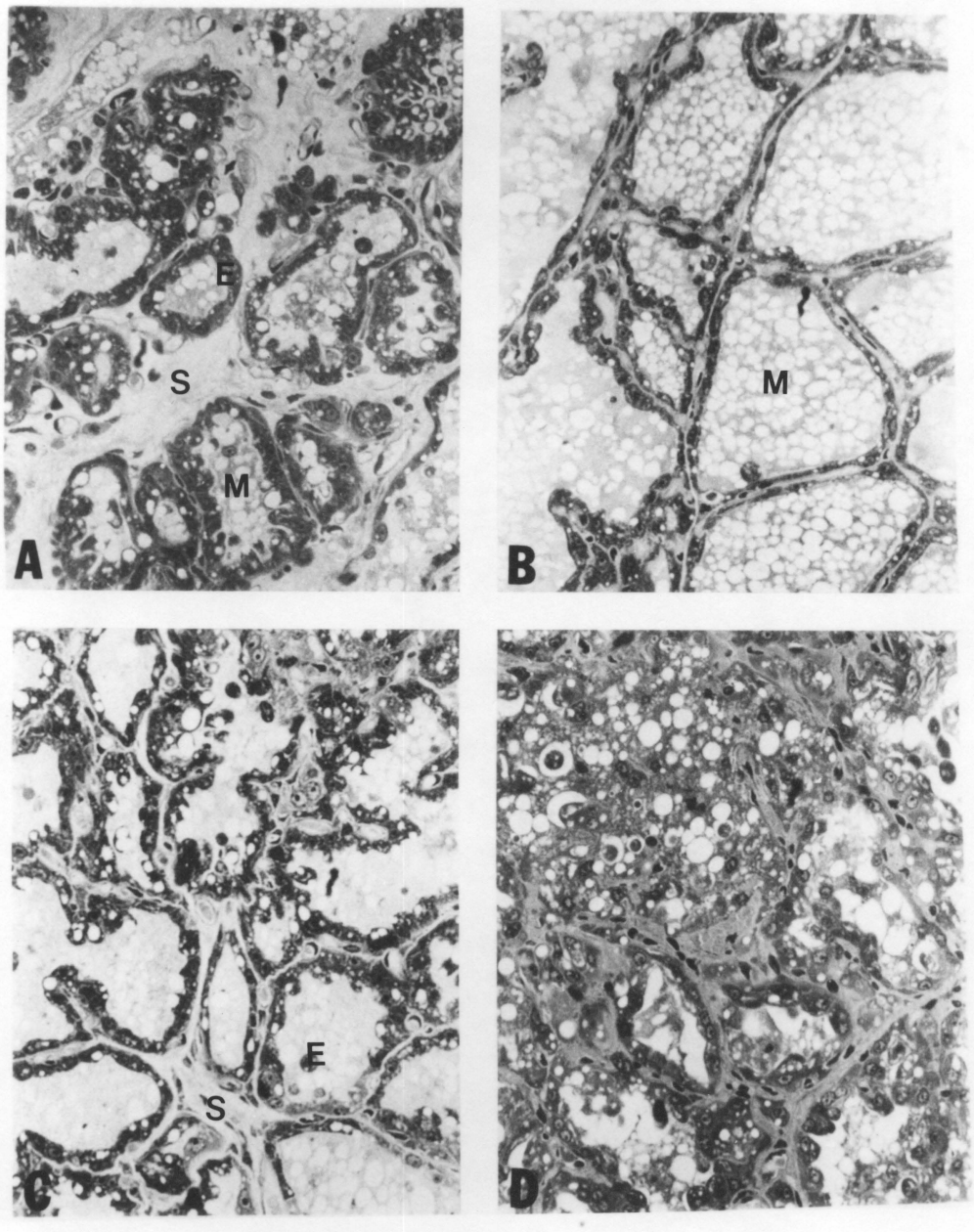
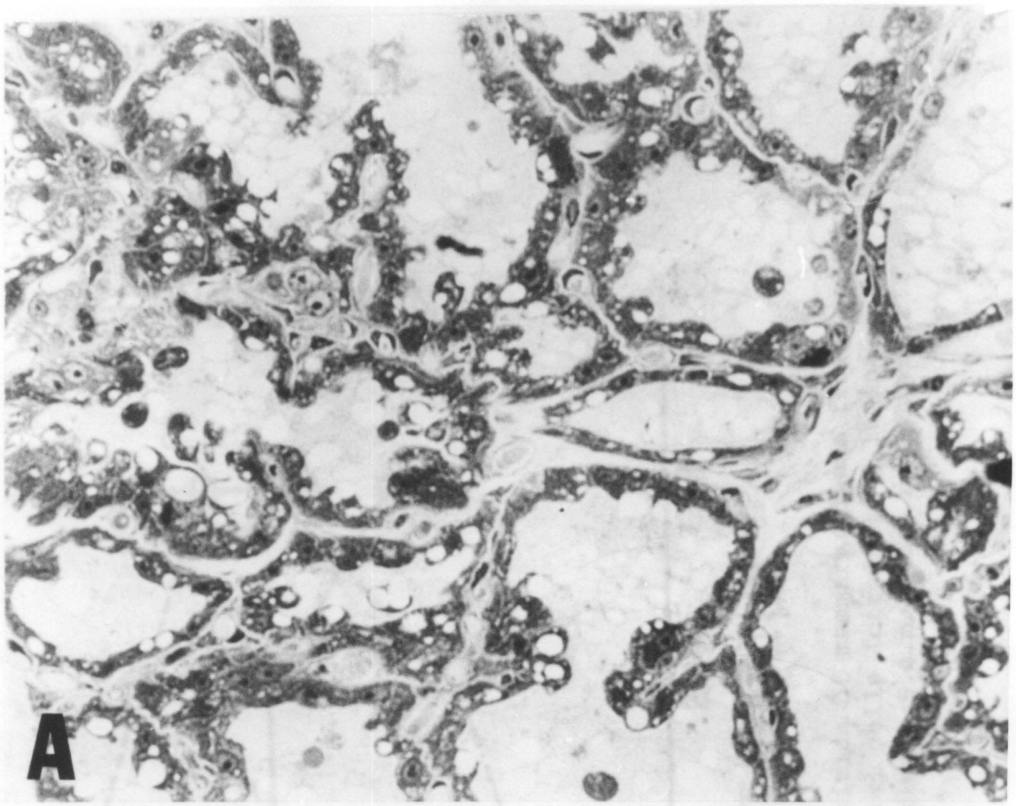
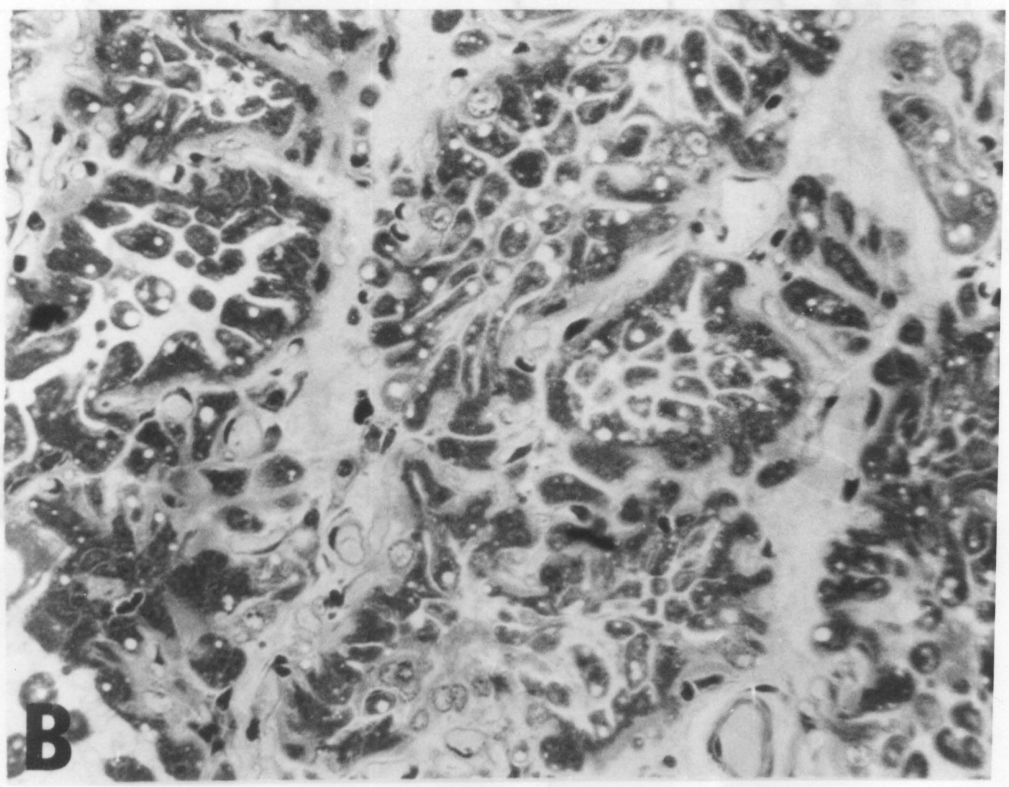

E
S
M
A
M
B
E
S
C
D

Fig. 2.5 Stage of development of the inguinal (a) and lateral (b) mammary glands at day 28 of lactation. Magnification=40X.


A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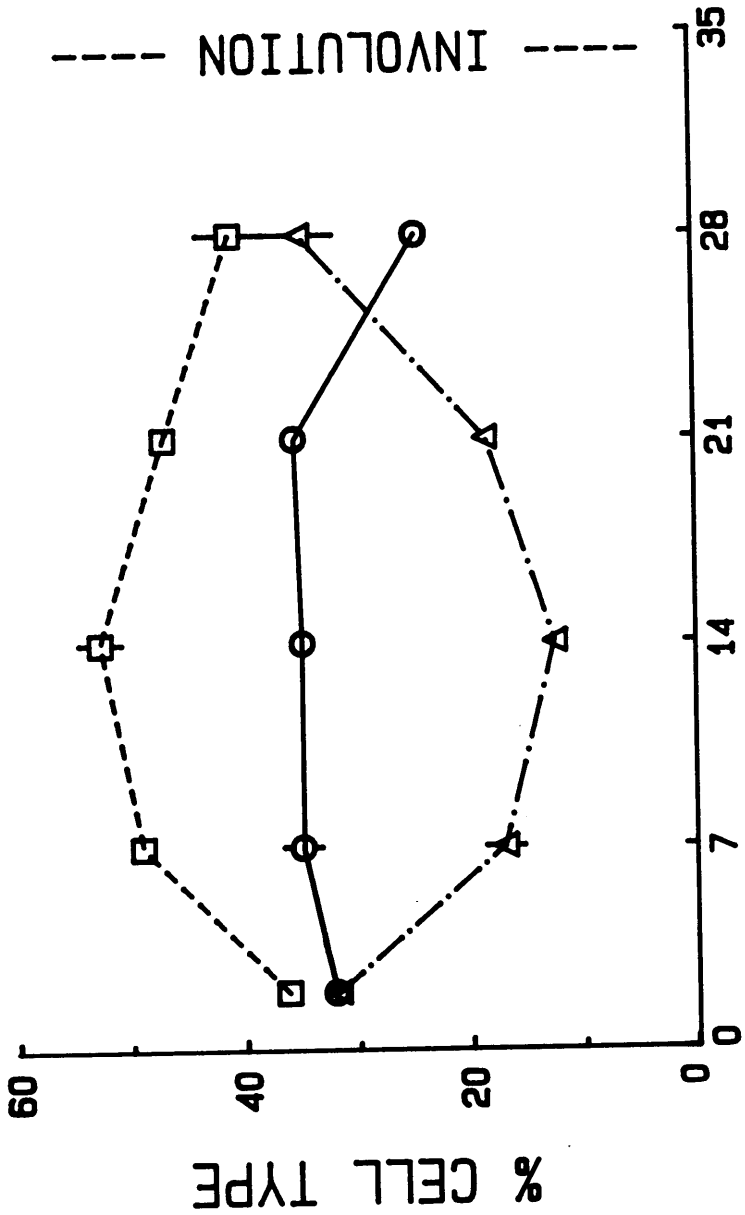

Fig. 2.6 Quantitative histological composition of mammary glands from day 2 through day 35 of lactation. % epithelial cells (circles), % luminal space (squares), % stromal tissue (triangles). S.e.m. where not shown is less than height of the symbol.

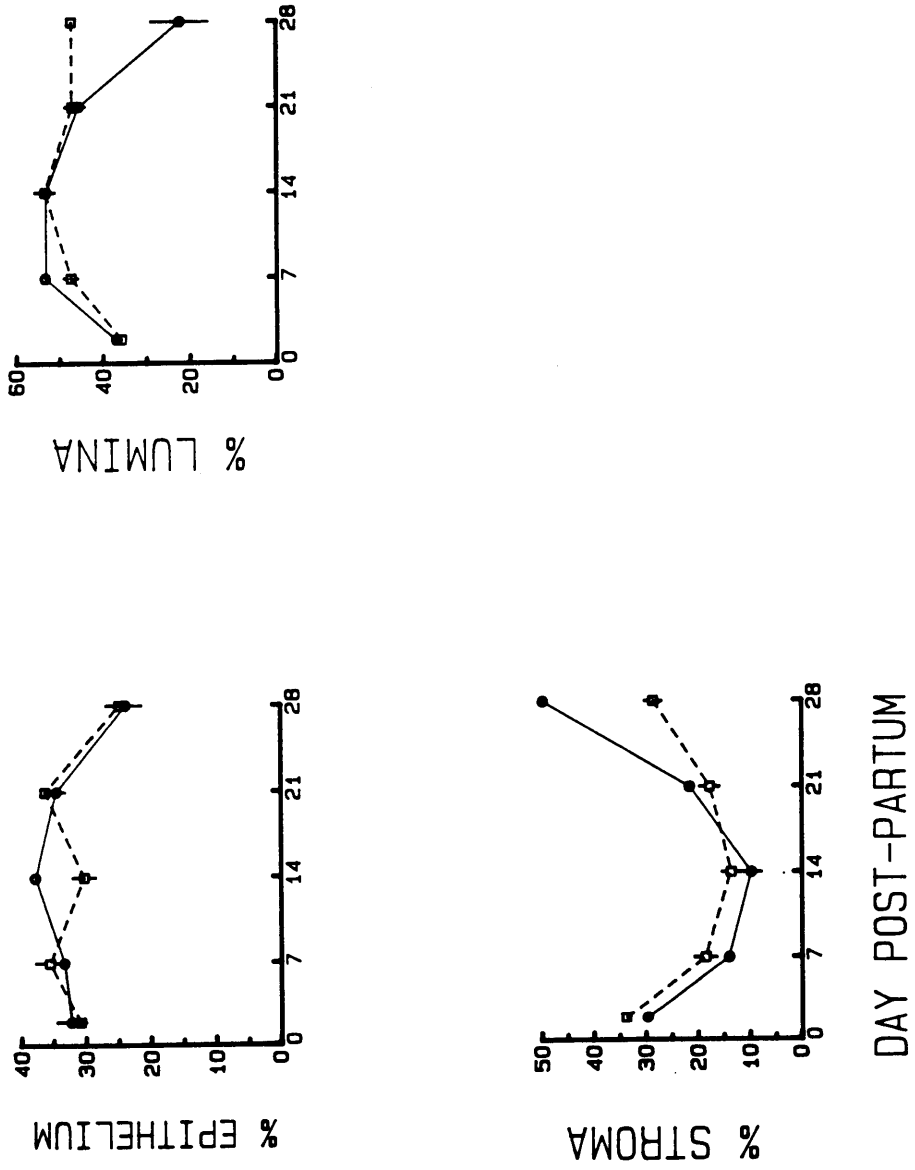

Fig. 2.7 Quantitative histological comparisons of the percent of epithelial cells, luminal space, and stromal tissue, between the inguinal (solid lines) and lateral (dashed lines) glands. S.e.m. where not shown is less than the height of the symbol.

SUMMARY

1. Milk composition is unusual for a rodent. Fat content is significantly higher and lactose is lower. Protein content is normal.

2. The nutritional suckling period of 28 day is longer than that previously reported for this and related species.

3. Milk yields peak at day 14 post-partum, decreases through day 21 and ceases by day 35.

   a. inguinal glands had greatest yield at early lactation, and ceased milk production by day 28.

   b. lateral glands yield peaked at day 14 and ceased milk production by day 35 post-partum.

4. High fat and low lactose content is possibly an adaptation to life in a xeric, unpredictable environment.

   a. increased milk fat would decrease potential stress on maternal water reserves.

   b. neonates would produce the greatest amount of metabolic water from the metabolism of high fat milk.

   c. the extended period of lactation may insure that both a source of nourishment is available to the neonates if little or none is available in the environment.

5. The total mass of inguinal glands was significantly less than the laterals.

6. When expressed on a mass specific basis, mammary gland mass changed significantly over time.

7. An inverse relationship between percent luminal space and percent stroma was found.

8. DNA concentration did not change from day 2 to day 35 post-partum indicates mammogenesis was complete by day 2 post-partum.

9. Increased milk production corresponded with a peak in total gland mass, RNA conc., RNA/DNA ratio, and percent alveoli.

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Appendix 1 - Assay procedures for experimental work presented in chapters 1 and 2.

Lactose Assay

(Modified from Perry and Doan, 1950)

1. Thaw skimmed milk sample.
2. Pipet 100 ul. of skimmed milk into a test tube.
3. Add 9900ul of picric acid to each tube and shake.
4. Centrifuge the tubes for 15 min. at 2,300 x g.
5. Transfer 2 ml of the supernatant to a 20 ml Meyers and Baily Sugar tube, containing 1 ml sodium bicarbonate.
6. Tubes are then shaken, stoppered with marble and placed in boiling water bath for 20 min.
7. A blank consisting of 2 ml of picric acid and 1 ml sodium bicarbonate is heated along with unknowns.
8. Remove and cool to room temperature (5 min) and dilute to 20 ml with distilled water.
9. A portion is then transferred to a colorimetric tube for reading at 520 nm.

Notes:

A standard curve of values- 0.625, 1.25, 2.5, 5.0, and 10% lactose (in distilled water) was used.

Sodium bicarbonate- 25 g. of annhydrous compound dissolved and brought up to 100 ml with distilled water (store at room temperature).

Modification of the Burton Method for Measurement of DNA in Tissues

1. Weigh mammary tissue (at least 1-2 gm.) and record weights. Keep tissue cold and chop into small pieces with razor blade.
2. Transfer the tissue to a square homogenizing vessel and add approx. 3 ml. of ice cold distilled water.
3. Homogenize tissue using Brinkman Polytron, two or three 15 sec. bursts at moderate speed.
4. Filter homogenate through small tea strainer to remove connective tissue.
5. Bring homogenate to total volume of 10 ml. with cold distilled water.
6. For use in Burton assay pipett 2 ml. of homogenate into each of 2 labeled test tubes.
7. Transfer remaining homogenate to labeled container and store in freezer.
8. At this point the samples may be frozen for susequent assay.

Nucleic Acid Extraction

9. Add 1.0 ml of 1N Perchloric Acid (PCA) ($HClO$) to each tube to be extracted on ice.
10. Allow samples to incubate on ice stirring every 10 min. using a glass rod-do not vortex.
11. Centrifuge tubes at 3000 rpm (approx. 1,800 x g.) for 20 min.

12. Carefully decant supernatant and discard.
13. Gently add 2.0 ml. of 0.25N PCA to wash pellet and discard wash. Repeat for 3 washes and 2 spins.
14. Add 2.0 ml. of 0.5 N PCA to the pellet, and resuspend, using glass rod.
15. Incubate tubes at 70 ℃ water bath for 20 min. Stir occasionally with glass rod.
16. Cool tubes in ice water bath for 10 min.
17. Centrifuge tubes at 3000rpm for 20 min.
18. Use pasteur pipette and transfer supernatant to a clean labeled test tube (16 x 100).
19. Add additional 2.0 ml of 0.5 N PCA to pellet, mix with glass rod.
20. Incubate tubes for 20 min. in 70°C water bath.
21. Cool tubes in ice water bath for 10 min. as before.
22. Centrifuge tubes at 3000 rpm for 20 min.
23. Remove supernatant and combine with supernatant obtained from first extraction.
24. At this point the DNA and RNA solution may be frozen for subsequent assay.

DNA Assay Diphenylamine Method of Burton
(Burton, 1956)

Standard DNA Solution

Stock solution is prepared by dissolving 40 mg calf thymus DNA in 100ml of 4.0 mM NaOH ie. 400 ug/ml. From this stock solution stds for assay are prepared by mixing equal volumes of the stock DNA with 1 N PCA and heating at 70 $^{\circ}$C for 30 min, Consequently, stds for actual will contain 200ug/ml. They can be stored for at least 6 months in the cold without apparent deterioration.

Standard curve should include volumes of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0 ml. These correspond to values of 5, 10, 20, 40, 80, 160, 220 ug of DNA. Blanks with 1 ml 0.5 N PCA must be included. Standards and unknowns are brought to 1 ml volume with .5 N PCA.

Diphenlyamine Reagent

Solution A: In a graduated cylinder (glass) dissolve 1.5 g diphenylamine in 100 ml of glacial acetic acid and add 1.5 ml of concentrated sulfuric acid. The solution is stored at room temperature in the dark.

Solution B: In a ventilated hood, using a glass pipette, pipet 2.0 ml ice cold acetaldehyde into a glass bottle containing 98 ml Double distilled H_2O. Store acetaldehyde (VOLATILE) and dilute acetaldehyde in refrigerator.

Immediately before assay add 0.1 ml of solution B to 20 ml solution A.

ASSAY-(use 16 x 100 mm glass tubes)

1. Construct a standard curve with 0.025-1.0 ml of DNA standard.
2. In duplicate pipet 250ul of each unknown into test tubes.
3. Adjust final volume of samples and standards to 1 ml with 0.5N PCA including at least 2 blanks of 0.5 N PCA.
4. In hood, pipet 2.0 ml of diphenylamine reagent per test tube.
5. Vortex tubes, cover with foil, incubate at 30°C for 16-20 hr.
6. Measure optical densities at 600 nm.

RNA Analysis-Orcinol Method
(modified from Cerotti, 1955)

Standard RNA Solution

1. Weigh out 0.0125 g highly polymerized calf liver RNA.
2. Add RNA to 50 ml volumetric flask and bring to volume with 0.5 N PCA for a conc. of 0.25 mg/ml.

Orcinol Reagent

Solution A: Dissolve 0.80 g FeCl*6H20 in 250 ml conc. HCl.

Reagent: Dissolve 1.0 g orcinol in 100 ml of solution A.

Assay- (use 16 x 100mm glass test tubes)

1. Construct standard curve with standard RNA solution:40, 100, 200, 400, 800, 1000 ul.
2. Pipet 250 ul of each unknown into test tubes.
3. Adjust final volume of all tubes to 1.0 ml with 0.5 N PCA, including 2 blanks of PCA.
4. Pipet 2.0 ml of the Orcinol reagent into each test tube (IN HOOD WEARING GLOVES).
5. Vortex tubes, cap with marbles and incubate in boiling bath for 30 min.
6. Cool tubes in cold water bath to room temperature.
7. Measure optical densities at 670 nm.

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