ABSTRACT

THE NORMAL MAMMARY GLAND DEVELOPMENT

OF RATS 10 TO 100

DAYS OF AGE

by Yagya Nand Sinha

Normal development of the rat mammary gland between 10 and 100 days of age was studied using mammary area and mammary deoxyribonucleic acid (DNA) content as indices of growth. Mammary area was measured from whole-mounts of the right abdominal mammary gland with a polar compensating planimeter. DNA was extracted by a modified Schmidt and Thannhauser procedure. Ribonucleic acid (RNA) and lipid content of the gland were estimated simultaneously.

The mammary gland area increased from 9.26 mm^2 at 10 days to 450.93 mm^2 at 100 days of age. This increase was allometric relative to either the body surface area (α =3.00) or body weight (α =1.95) of the animal.

The total DNA content of the mammary fat pad, containing the six abdominal-inguinal mammary glands increased from 0.33 mg at 10 days to 3.52 mg at 100 days of age. This increase was isometric (α =1.04) relative to body weight but allometric (α =1.56) relative to body surface area of the animal. No change in the slopes of log DNA plotted against either log (body weight) or log $(\text{body weight})^{2/3}$ was observed. This indicated a constant growth rate of the mammary fat pad between 10 and 100 days of age.

The DNA/100 g body weight increased from 1.53 mg at 10 days to 1.70 mg at 100 days of age. The slope of the linear regression curve was 0.001 which suggested that DNA/100 g body weight remained constant between

10 and 100 days of age.

The correlation of total mammary fat pad (TMFP)-DNA with mammary area was 0.82 ($P < 0.01$).

The total RNA content of the TMFP increased from 0.27 mg at 10 days to 2.56 mg at 100 days of age and these increases paralleled those of total DNA.

The RNA/DNA ratio tended to decline as age advanced suggesting that protein synthesis per mammary fat pad cell was retarded with advancing age.

The total lipid content of TMFP increased from 214.9 mg at 10 days to 2,163.1 mg at 100 days of age. The growth of the mammary gland appeared to be associated with a disappearance of the adipose tissue.

The total DNA content of the specific mammary area (SMA), containing the left and right abdominal mammary glands increased from 0.06 mg at 10 days to 0.53 mg at 35 days of age. This increase was 1.26 times faster than body weight increases and 1.89 times faster than body surface area increases. No change in the slopes of log DNA plotted against either log (body weight) or log $(\text{body weight})^{2/3}$ was observed which suggested that mammary gland growth, based on DNA, occurred at a constant rate between 10 and 35 days of age.

The DNA/100 g body weight of SMA increased from 0.28 mg at 10 days to 0.49 mg at 35 days of age and followed a pattern similar to the total DNA.

The correlation of SMA-DNA with mammary area was 0.77 ($P < 0.01$).

The total RNA content of the SMA increased from 0.05 mg at 10 days to 0.38 mg at 35 days of age and these increases paralleled those of total DNA.

The RNA/DNA ratio did not reveal any significant trend among the age groups, implying that the rate of protein synthesis remained almost constant between 10 and 35 days of age.

Dedicated to the memory of

my mother

Late Rajeshwari Sinha

THE NORMAL MAMMARY GLAND DEVELOPEMENT OF RATS 10 TO 100 DAYS OF AGE

By

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BIOGRAPHICAL SKETCH

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INTRODUCTION

In the past, many investigators have used rather subjective techniques to measure mammary gland growth. Recently, however, measurements of the mammary gland area or deoxyribonucleic acid (DNA) content have been used as quantitative indices of mammary gland growth. Mammary area measurements have been restricted primarily to studies of mammary growth before the onset of pregnancy, whereas DNA has been used to study mammary gland development under normal and experimental conditions of pregnancy, lactation, and involution. However, no systematic attempt has been made to study normal mammary gland development, using DNA as an index of growth, in young animals before the onset of pregnancy. Furthermore, mammary DNA and mammary area measurements have never been compared.

Experiments, therefore, were designed to determine the DNA content and the area of the mammary glands of rats between 10 and 100 days of age. Ribonucleic acid (RNA) and lipid content were estimated during this period of mammary gland growth to determine the changes in the protein synthetic activity and the role of the fat pad, respectively.

REVIEW OF LITERATURE

Normal Development of the Mammary Gland

Turner (1939), Folley (1952), Reece (1958), Mayer and Klein (1961), and Cowie and Folley (1961) have reviewed the growth of the mammary gland during different stages of sexual development.

Embryonic and fetal development. The embryonic and fetal development of the mammary gland of a large number of mammals, including man, was studied in the latter half of the nineteenth century by such workers as Huss (1873), Gegenbaur (1876), Rein (1881), and Klaatsch (1884). Later, Brouha (1905) studying bats and man, Lustig (1916) studying man, Myers (1917) studying rats, Gisler (1922) studying cats, Uehlinger (1922) studying horses, Turner (1930, 1931) studying cattle, and Turner and Gomez (1933a) studying mice, guinea pigs (1933b), and goats (1936) carried out extensive investigations of embryonic mammary development. In general, these studies indicated that the course of gland development was quite similar among species. The more important developmental stages included the formation of the mammary band or streak by a thickening of the ectoderm on either side of the ventral mid-line. The mammary streak was then transformed into the mammary line. Mammary buds appeared along this line and these buds determined the number of glands that eventually developed. The mammary bud invaginates from its proximal end to form the primary sprouts. The primary sprouts then were canalized. Secondary and tertiary sprouts proliferated from the proximal end of the primary sprouts. Henneberg (1900) observed in the albino rat the appearance of the mammary streak in eleven-day embryos. In the albino mouse, Turner and Gomez (1933a) observed that the appearance of the mammary streak occurred at 10 days, the mammary line at 12 days,

the mammary bud at 14 days, and the secondary sprouts at 20 days of age. Continued growth of these sprouts formed the duct system of the mammary gland. The extent of sprout development and the amount of canalization of the fetal mammary gland depended upon the length of the intra-uterine period of the fetus (Reece, 1958).

From birth to puberty. Puberty is defined as that age when the animal commences to release ova or becomes capable of bearing young. In primates, the accepted sign of puberty is the first menstruation, in rats, it is the vaginal introitus (Everett, 1961) and concurrent starting of the estrous cycle which occurs about the 35th to 42nd day of age. Great variations, however, in the onset of puberty are found according to breed, environment, and individuals within species.

At birth, the mammary gland is rudimentary and consists of a restricted duct system. From birth to puberty, the type of mammary gland growth appears to be species-specific. In the rabbit (Ancel and Bouin, 1911), cat (Turner and DeMoss, 1934), and dog (Turner and Gomez, 1934) the mammary gland retained its infantile characteristics exhibiting only a small degree of ductular growth between birth and puberty.

On the other hand, in species such as the rat (Myers, 1917), extensive growth of the duct system has been observed during the 4th and 5th weeks after birth although this observation was interpreted to be of doubtful significance. Similar growth patterns have been observed for the guinea pig (Turner and Gomez, 1933b), mouse (Gibson, 1930; Turner and Gomez, 1933a; Cole, 1933; Weiser, 1934), calf (Hammond, 1927), and rhesus monkey (Folley, Guthkelch, and Zuckerman, 1939).

Relative mammary growth analysis in monkeys (Folley et al., 1939),

rats (Cowie, 1949; Silver, 1953), and mice (Flux, 1954) revealed that the mammary duct growth during early life, as measured by mammary gland area, increased at the same rate as the body surface area increased (isometry). However, the mammary gland growth shifted in advance of puberty to a rate significantly greater than body surface area increases (allometry). In rats, this allometric growth occurred about the 23rd day of age. The mammary DNA content of rats increased in a linear manner between 21 and 42 days of age (Tucker, 1962).

Following puberty and before pregnancy. During this period, mammary gland development is influenced by the type and number of estrous cycles experienced by the species in question.

Myers (1917) using rats and Gardner and Strong (1935) using mice observed large increases in duct development about the 10th week of age. Very little further development was observed after this period.

Sutter (1921) observed a relationship in rats between the stage of the estrous cycle and appearance of the mammary gland. During estrus there was sprouting of the ducts as well as formation of new duct buds. Mammary regression occurred at metestrus although successive cycles exerted a cumulative effect on the gland growth. In the mouse, Bradbury (1932), Turner and Gomez (1933a), Cole (1933), and Wieser (1934) observed similar changes with the estrous cycle stage. During estrus, there was marked elongation of the end-buds and distension of the ducts with fluids, followed by regressive changes of the gland during metestrus.

In animals which have long estrous cycles (15-30 days), where one would expect some growth of the alveoli, very little, if any, alveolar proliferation was observed. Hammond (1927) did not mention finding

alveoli in virgin heifers. Turner and Gomez (1936) found extensive arborization of the ducts in goats, yet no true alveoli. Turner and Gomez (1933b) observed many duct buds but no formation of alveoli in nulliparous guinea pigs. Speert (1948) has described the cyclic changes in the mammary gland of rhesus monkeys with respect to the menstrual cycle, but no similar studies have been conducted in humans (Reece, 1958).

In species such as the rabbit, which are in constant estrus, indicating an indefinite follicular phase, marked development of the duct system with terminal enlargements occurred (Ancel and Bouin, 1911). However, in the ferret, Hammond and Marshall (1930) observed no significant development of the duct system.

During pregnancy. In primiparous animals, the extent and type of growth that occurs is influenced by the number of sexual cycles prior to conception (Reece, 1958). However, the pattern of growth is strikingly similar in most species. Lane-Claypon and Starling (1906), Ancel and Bouin (1911), Schil (1912), and Hammond and Marshall (1914) observed that growth of the duct system in primiparous rabbits continued throughout early gestation and this growth was followed by a rapid proliferation of the lobule-alveolar system which was completed by mid-pregnancy. In other species such as the rat (Turner and Schultze, 1931), mouse (Turner and Gomez, 1933a), guinea pig (Turner and Gomez, 1933b), cow (Hammond, 1927), and goat (Turner and Gomez, 1936) similar observations were recorded.

On the other hand, Cole (1933) and Jeffers (1935) studying mice and rats, respectively, found mammary gland hyperplasia to occur throughout pregnancy. Similarly, Reece and Warbritton (1953), using the colchicine

technique, observed mitotic activity throughout gestation in the rat, the maximum rate of mitosis occurring on the 10th day. DNA analyses have further confirmed this observation. Griffith and Turner (1959; 1961a) found that 20.9% of the total mammary growth in rats occurred during the first 10 days of pregnancy and 59.3% occurred by the 20th day of pregnancy. Development was 98.3% complete by the third day of lactation. Similarly, Tucker and Reece (1963a) observed consecutive increases in mammary DNA throughout pregnancy, representing a total 261% increase over virgin controls. In mice, Brookreson and Turner (1959) found that 30.6% of the total growth occurred during the first 12 days of pregnancy, 45.7% occurred during the last one-half of pregnancy, and 21.9% additional growth occurred between parturition and day 14 of lactation. In the rabbit, Denamur (1961) observed a rapid increase of mammary DNA during pregnancy. In multiparous guinea pigs, Nelson, Heytler, and Ciaccio (1962), however, found only a slight increase in the DNA content during pregnancy.

During lactation. Loeb and Hesselberg (1917) and Kuramitsu and Loeb (1921) found that mitotic activity ceased as soon as intense lactation commenced in guinea pig mammary glands. Cole (1933) detected no mitoses seven days after parturition in the mouse. Altman (1945) using cows, and Maeder (1922) and Weatherford (1929) using rats, rarely found mitoses during lactation, whereas Jeffers (1935) occasionally observed mitoses. Reece and Warbritton (1953) observed mitoses in less than 1% of the secretory cells on the fifth day of lactation in the rat. On the basis of these observations, Reece (1958) concluded that the increases in milk production during lactation were largely due to an increase in the

secretory rate rather than further growth of the mammary gland.

Recent DNA studies presented a somewhat different picture of lactational mammary growth. Greenbaum and Slater (1957) using rats found that the DNA-P content doubled between the end of pregnancy and the third day of lactation. Griffith and Turner (1959; 1961a) found that mammary hyperplasia was only 59.3% complete by the end of pregnancy, whereas mammary development by the third day of lactation was 98.3% complete. Tucker and Reece (1963b) using hooded Norway rats found that the total DNA/100 g of body weight increased 9.8% between the 20th day of pregnancy and the first day of lactation and increased 25.9% between the first and fourth day of lactation. Maximum mammary development occurred on the 12th day of lactation and 24.8% of the total mammary growth occurred during lactation. In other species such as the mouse (Lewin, 1957; Brookreson and Turner, 1959), rabbit (Denamur, 1961), and guinea pig (Nelson et al., 1962) a similar increase in DNA content during early lactation was observed.

After the peak of lactation has been reached, the amount of milk secreted gradually decreases. Histological studies suggested a decline in the secretory activity of the mammary parenchyma. Lenfers (1907), Kuramitsu and Loeb (1921), Hesselberg and Loeb (1937), Cole (1933), Jeffers (1935), and Blackburn and Macadam (1954) reported a gradual loss of secretory tissue during lactation.

The recent DNA studies of Smith and Richterich (1958), Brookreson and Turner (1959), Moon (1960), Turner (1960), Denamur (1961), and Nelson et al. (1962), in agreement with the histological studies, suggested a reduction in the number of cells with advancing lactation

but Tucker and Reece (1963b) observed no significant decrease in mammary DNA until after the 24th day of lactation when adequate suckling stimulus was provided.

During involution. The involutionary changes that occurred in the mammary gland have been studied using histologic techniques (Myers and Myers, 1921; Kuramitsu and Loeb, 1921; Maeder, 1922; Turner and Gomez, 1933a; Cole, 1933; Fekete, 1938; Hooker and Williams, 1940; Williams, 1942; Hesselberg and Loeb, 1937; Turner and Reineke, 1936; Reece and Warbritton, 1953). The mode of regression of the mammary glands in most species was quite similar (Turner, 1939). Following weaning, the gland became engorged with milk causing a rise in intramammary pressure. This engorgement persisted for 24 to 48 hours, then gradually decreased upon resorption of the milk. The alveoli collapsed, the epithelial cells lining the alveoli disintegrated, and the lobules gradually disappeared. The main difference among species was the length of time required for complete involution of the lobule-alveolar system to a duct system (Turner, 1939). Complete involution was observed 13 days after weaning in the mouse (Cole, 1933; Williams, 1942), 9 days in the rat (Maeder, 1922), 35 days in the guinea pig, and 75 days in the goat (Turner and Reineke, 1936).

Recently, biochemical changes occurring in the mammary tissue during involution have been studied. DNA estimations have generally agreed with the histologically observed loss of cells phenomenon (Kirkham and Turner, 1953; Turner, 1960; Griffith and Turner, 1961b). Slater (1962), on the other hand, observed an increase in the mammary DNA content during the first 48 hours after weaning and Tucker and

Reece (1963c), observed a slight but non-significant increase in DNA between the 21st day of lactation and the first day after weaning in the mammary gland of the rat. DNA decreased significantly only three days after weaning. McNaught (1956; 1957) suggested that on the basis of a decrease in oxygen uptake, respiratory quotient, glucose uptake, and an increase in lactic acid production, the involutionary changes could be seen as early as 8 to 12 hours after weaning.

Ribonucleic Acid Content of the Mammary Gland

The RNA content of tissues is considered to be intimately related to the biosynthesis of proteins by the tissues. However, rapid synthesis of proteins in the mammary gland tissue probably does not occur until the onset of pregnancy. The RNA content of the rat mammary gland before pregnancy has received little study. Kirkham and Turner (1953) reported that the total RNA and RNA/DNA ratios were low in undeveloped glands but increased gradually from pregnancy through lactation, reaching a peak at the end of lactation. Tucker (1962) observed that the RNA content of the specific area of the right abdominal gland increased from 0.14 mg at 21 days of age to 0.34 mg at 42 days of age. The RNA/DNA ratio, however, did not change significantly.

Lipid Content of the Mammary Gland

Studies on the lipid content of the mammary gland during various phases of sexual cycle are very scant. Harkness and Harkness (1956) determined the lipid content of the mammary glands of the albino rat during pregnancy, lactation, and involution. The lipid content diminished during lactation suggesting that the growth of the mammary

gland was associated with local disappearance of adipose tissue. Bitman et al. (1963a) determined that the lipid content of normal sheep mammary glands accounted for 27% of the total mammary gland weight in young animals, whereas in mature animals the lipid content accounted for about 12% of the gland weight.

Techniques of Assessing Mammary Development

Whole-mount preparation. The classical whole-mount preparation of the mammary gland was first introduced by Lane-Claypon and Starling (1906) in their study of the nature of the stimuli causing growth of the mammary gland. Later it was used extensively by Ancel and Bouin (1911) and is widely used even today. The method consists of mounting the entire gland between two glass slides after removing all muscular and adipose tissue and suitably staining the mammary paranchymal tissue. However, whole-mounts can be applied only to those species such as the rat, mouse, rabbit, and monkey in which the mammary gland is a relatively flat sheet of tissue. In species such as the guinea pig, cow, sheep, goat, and human, the gland develops equally in three dimensions and this technique has much less value. In such cases, thick histological sections of the gland have been used to examine the tissue.

Thick histological sections. This technique consists of cutting rough, serial slices of the tissue, ranging from 0.5 - 5.0 mm in thickness, followed by staining the tissue. Dabelow (1941) used thick sections to study the human mammary gland. Graumann (1952) and Pfaltz (1949) used this technique to study neo-natal human mammary glands while Schalm and Haring (1939) have used this to study the bovine gland.

Richardson (1947) suggested that the gland, while it is in a state of full distension with stored milk, should be fixed by vascular perfusion prior to obtaining the sample because handling of an unfixed gland caused considerable distortion of the cell structure.

Mammography. Mammography is soft tissue roentgenography of the breast. This clinical technique evaluates quantitative changes in the mammary structure of the human breast. Warren (1930) has reported on the clinical application of mammography. Leborgne (1951) refined the technique and subsequently Egan (1960), Ingleby, Moore, and Gershon-Cohen (1957), Engleby and Gershon-Cohen (1960), and Egan (1963) have made considerable use of this technique.

Total mammary gland area. The mammary area covered by the duct and lobule-alveolar systems of whole-mount preparations have been quantitatively measured. Folley et al. (1939) determined the mammary area of rhesus monkeys by tracing the periphery of the whole-mounted glands onto xylol-soaked papers and measuring the area either with a planimeter or squared paper. Cowie and Folley (1947), Cowie (1949), and Silver (1953) using rats and Flux (1954) using mice have obtained mammary gland areas by this method. Macdonald and Reece (1960) used a technique to measure mammary gland area which eliminated the use of the planimeter or counting the squares. These workers took negative pictures of the whole-mount preparations, cut the pictures around the periphery of the gland, weighed the resulting photograph and compared it to a 1 cm^2 standard. Using this technique, they studied the response of rat mammary glands to various mammogens (Macdonald and Reece, 1962; 1963).

Analysis of degree of arborescence. The area of the mammary gland alone does not indicate the morphologic differences within the area, and therefore, Cowie and Folley (1947) devised a scoring system to quantitate the arborescence of the duct system, side-buds, end-buds, and alveoli. Silver (1953) in determining the degree of arborescence of the rat mammary gland adopted the grid technique of Short (1950) which was initially developed for measuring the internal surface area of lung alveoli. In this technique, the number of intersections made by side-buds and ducts per cm of grid were counted. Flux (1954) counted the number of junctions between the ducts in estimating the arborescence of the mouse mammary gland, whereas Benson et al. (1957) devised a scoring system for the duct and alveolar development of guinea pigs.

Total surface area of the secretory epithelium. Attempts were made by Cowie et al. (1952) to estimate the total internal surface area of the mammary alveoli and to correlate this with milk yield. Richardson (1953) applied the method of Short (1950) and Chalkley (1943) to determine the surface area of the secretory epithelium of the goat mammary gland.

Volume of glandular tissue. Benson et al. (1957) used a procedure in guinea pigs to determine the volume of the glandular tissue from area measurements of serial sections of the gland. This procedure, though useful for three-dimensional glands, is extremely time-consuming (Cowie and Folley, 1961).

Relative growth analysis. The concept of relative growth analysis, as developed by Huxley (1924) whereby the rate of growth of a particular organ is related to the growth of the animal as a whole was first applied to the mammary gland by Folley et al. (1939). The body surface area is

approximately proportional to the function (body weight)$^{2/3}$, and this function was used as a standard of reference to which the changes in mammary area were related. Cowie (1949) and Silver (1953) using rats and Flux (1954) using mice applied this technique to the study of normal and experimental mammary growth. Aberle (1934) compared the rate of growth of the mammary gland in rhesus monkeys with the rate of weight increase of the kidney, the kidney serving as a reference standard.

Deoxyribonucleic acid content. Davidson and Leslie (1950a,b) suggested that the DNA content may be used as a measure of cell numbers in a tissue. Kirkham and Turner (1953) were the first workers to use the DNA content as a measure of mammary gland growth. Tucker and Reece (1962) observed that the DNA content per nucleus was constant in mammary tissue of pregnant and lactating rats, indicating that DNA analysis may be used directly as an index of gland growth. Numerous publications have appeared using DNA as a measure of normal (Griffith and Turner, 1957; Brookreson and Turner, 1959; Griffith and Turner, 1961a; and Tucker, 1962) as well as experimentally induced gland growth (Kirkham and Turner, 1954; Anderson, Brookreson, and Turner, 1961; Moon, Griffith, and Turner, 1959; Yamamoto and Turner, 1956; and Wada and Turner, 1959a,b).

Alkaline phosphatase and iron content. Huggins and Mainzer (1957; 1958) determined the alkaline phosphatase content of the mammary gland and suggested its use as a method of assessing mammary development. Rawlinson and Pierce (1950) have suggested that the iron content of the gland measured the gland development. However, neither of these techniques has received widespread application.

MATERIALS AND METHODS

Animals

Female rats of the Sprague-Dawley strain were used throughout this study. The animals were housed in a room maintained at a temperature of 24° ± 1° C and illuminated with fluorescent lights from 8:00 AM to 8:00 PM. The animals were given ad libitum water and a ration consisting of:

Ground shell corn	460 lb
Soybean oil meal, 44% protein	200 lb
Fishmeal, 65% protein	100 lb
Alfalfa meal dehydrated, 17% protein	50 lb
Dried skim milk powder	100 lb
Sugar (as beet sugar-sucrose)	50 lb
Corn oil	30 lb
Trace mineral salt	5 lb
Vitamin B complex	1 lb
1. Riboflavin, 2,000 mg/lb	
2. Pantothenic acid, 4,000 mg/lb	
3. Niacin, 9,000 mg/lb	
4. Choline chloride, 90,000 mg/lb	
Vitamin A and D mixture	0.5 lb
1. 80% A, 10,000 IU/g	
2. 20% D, 9,000 IU/g	
Vitamin B_{12}, 6 mg/lb	3.75 lb

Rats for these experiments were obtained from litters adjusted within 2 days of birth to six pups per mother. The rat pups were weaned at 20 days of age and were then housed 20 rats (maximum) per cage (cage area = 22 x 20 in.) until 50 days of age. At this time rats

were transferred to another cage (cage area = 25.5 x 9.5 in.) and were housed 10 rats per cage.

Mammary gland area experiment. Rats were sacrificed at 10, 20, 23, 25, 30, 33, 35, 40, 60, 80, and 100 days of age and the skin containing the right abdominal mammary gland was removed for mammary area measurements. Vaginal smears from rats 60 days of age and older were prepared prior to sacrifice for ascertaining the stage of the estrous cycle.

Total mammary fat pad (TMFP) experiment. Rats were sacrificed at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 days of age and the entire fat pad containing the two abdominal and four inguinal mammary glands was removed for nucleic acid analyses. Vaginal smears of the rats were prepared at the time of sacrifice from rats 40 days of age and older with the exception of the 50-day group.

Specific mammary area (SMA) experiment. Rats were sacrificed at 10, 15, 20, 21, 22, 23, 24, 25, 30, 31, 32, 33, 34, and 35 days of age. The two areas containing the right and left abdominal mammary glands were removed for nucleic acid analyses. Preliminary studies of whole mounts revealed the location of this specific area. The lymph nodes, which served as a landmark for this specific area, were removed prior to analysis.

Mammary Gland Area Measurement

The skin-mammary gland complex was removed from the rat, pinned on a cork board, and fixed in Bouin's fluid for at least 24 hours. The tela subcutanea, containing the mammary gland, was separated from the skin, and with the aid of a binocular microscope, the major lymph nodes, blood vessels, and nerves were dissected out. The Bouin's fluid was

removed from the gland by washing several times with 2% NH_4OH in 70% ethanol. The fat was removed by placing the gland in 95% ethanol for several days. The gland was subsequently stained with Meyer's hemalum for 12-24 hours, washed several times with 1% potassium alum and differentiated in acid-alcohol (2.0% HCl in 70% ethanol) until the ducts and alveoli of the gland presented a sharp contrast to the matrix. The gland was further dehydrated in 80%, 95%, and absolute alcohol for five, five, and eight minutes, respectively; cleared in xylene-ethanol (1:1) followed by 100% xylene for three and five minutes, respectively; and then mounted in permount (Fisher Scientific Co.).

The whole-mount was placed in the negative chamber of a photographic enlarger and the periphery of the enlarged image was traced onto paper by two persons. The indentations of the gland were followed only where the indentation was twice as wide as the length of the adjacent duct. The outline of the gland thus obtained was then traced with a planimeter (Keuffel and Esser Co. Compensating Polar Planimeter Model No. 620015) by two persons. The area obtained was corrected for magnification (the magnification was about 8 for glands under 30 days of age and about 3.2 for glands of 30 days and above) and the average area in mm^2 for each gland was calculated.

Nucleic Acid Analyses

The animals were sacrificed by cervical dislocation and the entire fat pad (TMFP) containing the six abdominal-inguinal glands, or the specific area (SMA) containing the left and right abdominal glands were removed and placed immediately in ice-cold 0.25 M sucrose. The tissue was stored at -20° C until assayed for nucleic acid content.

The nucleic acid extraction was performed by the Schmidt and Thannhauser (1945) procedure as modified by Tucker (1964). The tissue was thawed, blotted on paper towels, and weighed on an analytical balance. The entire TMFP or SMA was cut into small pieces with scissors and suspended in ice-cold glass distilled water in the ratio of 1 g per 20 ml of TMFP and 1 g per 40 ml of SMA. The tissue was homogenized for 2 minutes in either the 1000 ml Pyrex glass container or the 25 to 250 ml semi-micro metal (Cenco 17246-2) container of a Cenco PB-5 Waring Blender. Fat pads from animals 70 to 100 days of age were, however, homogenized for 3 to 4 minutes to obtain a satisfactory homogenate.

Duplicate 2 ml or 4 ml samples of the homogenate, depending on the dilution, were removed into polypropylene tubes and 8 ml or 6 ml, respectively, of 95% alcohol was added. The samples were incubated at least 12 hours at room temperature with continuous shaking and were then centrifuged in a Servall RC-2 centrifuge at 1-3° C. All centrifugations were performed at 32,000 x g for 20 minutes. The supernatant fluid was poured off[1] and nine ml of methanol-chloroform (2:1) was added to the residue. After incubation at room temperature for at least 24 hours, the samples were centrifuged, supernatant fluids poured off[1], and nine ml of ether was added to the residue. The samples were then incubated for at least 12 hours at room temperature, centrifuged, and the ether fraction removed[1].

The residue was extracted twice with 5 ml of 10% ice-cold trichloroacetic acid which was removed by washing with 5 ml of ice-cold 95% ethanol saturated with sodium acetate.

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[1]See lipid analysis, page 18.

The samples were digested with 2 ml of 1N KOH for 15 hours at 37° C. The digest was acidified with 0.3 ml of ice-cold 6N HCl and 2.5 ml of 10% perchloric acid (PCA). The samples were centrifuged and washed twice with 2 ml each of ice-cold 5% PCA. These combined acid supernatant fluids, containing the RNA, were adjusted to 10 ml and were analyzed for RNA-ribose by the colorimetric orcinol procedure of Mejbaum (1939) as described by LePage (1957). This procedure consisted of mixing a 3 ml aliquot of the above supernatant fluids with 3 ml of a 1.0% solution of orcinol in 0.1% $FeCl_3 \cdot 6\ H_2O$ dissolved in concentrated HCl. The reaction mixture was boiled in a water bath for 30 minutes, and the resulting color development was determined in a Beckman D B Spectrophotometer at 670 Mμ. The RNA content was calculated from a standard curve obtained using highly purified yeast RNA (Worthington Biochemical Corp.).

The DNA was extracted from the residue, remaining after cold PCA treatment, with 5 ml of 5% PCA heated to 70° C for 15 minutes. After centrifuging, the residue was washed twice with 2 ml of ice-cold 5% PCA. These DNA-containing supernatant fluids were combined and adjusted to 10 ml with 5% PCA. The absorbancy of the DNA-containing solution was read at 268 Mμ in a Beckman DB Spectrophotometer. Highly polymerized DNA (Worthington Biochemicals Corp.) was used as the standard.

Lipid Analysis

The alcohol, methanol-chloroform, and ether extracts of the tissue, obtained in the process of extracting the nucleic acids were used to estimate the lipid content of the total mammary fat pad. The three lipid-containing extracts were pooled into tared beakers and allowed to

evaporate for 24 hours at room temperature. Such treatment removed most of the ether. The remaining lipid solvents and moisture were evaporated at 65° C on a hot plate. The beakers containing the lipid fractions were placed into a dessicator for at least 24 hours and then weighed on an analytical balance.

Relative Growth Analyses

Several studies (Cowie, 1949; Silver, 1953; Flux, 1954; Folley, 1956) have related mammary gland development with the growth of the body or the increase in the body surface area. It was of interest, therefore, to present an analysis of mammary development relative to increases in body weight and/or body surface area.

The simple allometric growth law (Huxley, 1924) is usually expressed as $y=bx^{\alpha}$, where y = the quantity undergoing allometric growth, x = the reference quantity, and b and α are constants, the more biologically significant of which, termed the "equilibrium constant" is α . This relation may also be expressed in the form $\log y = b + \alpha \log x$. When data relating to growth rates conform to this law, a straight line is obtained by plotting log y against log x. The slope of such a straight line is equal to α . In the case of isometric growth, which may be considered a special case of allometric growth, the second expression becomes $\log y = \log b + \log x$ and the straight line obtained by logarithmic plotting of the data will have a slope equal to unity.

The function $(\text{body weight})^{2/3}$, which is approximately proportional to body surface area, was the reference standard of choice to which mammary area was related. However, mammary area was also related to

the body weight increases of the animal for comparing with mammary DNA data.

On the other hand, total body DNA, a measure of body cell numbers, was probably the most reasonable reference standard with which changes in mammary DNA could be related. However, since body DNA measurements would be difficult to obtain, the body weight of the animal was used as the reference standard. It was assumed that the rate of increase in the body weight was approximately proportional to the rate of increase in body DNA. The mammary DNA was also related to $(\text{body weight})^{2/3}$, a function of body surface area, for comparing with mammary area data.

Statistical Analyses

Standard errors of means, coefficients of correlation, linear regression analysis, and analysis of variance were computed (Snedecor, 1956; Dixon and Massey, 1957).

RESULTS AND DISCUSSION

Area of Mammary Glands

The area in mm^2 of the right abdominal mammary gland of rats 10 to 100 days of age is presented in Table 1. The abdominal mammary gland area of rats increased from 9.26 mm^2 at 10 days of age to 494.57 mm^2 at 80 days of age, representing a 5,241% increase. The mammary gland area increased 65% between 10 and 20 days, 1,857% between 20 and 40 days, and 65% between 40 and 80 days. The area then declined 8.8% between 80 and 100 days of age. Inspection of the growth curve (Fig. 1) indicated that the acceleration in the growth rate of the mammary gland area was established in the Sprague-Dawley rat as early as 20 days of age, whereas Cowie (1949), using hooded Norway rats, observed this rapid increase in mammary growth after 30 days of age. This divergence of results may represent strain differences. Moreover, Cowie (1949) measured the area of all 12 mammary glands, whereas in the present experiment only the area of the right abdominal mammary gland was measured.

The average mammary area of rats exhibiting proestrus or estrus and metestrus or diestrus is presented in Table 2. Average mammary area of 100-day old rats exhibiting proestrus or estrus was 458.83 mm^2 compared with 345.14 mm^2 in rats of the same age group exhibiting metestrus or diestrus. But the mammary area of 60- and 80-day old rats exhibiting proestrus or estrus was less than the mammary area of rats of the corresponding age groups exhibiting either metestrus or diestrus. These observations failed to support the phenomenon of increased duct proliferation during estrus reported by several workers using histological techniques. This contradictory finding was attributed to the presence of

Table 1. Mammary gland area of rats 10 to 100 days of age.

Age	No. of rats	Avg. body wt. (g)	Area (mm^2)
10	12	22.0	9.26 ± 0.94
20	12	42.0	15.29 ± 1.42
23	12	57.0	35.54 ± 3.27
25	12	62.0	48.75 ± 3.99
30	12	68.0	67.06 ± 4.32
33	12	92.0	138.02 ± 6.65
35	12	99.0	178.31 ± 8.72
40	12	122.0	299.22 ± 14.82
60	12	159.0	415.90 ± 12.90
80	12	188.0	494.57 ± 27.39
100	12	217.0	450.93 ± 24.07

Table 2. Mammary gland area of rats in various stages of the estrous cycle.

Age	No. of rats	*Rats in P or E	Avg. area (mm^2)	*Rats in M or D	Avg. area (mm^2)
60	12	5	409.96	7	420.15
80	12	9	452.50	3	620.75
100	12	8	458.83	4	435.14

* P = proestrus, E = estrus, M = metestrus, D = diestrus.

several uncontrolled variables in the present experiment, such as the body size of the animal, the number of estrous cycles experienced by each individual animal, and the individual rate of mammary growth of the animal.

The agreement of the data with the law of simple allometry was

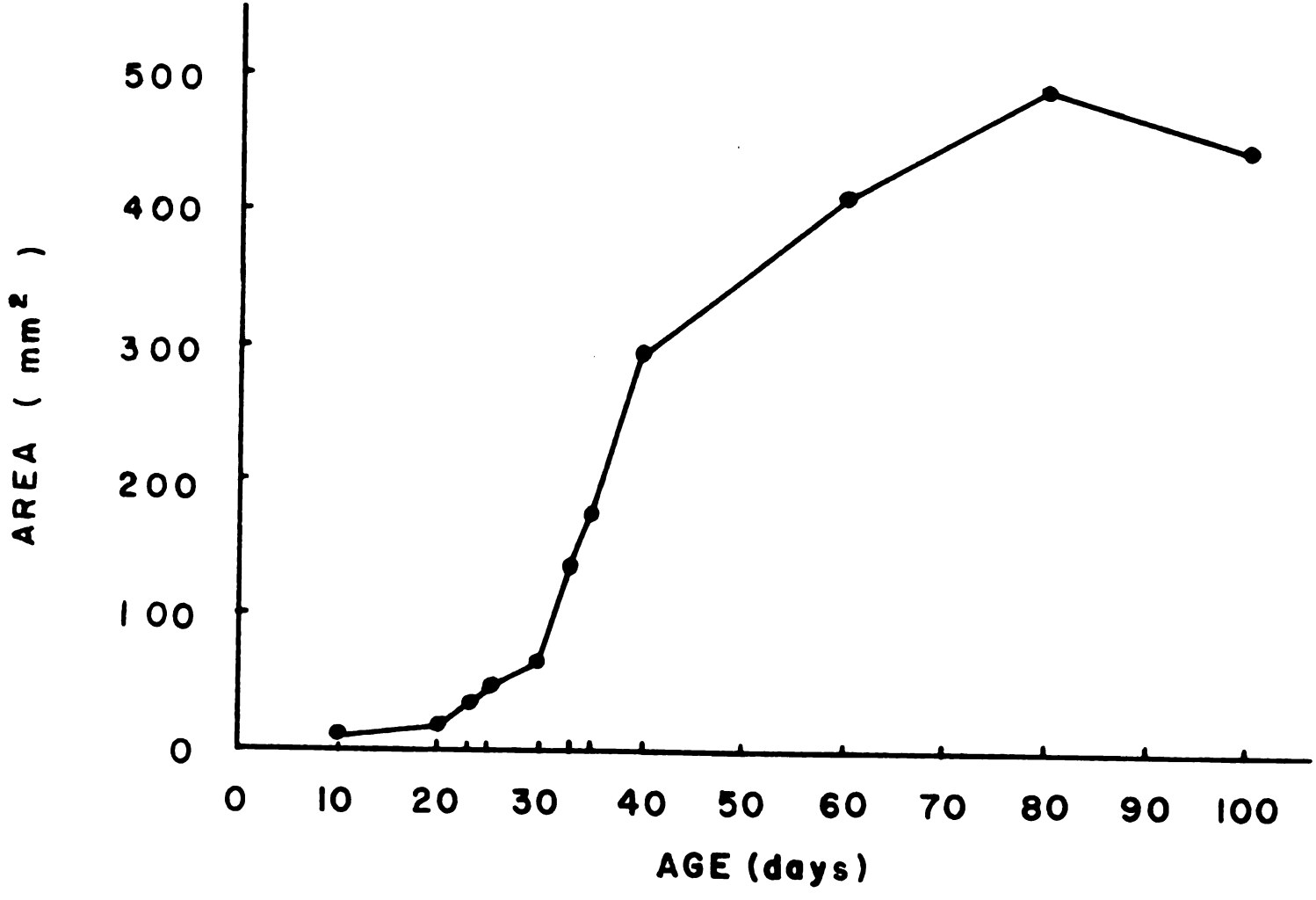

FIG. 1. MAMMARY GLAND AREA OF RATS 10 TO 100 DAYS OF AGE.

tested by plotting log mammary area against log (body weight)$^{2/3}$, a function of body surface area (Fig. 2). Since Cowie (1949) fitted similar data by two straight lines, intersecting arbitrarily in the neighborhood of log (body weight)$^{2/3}$ = 1.1, it was of interest, to fit the data with three straight lines, as follows: (a) including all the points; (b) including points from log (body weight)$^{2/3}$ = 0 to log (body weight)$^{2/3}$ = 1.1, which represented rats 10 to 20 days of age; and (c) including points above log (body weight)$^{2/3}$ = 1.1, which represented rats 23 to 100 days of age. The respective coefficients of equilibrium (α) for the three linear regression lines, calculated by the method of least squares, were 3.00 ($P < 0.01$), 1.13 ($P < 0.01$) and 3.00 ($P < 0.01$), respectively. The latter two values agreed very well with the corresponding values of 1.10 and 3.03, respectively, reported by Cowie (1949). Thus, the mammary gland area increased three times faster than the body surface area between 10 and 100 days of age inclusive but the mammary gland area increased only 1.13 times faster than body surface area between 10 and 20 days of age. This observation agreed very well with the data of Cowie (1949) which suggested that allometry commenced at day 23 of age. Inspection of the plot (Fig. 2) showed that most of the data could be fitted to a single straight line. On the other hand, the data would probably have been best fitted by a cubic curve. The lower portion of this curve is explained by the α value of 1.13 as discussed above. On the other hand, it would appear that at some point approximating 1.4 to 1.5 log (body weight)$^{2/3}$ the mammary gland area did not increase further despite increases in surface area.

In addition, the growth rate of the mammary gland area was related

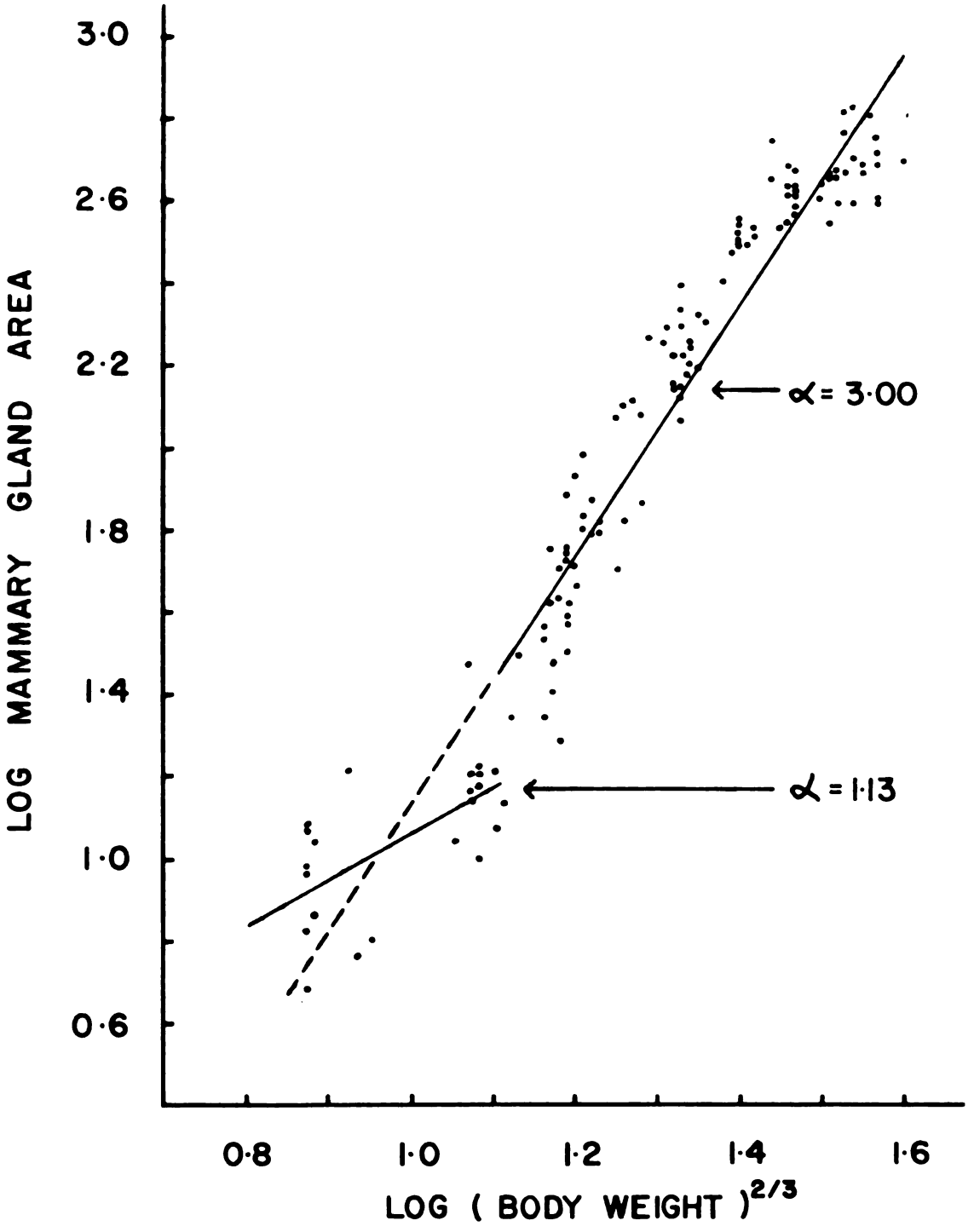

FIG. 2. RELATION BETWEEN MAMMARY GLAND AREA AND (BODY WEIGHT)$^{2/3}$ OF RATS 10 TO 100 DAYS OF AGE.

to the body weight of the animal. The plot of log mammary gland area against log body weight appeared to fit a straight line (Fig. 3) although a cubic curve would probably more closely fit the data. The equilibrium constant α, calculated by the method of least squares, was 1.95 which was 35% less than the corresponding α value obtained in the relative growth analysis of mammary area with respect to body surface area. Thus, the mammary gland area between 10 and 100 days of age increased about two times faster than the body weight.

This data also showed that the two reference standards, body surface area and body weight of the animal, did not increase with advancing age at the same rate; body weight tended to increase more rapidly than body surface area. This observation was in agreement with the geometric relationships between weight and surface area as discussed by Brody (1945).

Nucleic Acid Content of Mammary Glands

Total mammary fat pad (TMFP). The nucleic acid content of the entire mammary fat pad containing the six abdominal-inguinal mammary glands of rats 10 to 100 days of age is presented in Table 3. DNA is expressed as total mg of DNA and mg of DNA per 100 g of body weight. RNA is expressed as total mg of RNA. The total DNA content of TMFP increased from 0.33 mg at 10 days of age to 3.57 mg at 90 days of age. The mammary fat pad increased in a linear manner to 50 days of age (Fig. 4), confirming the observations of Tucker (1962), but declined 10%, 21%, and 17% at 60, 70, and 80 days of age, respectively, relative to 50 days. The differences among means of 50-, 60-, 70-, and 80-day groups, however, were not statistically significant ($P > 0.05$).

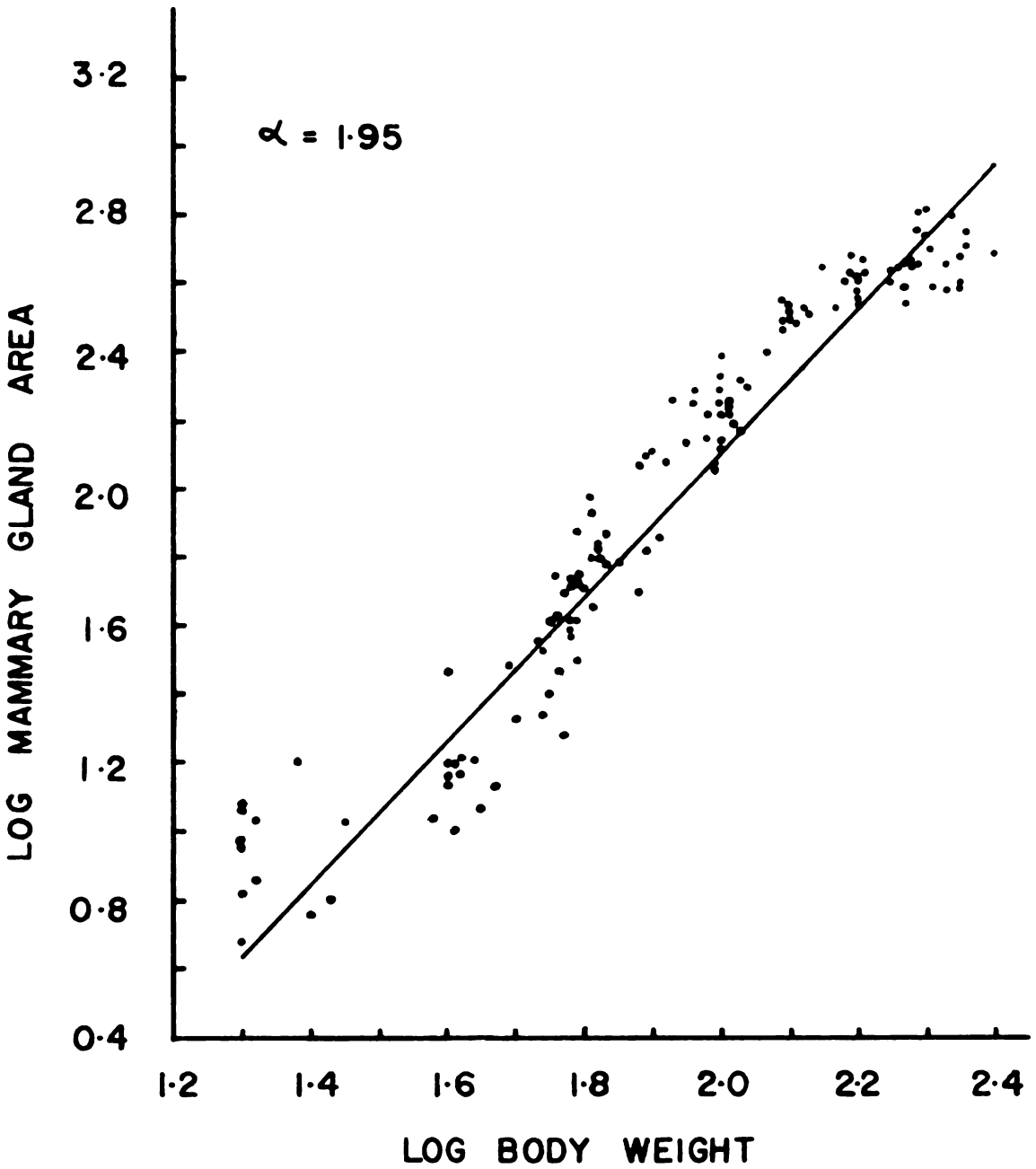

FIG. 3. RELATION BETWEEN MAMMARY GLAND AREA AND BODY WEIGHT OF RATS 10 TO 100 DAYS OF AGE.

Table 3. Nucleic acid content of the total mammary fat pad of rats 10 to 100 days of age.

Age	No. of rats	Body wt. (g)	Gland wt. (g)	Total DNA (mg)	DNA/100 g (mg)	Total RNA (mg)	RNA/DNA
10	12	22.0	0.9846	0.33 ± 0.01	1.53 ± 0.05	0.27 ± 0.01	0.82 ± 0.02
20	12	42.0	1.0540	0.53 ± 0.06	1.26 ± 0.13	0.42 ± 0.04	0.81 ± 0.04
30	12	77.0	2.0525	1.25 ± 0.06	1.63 ± 0.04	1.13 ± 0.08	0.87 ± 0.03
40	12	125.0	2.8792	2.24 ± 0.20	1.78 ± 0.10	1.70 ± 0.10	0.76 ± 0.02
50	12	140.0	3.2742	2.81 ± 0.20	1.99 ± 0.10	1.91 ± 0.10	0.72 ± 0.06
60	12	173.0	4.4657	2.55 ± 0.07	1.49 ± 0.04	1.94 ± 0.03	0.78 ± 0.03
70	12	180.0	4.9472	2.33 ± 0.20	1.30 ± 0.10	1.63 ± 0.10	0.73 ± 0.04
80	12	196.0	6.1271	2.41 ± 0.05	1.22 ± 0.07	1.49 ± 0.04	0.62 ± 0.03
90	12	198.0	4.3887	3.57 ± 0.20	1.80 ± 0.10	2.34 ± 0.20	0.65 ± 0.02
100	12	208.0	5.2175	3.52 ± 0.17	1.70 ± 0.08	2.56 ± 0.10	0.74 ± 0.03

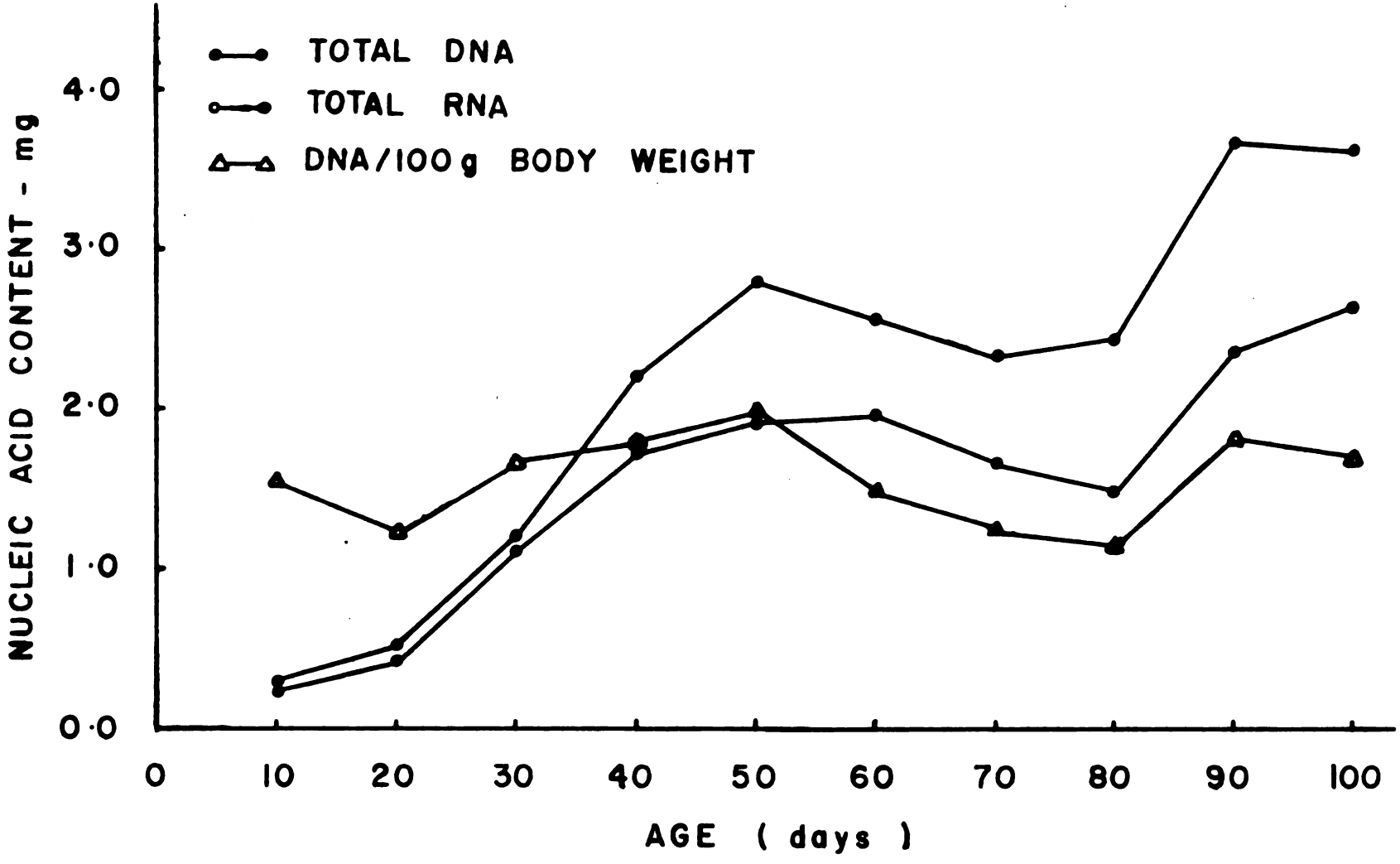

FIG. 4. NUCLEIC ACID CONTENT OF THE TOTAL MAMMARY FAT PAD OF RATS 10 TO 100 DAYS OF AGE.

The constancy in DNA content between 50 and 80 days of age may be attributed to an error in sampling. It was also possible that the stage of the estrous cycle of rats in these age groups influenced the average TMFP-DNA content, because several workers (Turner and Gomez, 1936b; Cole, 1933; Sutter, 1921) have reported increased duct proliferation during estrus. The average TMFP-DNA content for proestrus or estrus and metestrus or diestrus rats within age groups is presented in Table 4. The 40- and 70-day old rats exhibiting proestrus or estrus contained more DNA than the rats of the same age groups exhibiting metestrus or diestrus. On the other hand, the 60-, 80-, 90-, and 100-day age groups were just the reverse. Rats exhibiting metestrus or diestrus contained more DNA than rats exhibiting proestrus or estrus. Thus, this TMFP-DNA estrous cycle data, in agreement with the mammary area estrous cycle data, failed to support the histological findings that the estrous cycle consistently influences mammary growth. The TMFP data, however, can not be interpreted without reservation because the number of estrus cycles experienced by each individual of the same age group was not known, and the contribution of extraneous fat pad tissue may have masked any effects on the mammary parenchyma _per se_. Furthermore, the number of animals per stage of the estrus cycle within age groups was often quite low.

DNA/100 g body weight declined from 1.53 mg at 10 days of age to 1.26 mg at 20 days of age, increased between 20 and 50 days to 1.99 mg, declined between 50 and 80 days to 1.22 mg and increased again to 1.80 mg at 90 days of age. When a linear regression curve was fitted to the plot of DNA/100 g body weight against age (Fig. 4), the slope of the line

Table 4. DNA content of the total mammary fat pad of rats in various stages of the estrous cycle.

Age	No. of rats	*Rats in P or E	Avg. DNA (mg)	*Rats in M or D	Avg. DNA (mg)
40	12	8	2.48	3	1.52
60	12	7	2.43	5	2.72
70	12	4	2.75	7	2.00
80	12	7	2.16	5	2.75
90	12	8	3.52	3	3.84
100	12	7	3.48	5	3.58

* P = proestrus, E = estrus, M = metestrus, D = diestrus.

Table 5. Lipid content of the total mammary fat pad of rats 10 to 100 days of age.

Age	Body wt. (g)	Gland wt. (g)	Total lipid (mg)	Lipid as % of fresh gland wt.
10	22.0	0.9846	214.9 ± 15.2	40.5 ± 3.5
20	42.0	1.0540	451.4 ± 62.8	42.1 ± 4.0
30	77.0	2.0525	888.3 ± 74.4	43.8 ± 3.6
40	125.0	2.8792	1161.7 ± 62.0	40.6 ± 3.6
50	140.0	3.2742	1719.2 ± 198.0	52.1 ± 3.2
60	173.0	4.4657	2616.5 ± 188.3	58.9 ± 2.9
70	180.0	4.9472	1576.3 ± 89.0	32.1 ± 1.2
80	196.0	6.1271	3413.1 ± 240.5	56.0 ± 1.0
90	198.0	4.3887	1959.9 ± 210.5	44.3 ± 3.7
100	208.0	5.2175	2163.1 ± 95.1	41.7 ± 1.3

was found to be 0.001 which suggested that DNA/100 g body weight remained constant between 10 and 100 days of age. In other words, TMFP-DNA increased at the same rate as the body weight during this period. This observation was similar to the findings of the growth analysis of mammary DNA relative to body weight as discussed below.

The agreement of the increases in TMFP-DNA with the law of simple allometry was tested by plotting log DNA against log body weight of the animal (Fig. 5). Inspection of the plot showed that the data appeared to fit a straight line. The slope, or equilibrium constant (α) of the best fit linear regression, was 1.04 which did not differ significantly from unity ($P > 0.05$). When log DNA was plotted against log (body weight)$^{2/3}$, the data again appeared to fit a single straight line (Fig. 6) and the slope of that line was found to be 1.56 which was significantly greater than unity ($P < 0.01$). These data suggested that the increase in the DNA of the mammary fat pad between 10 and 100 days of age was isometric with respect to body weight and allometric with respect to the body surface area of the animal. This also implied, in agreement with the observation of the relative growth analysis of mammary area, that body surface area and body weight of the animal increased at different rates, body weight increasing faster than body surface area. There was no change with age in the slopes of the plot of log DNA against log body weight or log (body weight)$^{2/3}$. These observations suggested that the abrupt increase in mammary area, occurring around day 23, did not occur between 10 and 100 days of age based on the DNA of the total mammary fat pad.

The correlation coefficient of the total DNA content of the TMFP

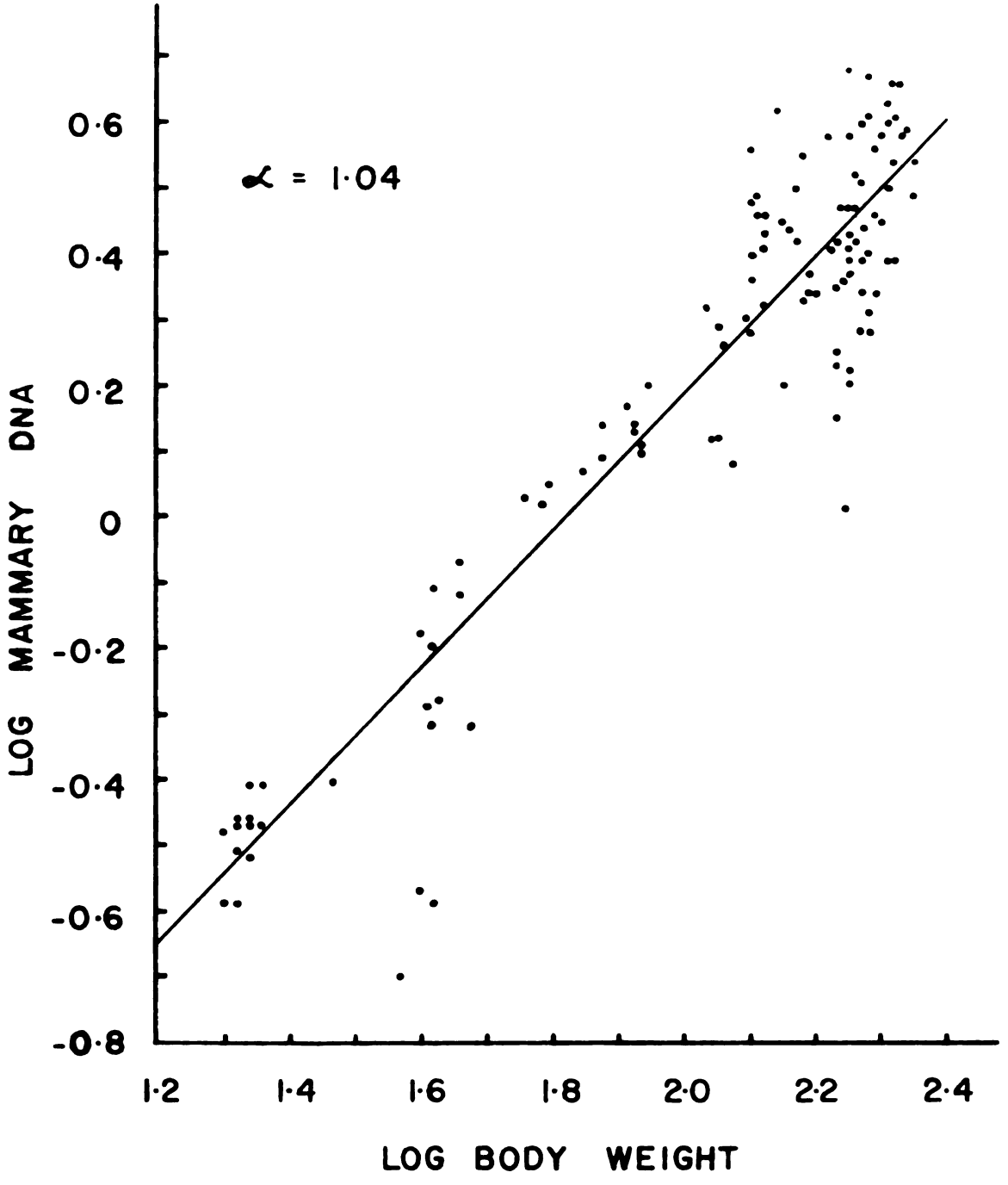

FIG. 5. RELATION BETWEEN DNA OF TOTAL MAMMARY FAT PAD AND BODY WEIGHT OF RATS 10 TO 100 DAYS OF AGE.

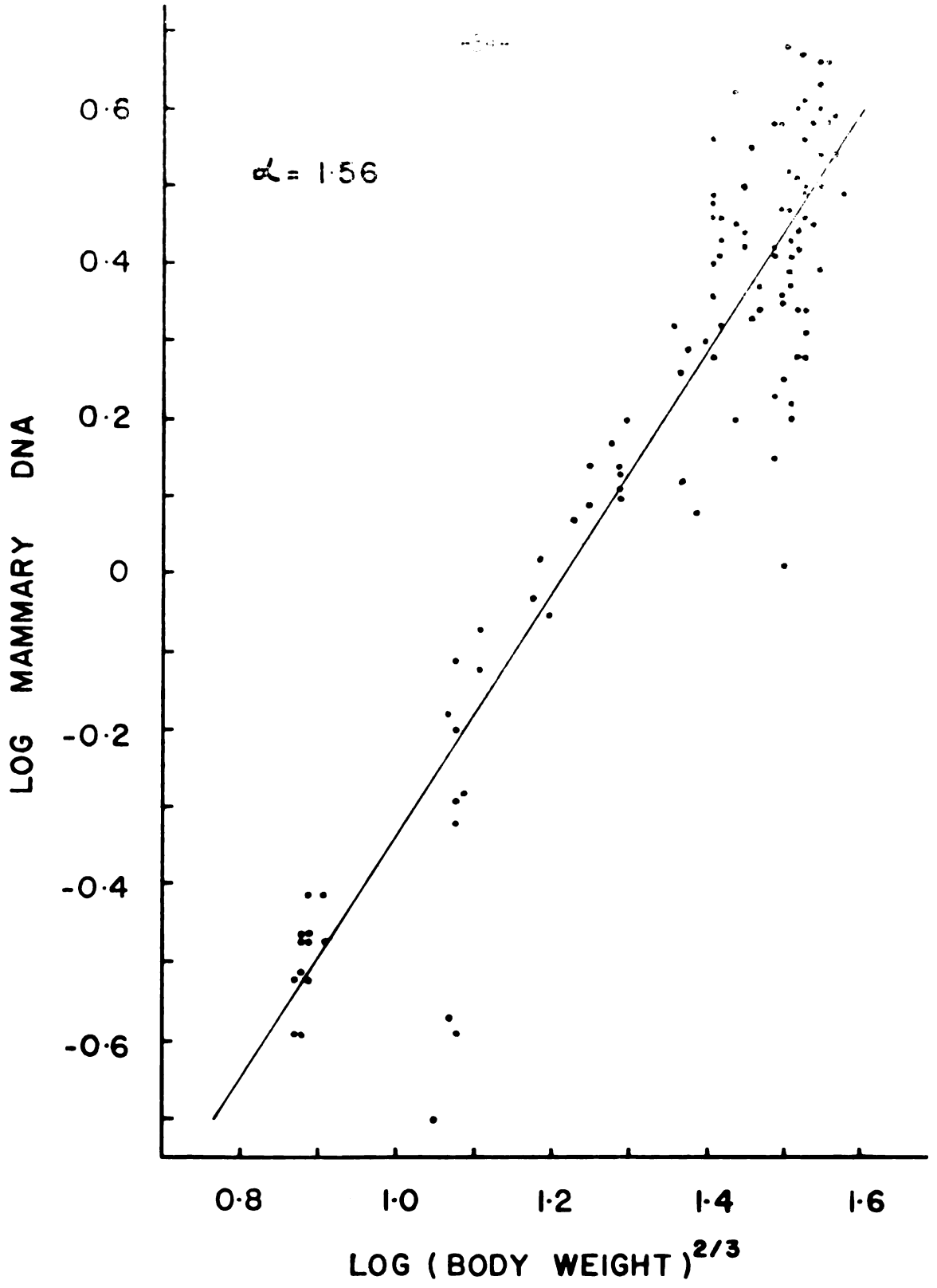

FIG. 6. RELATION BETWEEN DNA OF TOTAL MAMMARY FAT PAD AND (BODY WEIGHT)$^{2/3}$ OF RATS 10 TO 100 DAYS OF AGE.

with mammary gland area was 0.82 ($P < 0.01$).

The total RNA content of the TMFP increased from 0.27 mg at 10 days of age to 2.56 mg at 100 days of age. The increase in total RNA followed a pattern similar to the total DNA (Fig. 4). The ratio of RNA to DNA tended to decline as age advanced implying that protein synthesis per mammary fat pad cell was retarded with advancing age.

The lipid content of the TMFP is presented in Table 5 and is expressed in total mg of lipid and lipid as percent of fresh gland weight. The total lipid content increased from 214.9 mg at 10 days of age to 3,413.1 mg at 80 days of age. The lipid content represented 40.5% of the mammary gland weight at 10 days of age and 58.9% at 60 days of age. The lipid fraction of the gland declined from 56.0% to 44.3% of the gland weight between 80 and 90 days of age during which period there was a 48% increase in the DNA content of the TMFP (Table 3). This may be interpreted as suggesting that the growth of the mammary gland was associated with a disappearance of the adipose tissue as reported by Harkness and Harkness (1956) in mammary glands of albino rats. The role of the fat pad in the growth of the mammary gland has not been studied extensively. However, some unpublished observations in our laboratory indicated that when the surrounding fat pad was removed surgically from rats at an early age, the mammary gland failed to grow under the stimulus of pregnancy as compared with the contralateral, intact glands. These observations suggested that the presence of the fat pad was necessary for the mammary gland to grow and that the amount of development of the fat pad may limit the extent of growth of the mammary gland.

Specific mammary area (SMA). The preceding data were observations on the growth of the entire mammary fat pad containing the mammary glands as well as considerable amounts of extraneous tissue including adipose tissue, other connective tissue, and lymph nodes. It was of interest, therefore, to determine the growth of the mammary tissue without extraneous fat pad tissue. For this purpose, the nucleic acid content was determined in the two specific mammary areas containing the right and left abdominal mammary glands. Whole-mount preparations of glands showed that it was not possible to remove such specific mammary areas after 35 days of age because of the overlapping of the abdominal and the first inguinal mammary glands.

The nucleic acid content of rats 10 to 35 days of age is presented in Table 6. Total DNA increased from 0.06 mg at 10 days of age to 0.34 mg at 30 days of age, representing a 467% increase. The DNA declined, however, between 31 and 33 days of age to 0.27 mg and increased again between 34 and 35 days of age to 0.53 mg. The differences between the total DNA content of 30-, 31-, 32-, and 33-day groups was not statistically significant ($P > 0.05$). The failure to observe an increase in the DNA content between 31 and 33 days of age could be due to error in removing the SMA from the fat pad. Another more likely possibility was that rats at this age were influenced by approaching puberty. The external signs of estrus were not apparent until after the 34th or 35th day but a 62% increase in mammary DNA between 25 and 30 days of age suggested that the mammary gland was already under the influence of the mammogenic hormone(s). The regression of mammary DNA between the 31st and 33rd days was probably caused by a lack of the ovarian hormones,

Table 6. Nucleic acid content of the specific mammary area of rats 10 to 35 days of age.

Age	No. of rats	Body wt. (g)	Gland wt. (g)	Total DNA (mg)	DNA/100 g (mg)	Total RNA (mg)	RNA/DNA
10	12	21.0	0.1175	0.06 ± 0.004	0.28 ± 0.01	0.05 ± 0.003	0.87 ± 0.03
15	12	32.0	0.2057	0.09 ± 0.007	0.28 ± 0.02	0.07 ± 0.004	0.83 ± 0.05
20	12	44.0	0.2700	0.13 ± 0.002	0.29 ± 0.01	0.10 ± 0.004	0.81 ± 0.02
21	12	42.0	0.2179	0.12 ± 0.01	0.30 ± 0.02	0.10 ± 0.009	0.82 ± 0.02
22	12	43.0	0.2144	0.14 ± 0.009	0.33 ± 0.02	0.12 ± 0.008	0.83 ± 0.02
23	12	49.0	0.2485	0.16 ± 0.01	0.32 ± 0.02	0.12 ± 0.01	0.73 ± 0.03
24	12	52.0	0.2485	0.18 ± 0.002	0.34 ± 0.01	0.15 ± 0.002	0.82 ± 0.03
25	12	55.0	0.6540	0.21 ± 0.01	0.41 ± 0.03	0.19 ± 0.01	0.90 ± 0.03
30	12	79.0	0.5549	0.34 ± 0.02	0.44 ± 0.03	0.27 ± 0.02	0.78 ± 0.02
31	12	84.0	0.5000	0.30 ± 0.02	0.35 ± 0.02	0.26 ± 0.01	0.91 ± 0.07
32	12	87.0	0.5720	0.29 ± 0.02	0.33 ± 0.01	0.27 ± 0.02	0.97 ± 0.05
33	12	90.0	0.4633	0.27 ± 0.02	0.30 ± 0.02	0.20 ± 0.01	0.74 ± 0.03
34	12	93.0	0.5393	0.38 ± 0.02	0.41 ± 0.02	0.29 ± 0.01	0.78 ± 0.02
35	12	108.0	0.6009	0.53 ± 0.03	0.49 ± 0.03	0.38 ± 0.02	0.73 ± 0.01

whereas the rapid increase between 34 and 35 days of age could be attributed to the presence of hormones involved in controlling the estrous cycle. This cyclic effect on SMA-DNA before puberty agrees very well with the histological observations relating estrous cycle to mammary development (Turner and Gomez, 1936; Cole, 1933; Sutter, 1921). The mammary area data of the present experiment and that of Cowie (1949) suggested an allometric mammary growth rate at least two weeks before puberty whereas the SMA-DNA data indicated that this prepubertal growth of the gland is cyclic as well as cumulative.

The DNA/100 g body weight increased from 0.28 mg at 10 days of age to 0.49 mg at 35 days of age, representing a 75% increase, and followed almost the same pattern of increase with age as did total DNA (Fig. 7).

The agreement of the increases in the DNA content of SMA with the law of simple allometry was tested by plotting log DNA against log body weight of the animal (Fig. 8). From inspection of the plot, the data appeared to fit a straight line. The slope (α) of the best fit linear regression was found to be 1.26 which was significantly greater than unity ($P < 0.01$). When DNA was related to the body surface area, by plotting log DNA against log $(\text{body weight})^{2/3}$, the data again appeared to fit a straight line (Fig. 9). The slope of this line was found to be 1.89 which was significantly greater than unity ($P < 0.01$). These α values suggested that the increase in the DNA content of SMA between 10 and 35 days of age was only slightly allometric with respect to the body weight increases but considerably allometric with respect to the body surface area changes of the animal. The difference in the two α values again suggested that the body weight increased faster than the body

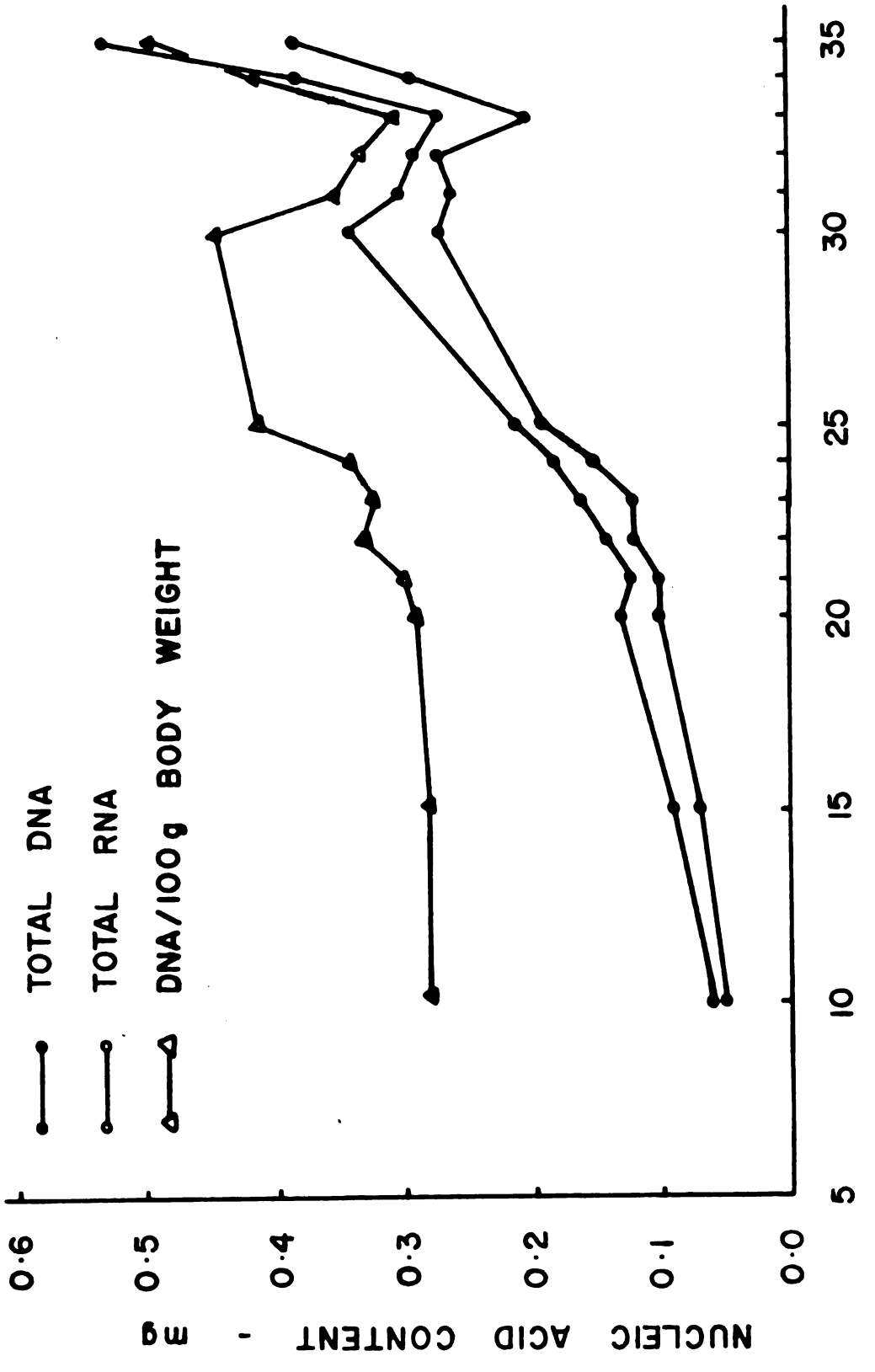

FIG. 7. NUCLEIC ACID CONTENT OF THE SPECIFIC MAMMARY AREA OF RATS 10 TO 35 DAYS OF AGE.

surface area.

Inspection of the plot of log DNA against log body weight (Fig. 8) and log DNA against log (body weight)$^{2/3}$ (Fig. 9) did not reveal any change in the slopes of the curves at any point between 10 and 35 days of age which again suggested that mammary gland growth, as measured by the DNA content of the gland, continued at a constant rate between 10 and 35 days of age.

The correlation coefficient of the total DNA content of the SMA with mammary gland area was 0.77 ($P < 0.01$).

Total RNA of SMA increased from 0.05 mg at 10 days of age to 0.38 mg at 35 days of age. The increases in total RNA were similar to the increases in total DNA (Fig. 7). The ratio of RNA to DNA did not reveal any significant trend among the age groups which suggested that the rate of protein synthesis remained constant between 10 and 35 days of age.

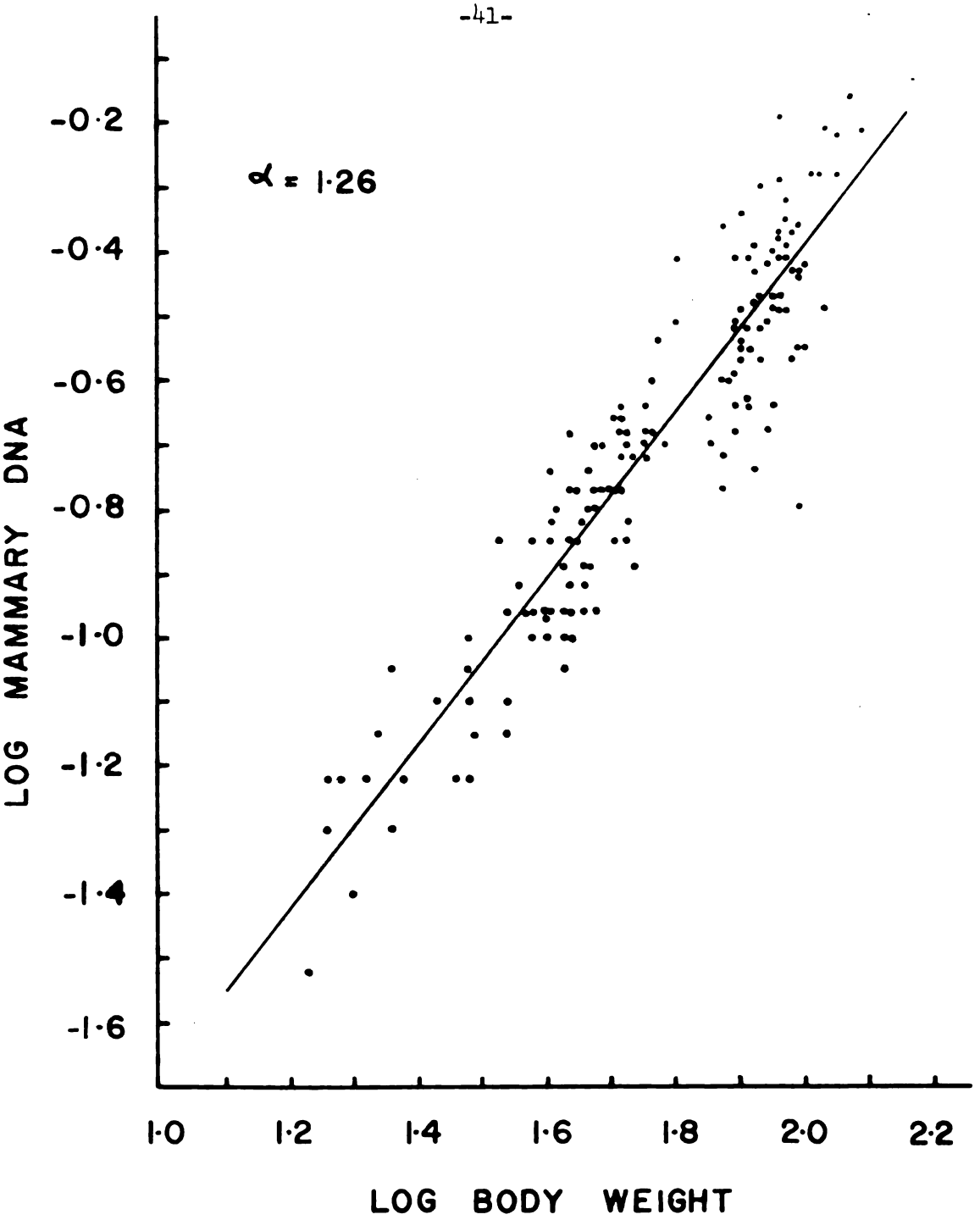

FIG. 8. RELATION BETWEEN DNA OF THE SPECIFIC MAMMARY AREA AND BODY WEIGHT OF RATS 10 TO 35 DAYS OF AGE.

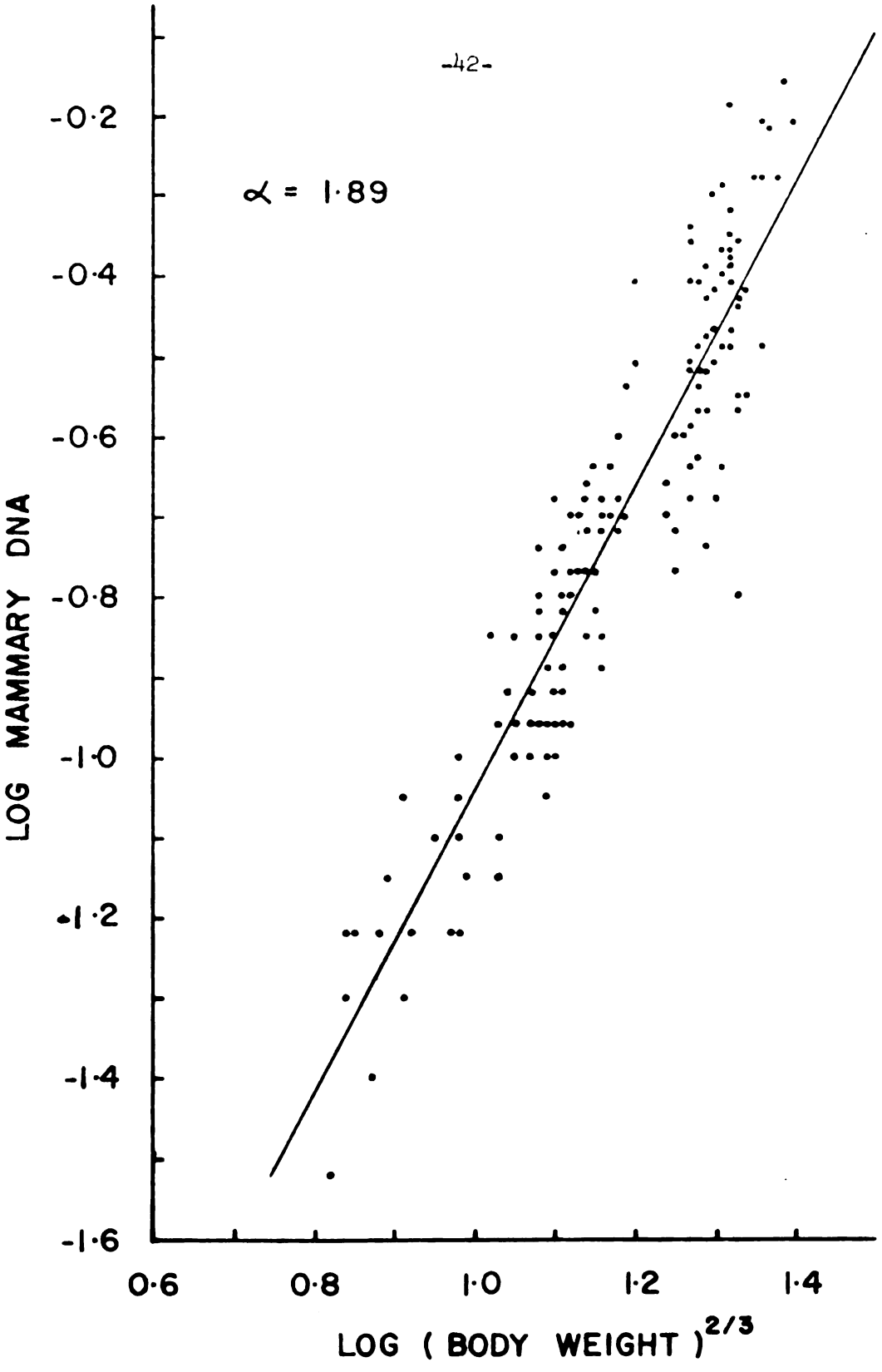

FIG. 9. RELATION BETWEEN DNA OF THE SPECIFIC MAMMARY AREA AND (BODY-WEIGHT)$^{2/3}$ OF RATS 10 TO 35 DAYS OF AGE.

SUMMARY

1. The mammary gland area of rats increased from 9.26 mm^2 at 10 days to 450.93 mm^2 at 100 days of age. This increase was allometric relative to either the body surface area ($\alpha = 3.00$) or body weight ($\alpha = 1.95$) of the animal. Arbitrarily fitting a regression curve to include animals between 10 and 20 days and between 23 and 100 days of age suggested that mammary area increased only 1.13 times as fast as surface area before 20 days of age and 3.00 times faster after 23 days of age. The mammary area of the older age groups did not increase further with increasing surface area.

2. The total DNA content of the mammary fat pad, containing the six abdominal-inguinal mammary glands, increased from 0.33 mg at 10 days to 3.52 mg at 100 days of age. This increase was isometric ($\alpha = 1.04$) relative to body weight but allometric ($\alpha = 1.56$) relative to body surface area of the animal. No change in the slopes of log DNA plotted against either log body weight or log (body weight)$^{2/3}$ was observed. The mammary gland complex thus appeared to grow at a constant rate based on DNA, between 10 and 100 days of age.

3. The DNA/100 g body weight increased from 1.53 mg at 10 days to 1.70 mg at 100 days of age. The slope of the linear regression curve was 0.001 which suggested that DNA/100 g body weight remained constant between 10 and 100 days of age.

4. The correlation of TMFP-DNA with mammary area was 0.82 ($P < 0.01$).

5. The total RNA content of the TMFP increased from 0.27 mg at 10 days to 2.56 mg at 100 days of age and these increases paralleled those of total DNA.

6. The RNA/DNA ratio tended to decline as age advanced implying that protein synthesis per mammary fat pad cell was retarded with advancing age.

7. The total lipid content increased from 214.9 mg at 10 days to 2,163.1 mg at 100 days of age. The growth of the mammary gland appeared to be associated with a disappearance of the adipose tissue.

8. The total DNA content of the specific mammary area containing the left and right abdominal mammary glands increased from 0.06 mg at 10 days to 0.53 mg at 35 days of age. This increase was 1.26 times faster than body weight and 1.89 times faster than body surface area of the animal. No change in the slopes of log DNA plotted against either log body weight or log $(\text{body weight})^{2/3}$ was observed, which again suggested that relative mammary gland growth, based on DNA, occurred at a constant rate between 10 and 35 days of age. A cyclic mammary growth period was noted between 30 and 35 days which suggested that the mammogenic hormones were cycling and effecting mammary development before puberty.

9. The DNA/100 g body weight of the SMA increased from 0.28 mg at 10 days to 0.49 mg at 35 days of age and followed a pattern similar to the total DNA.

10. The correlation of SMA-DNA with mammary area was 0.77 ($P < 0.01$).

11. The total RNA content of the SMA increased from 0.05 mg at 10 days to 0.38 mg at 35 days of age and these increases paralleled those of total DNA.

12. The RNA/DNA ratio did not reveal any significant trend among the age groups implying that the rate of protein synthesis remained

almost constant between 10 and 35 days of age.

BIBLIOGRAPHY

Aberle, S. B. D. 1934. Growth of the mammary gland in the rhesus monkey. Proc. Soc. Exp. Biol. and Med., 32:249.

Altman, A. D. 1945. Izmeneniya v vimeni korov v protsesse razdoya. Vestn. Zhivotnov., No. 1, 85. As abstracted in Dairy Sci. Abst., 9:287. 1948.

Ancel, P. and Bouin, P. 1911. Recherches sur les fonctions du corps jaune gestatif. II. Sur le determinisme du developpement de la gland mammaire au cours de la gestation. J. de Physiol. et de Path. Gen., 13:31.

Anderson, R. R., Brookreson, A. D., and Turner, C. W. 1961. Experimental growth of mammary gland in male and female mice. Proc. Soc. Exp. Biol. and Med., 106:567.

Benson, G. K., Cowie, A. T., Cox, C. P., and Goldzveig, S. A. 1957. Effects of estrone and progesterone on mammary development in the guinea pig. J. Endocrinol., 15:126.

Bitman, J., Cecil, H. C., Gilliam, D. R., and Wrenn, T. R. 1963a. Chemical composition of sheep mammary gland. J. Dairy Sci., 46:941.

Blackburn, P. S. and Macadam, I. 1954. The cells in bovine milk. J. Dairy Res., 21:31.

Bradbury, J. T. 1932. Study of the factors influencing mammary development and secretion in the mouse. Proc. Soc. Exp. Biol. and Med., 30:212.

Brody, S. 1945. Bioenergetics and Growth. Reinhold Publishing Corporation, New York.

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

Brouha. 1905. Recherches sur les diverses phases du developpement et de l'activite de la mammelle. Arch. de biol., 21:459.

Chalkley, H. W. 1943. Method for the quantitative morphologic analysis of tissues. J. Nat. Cancer Inst., 4:47.

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

Cowie, A. T. 1949. The relative growth of the mammary gland in normal, gonadectomized, and adrenalectomized rats. J. Endocrinol., 6:145.

Cowie, A. T. and Folley, S. J. 1947. The role of the adrenal cortex in mammary development and its relation to the mammogenic action of the anterior pituitary. Endocrinology, 40:274.

Cowie, A. T. and Folley, S. J. 1961. The mammary gland and lactation. In Sex and Internal Secretions, 3rd ed., William C. Young, Ed., Vol. I, Ch. 10. The Williams and Wilkins Co., Baltimore.

Cowie, A. T., Folley, S. J., Malpress, F. H., and Richardson, K. C. 1952. Studies on the hormonal induction of mammary growth and lactation in the goat. J. Endocrinol., 8:64.

Dabelow, A. 1941. Morph. Jb., 85:361. As cited by Mayer, G. and Klein, M. 1961. Histology and Cylology of the mammary gland. In Milk: the Mammary Gland and Its Secretion, S. K. Kon and A. T. Cowie, Eds., Vol. I, Ch. 2. Academic Press, Inc., New York.

Davidson, J. N. and Leslie, I. 1950a. A new approach in the biochemistry of growth and development. Nature, 165:49.

Davidson, J. N. and Leslie, I. 1950b. Nucleic acids in relation to tissue growth:a review. Cancer Research, 10:587.

Denamur, R. 1961. Etude de la glande mammaire de la lapine pendant la gestation et la lactation. Ann. Endocrinol., 22:768.

Dixon, W. J. and Massey, F. J. 1957. Introduction to Statistical Analysis. 2nd ed. McGraw-Hill Book Company, Inc., New York.

Egan, R. L. 1960. Experience with mammography in a tumor institution; evaluation of 1,000 studies. Radiology, 75:894.

Egan, R. L. 1963. Mammography. Am. J. Sur., 106:421.

Everett, J. W. 1961. The mammalian female reproductive cycle and its controlling mechanisms. In Sex and Internal Secretions, 3rd ed., William C. Young, Ed., Vol. I, Ch. 8. The Williams and Wilkins Co., Baltimore.

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.

Flux, D. A. 1954. Growth of the mammary duct system in intact and ovariectomized mice of the CHI strain. J. Endocrinol., 11:223.

Folley, S. J. 1952. Lactation. In Marshall's Physiology of Reproduction, 3rd ed., A. S. Parkes, Ed., Vol. II, Ch. 20. Longmans, Green and Co., London, New York, and Toronto.

Folley, S. J. 1956. The Physiology and Biochemistry of Lactation. Oliver and Boyd, Ltd., Edinburgh and London.

Folley, S. J., Guthkelch, A. N., and Zuckerman, S. 1939. The mammary gland of the rhesus monkey under normal and experimental conditions. Proc. Roy. Soc., B., 126:469.

Gardner, W. U. and Strong, L. C. 1935. The normal development of the mammary glands of virgin female mice of ten strains varying in susceptibility to spontaneous neoplasm. Am. J. Cancer, 25:282.

Gegenbaur, C. 1876. Zur genauren Kenntniss der Zitzen der saugethiere. Morph. Jahrb., 1:266.

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.

Gisler, E. 1922. Die Entwicklung der Milchdruse bei der Katze. Inaug. Diss. Zurich. As cited by Turner, C. W. and Gomez, E. T. 1933. The normal development of the mammary gland of the male and female guinea pig. Mo. Agr. Exp. Sta. Res. Bul. 194.

Graumann, W. 1952. Z. mikr.-anat. Forsch., 58:358. As cited by Mayer, G. and Klein, J. 1961. Histology and Cylology of the mammary gland. In Milk:the Mammary Gland and Its Secretion S. K. Kon, and A. T. Cowie, Eds., Vol. I, Ch. 2. Academic Press, Inc., New York.

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

Griffith, D. R. and Turner, C. W. 1957. Deoxyribonucleic acid (DNA) content of mammary gland during pregnancy and lactation. Proc. Soc. Exp. Biol. and Med., 95:347.

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

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

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

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

Hammond, J. and Marshall, F. H. A. 1914. The functional correlation between the ovaries, uterus, and mammary glands in the rabbit, with observation on the estrous cycle. Proc. Roy. Soc., B., 87:422.

Hammond, J. and Marshall, F. H. A. 1930. Oestrus and pseudo-pregnancy in the ferret. Proc. Roy. Soc., B., 105:607.

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.

Henneberg, Brunno. 1900. Die erste Entwickelung der Mammarorgane bei der ratte. Anat. Hefte., 13:1.

Hesselberg, 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. and Med., 12:559.

Huggins, C. and Mainzer, K. 1957. Hormonal influences on mammary tumors of the rat. II. Retardation of growth of a transplanted fibroadenoma in intact female rats by steroids in the androstane series. J. Exp. Med., 105:485.

Huggins, C. and Mainzer, K. 1958. In Endocrine Aspects of Breast Cancer, A. R. Currie, Ed., pp. 297-304. E. and S. Livingstone, Ltd., Edinburgh, London.

Huss, M. 1873. Beiträge zur Entwickclungsgeschichte der Milchdrüse beim Menschen und bei Wiederkauern. Jena Zeitsch. f. Med. u. Naturw., 7:176.

Huxley, J. S. 1924. Nature, 114:895. As cited by Cowie, A. T. 1949. The relative growth of the mammary gland in normal, gonadectomized, and adrenalectomized rats. J. Endocrinol., 6:145.

Ingleby, H. and Gershon-Cohen, J. 1960. Comparative Anatomy, Pathology, and Roentgenology of the Breast. 1960. University of Pennsylvania Press, Philadelphia.

Ingleby, H., Moore, L., and Gershon-Cohen, J. 1957. Gestational Breast changes. Obst. and Gynec., 10:149.

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

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

Kirkham, W. R. and Turner, C. W. 1954. Induction of mammary growth in rats by estrogen and progesterone. Proc. Soc. Exp. Biol. and Med., 87:139.

Klaatsch, H. 1884. Zur Morphologic der Saugethierzitzen. Morphol. Jahrb., 9:253.

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.

Lane-Claypon, J. E. and Starling, E. H. 1906. An experimental inquiry into the factors which determine the growth and activity of the mammary glands. Proc. Roy. Soc., B., 77:505.

Leborgne, R. 1951. Diagnosis of tumors of the breast by simple roentgenography:calcifications in carcinomas. Am. J. Roentgenol., 65:1.

Lenfers, P. 1907. Zur Histologie der Milchdrüse des Rindes. Z. Fleisch. u. Milchhyg., 17:340, 383, 424.

LePage, G. A. 1957. Methods for the analysis of phosphorylated intermediates. In Mamometric Techniques, 3rd ed., W. W. Umbreit, R. H. Burris, and J. F. Stauffer, Eds., Ch. 16. Burgess Publishing Co., Minneapolis, Minn.

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

Loeb, L. and Hesselberg, C. 1917. The cyclic changes in the mammary gland under normal and pathological conditions. II. The changes in the pregnant guinea pig, the effect of lutein injections, and the correlation between the cycle of the uterus and ovaries and the cycle of the mammary gland. J. Exp. Med., 25:305.

Lustig, H. 1916. Zur Entwicklungsgeschichte der menschlichen Brustdrüse. Arch. f. mikr. Anat., 87:38.

Macdonald, G. J. and Reece, R. P. 1960. Area measurement of rat mammary glands. J. Dairy Sci., 43:1658.

Macdonald, G. J. and Reece, R. P. 1962. Quantitative response of rat mammary glands to mammogens. I. Estrogen alone and with progesterone. Proc. Soc. Exp. Biol. and Med., 110:647.

Macdonald, G. J. and Reece, R. P. 1963. Quantitative response of rat mammary glands to mammogens. II. Anterior pituitary tissue alone and with estrogen. Proc. Soc. Exp. Biol. and Med., 112:669.

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.

Mayer, G. and Klein, M. 1961. Histology and Cytology of the mammary gland. In Milk: the Mammary Gland and Its Secretion, S. K. Kon, and A. T. Cowie, Eds., vol. I, Ch. 2. Academic Press, Inc., New York.

McNaught, M. L. 1956. National Institute for Research in Dairying, Report 1956, p. 59.

McNaught, M. L. 1957. National Institute for Research in Dairying, Report 1957, p. 58.

Mejbaum, W. 1939. Uber die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsaure. Z. physiol. chem., Hoppe-Seyler's, 258:117.

Moon, R. C., 1960. Mammary gland status in declining lactation. Federation Proc., 19:149.

Moon, R. C., Griffith, D. R., and Turner, C. W. 1959. Normal and experimental growth of rat mammary gland. Proc. Soc. Exp. Biol. and Med., 101:788.

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.

Myers, J. A. 1917. Studies on the mammary gland. III. A comparison of the developing mammary glands in male and female albino rats from the late fetal stages to ten weeks of age. Anat. Rec., 13:205.

Nelson, W. L., Heytler, P. G., and Ciaccio, E. I. 1962. Guinea pig mammary gland growth changes in weight, nitrogen and nucleic acids. Proc. Soc. Exp. Biol. and Med., 109:373.

Pfaltz, C. R. 1949. Das embryonale und postnatale verhalten der männlichen Brüstdrüse beim Menschen. II. Das Mammarorgan im kindes-, Junglings -, Mannes -, und Greisenalter. Acta anat., 8:293.

Rawlinson, H. E. and Pierce, G. B. 1950. Iron content as a quantitative measurement of the effect of previous pregnancies on the mammary gland of mice. Endocrinology, 46:426.

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.

Rein, G. 1881. Untersuchungen über die embryonale entwicklungsgeschichte der milchdrüe. Archiv. fur mikr. Anatomie, 20:431.

Richardson, K. C. 1947. Some structural features of the mammary tissues. Brit. Med. Bull,,5:123.

Richardson, K. C. 1953. Measurement of the total area of secretory epithelium in the lactating mammary gland of the goat. J. Endocrinol., 9:170.

Schalm, W. O. and Haring, C. M. 1939. J. Tech. Meth. p. 1997. As cited by Mayer, G. and Klein, M. 1961. Histology and Cytology of the mammary gland. In Milk:the Mammary Gland and Its Secretion, S. K. Kon, and A. T. Cowie, Eds., vol. I, Ch. 2. Academic Press, Inc. New York.

Schil, L. 1912. Recherches sur la glande mammaire; sur les phases qu'elle presente au cours de son evolution et leur determinisme. These, Nancy, 262.

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

Short, R. H. D. 1950. Alveolar epithelium in relation to growth of the lung. Phil. Tr. Roy. Soc. London, Ser. B., 235:35.

Silver, M. 1953. A quantitative analysis of the role of oestrogen in mammary development in the rat. J. Endocrinol., 10:17.

Slater, T. F. 1962. Studies on mammary involution. I. Chemical changes. Arch. intern. physiol. et. biochem., 70:167.

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

Snedecor,G. W. 1956. Statistical Methods. 5th ed. The Iowa State College Press, Ames, Iowa.

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

Sutter, Monroe. 1921. Cyclic changes in the mammary gland of the rat associated with the estrous cycle. Proc. Am. Assn. Anatomists, Anat. Rec., 21:59.

Tucker, H. A. 1962. The Nucleic Acid Content of Rat Mammary Glands. Ph. D. Thesis. Rutgers University, New Brunswick, New Jersey.

Tucker, H. A. 1964. Influence of number of suckling young on nucleic acid content of lactating rat mammary gland. Proc. Soc. Exp. Biol. and Med., 116, 1964, (In press).

Tucker, H. A. and Reece, R. P. 1962. Nucleic acid estimates of mammary tissue and nuclei. Proc. Soc. Exp. Biol. and Med., 111:639.

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

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

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

Turner, C. W. 1930. The anatomy of the mammary gland of cattle. I. Embryonic development. Mo. Agr. Exp. Sta. Res. Bul. 140.

Turner, C. W. 1931. The anatomy of the mammary gland of cattle. II. Fetal development. Mo. Agr. Exp. Sta. Res. Bul. 160.

Turner, C. W. 1939. The mammary glands. In Sex and Internal Secretions., 2nd ed., E. Allen, C. H. Danforth, and E. A. Doisy, Eds., Ch. 11. The Williams and Wilkins Co., Baltimore.

Turner, C. W. 1960. DNA as a quantitative measure of mammary gland growth. Paper presented at 1st Intern. Congr. Endocrinol., Copenhagen.

Turner, C. W. and DeMoss, W. R. 1934. The normal and experimental development of the mammary gland. I. The male and female domestic cat. Mo. Agr. Exp. Sta. Res. Bul. 207.

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. 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. 196.

Turner, C. W. and Gomez, E. T. 1934. The normal and experimental development of the mammary gland. II. The male and female dog. Mo. Agr. Exp. Sta. Res. Bul. 207.

Turner, C. W. and Gomez, E. T. 1936. The development of the mammary glands of the goat. Mo. Agr. Exp. Sta. Res. Bul. 240.

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. 235.

Turner, C. W. and Schultze, A. B. 1931. A study of the causes of the normal development of the mammary glands of the albino rat. Mo. Agr. Exp. Sta. Res. Bul. 157.

Uehlinger, P. 1922. Studies zur Entwicklung der Milchdrüse des Pferdes. Inaug. Diss. Zurich. Basel, Kreis and Co. As cited by 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. 194.

Wada, H. and Turner, C. W. 1959a. Effect of relaxin on mammary gland growth in female mice. Proc. Soc. Exp. Biol. and Med., 101:707.

Wada, H. and Turner, C. W. 1959b. Effect of relaxin on mammary gland growth in the female rat. Proc. Soc. Exp. Biol. and Med., 102:568.

Warren, S. L. 1930. Roentgenologic study of the breast. Am. J. Roentgenol., 24:113.

Weatherford, H. L. 1929. A cytological study of the mammary gland: golgi apparatus, trophospongium, and other cytoplasmic canaliculi, mitochondria. Am. J. Anat., 44:199.

Weiser, C. 1934. Untersuchtungen uber die Mausebrustdruse und ihre physiologischen und pathologischen Veranderungen. Arch. f. Gynak., 156:534.

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.

Yamamoto, H. and Turner, C. W. 1956. Experimental mammary gland growth in rabbits by estrogen and progesterone. Proc. Soc. Exp. Biol. and Med., 92:130.