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Hormones Stimulating Mammary Gland Growth of the Rat

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In recent years, in addition to station support, generous grants-in-aid have come from the United States Department of Health, Education, and Welfare under Grant No. A 299. The American Cancer Society, for a number of years, also supported this project.

The advances in our knowledge of the hormones involved in the normal growth of the mammary glands of domestic and experimental mammals have been due to the fine group of graduate students and post-doctorate Fellows who have come to the University of Missouri from many states and foreign countries to work on this project. Credit for their contributions is indicated in the citation to the reviews of their research.

In our early studies of the normal anatomy and growth of the mammary glands we were handicapped by the lack of quantitative measures of gland growth. In 1952 Dr. W. R. Kirkham who had received his Ph.D. degree in Biochemistry at the University of Missouri came to work in our laboratory as a Post-Doctorate Fellow. He was familiar with the suggestions that the newly discovered nucleic acid deoxyribonucleic acid (DNA) was a constant component of each cell. Thus the chemical measurement of DNA would be an excellent measure of cell numbers. His study of the DNA of the mammary gland initiated studies which have continued to the present time. The present report summarizes this research concerning the hormones stimulating mammary gland growth of the rat.

While knowledge of the hormones stimulating mammary gland growth is of great importance, possibly an even more important result of the project has been the training of graduate students and post-doctorate fellows in the field of animal physiology and endocrinology.

These students have obtained responsible positions in colleges, experiment stations, and commercial research laboratories both in the United States and abroad. Their training in both coursework and research at the University of Missouri with the opportunity for similar research in their present locations is having an ever widening influence upon graduate education in animal physiology and endocrinology.

The writer is indebted to Dr. Ralph Anderson of the Missouri Station, to Dr. R.P. Reece of the New Jersey Station and to Dr. H.A. Tucker of the Michigan Station for reading the manuscript and making a number of valid suggestions for its improvement.

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For the past 40 years, the Milk Secretion Laboratory of the Department of Dairy Husbandry has studied the normal mammary gland growth of domestic and laboratory animals. In 1933 this material was assembled in mimeograph form and in 1939 was published with illustrations (Turner, 1939). Later, in 1952, material on the mammary glands of domestic animals was published (Turner, 1952). Information on the mammary glands of experimental animals is currently being prepared for publication (Turner, 1971).

The investigation of hormones stimulating mammary gland growth in various experimental animals was begun when estrogen and progesterone became available. Later, as other hormones became available their effect upon mammary gland growth was also studied. The present report concerns these studies using rats as the experimental animal.

The Rat Mammary Gland

In the rat, the 12 normal mammary glands consist of a flat sheet with the major ducts and their branches lying in a single plane. In our earlier work, the normal growth of the duct system was observed during recurring estrous cycles in virgin animals by means of the whole-mounts of the glands (Turner and Schultze, 1931).

During pseudo- and normal pregnancy the ducts developed lobule-alveolar systems with the initiation of milk secretion, occurring at about the time of parturition. The anatomical alteration from duct growth to lobular alveolar growth was easily recognized by whole-mount preparations.

In our early studies, it was shown that the duct system of the ovariectomized rat could be stimulated to extensive growth by the estrogenic hormone. Further, the lobule-alveolar system could be stimulated by the combination of estrogen and progesterone (Turner and Frank, 1931.)

Quantitative Measures of Mammary Gland Growth

In the experimental growth of the duct and lobule-alveolar systems, the qualitative observation of duct and lobule-alveolar growth was noted but it was difficult to estimate the extent of this growth qualitatively by visual observations of whole-mounts. Cowie and Folley (1947) evolved a semi-quantitative method of estimating the degree of duct branching and alveolar formation in rats. Flux

(1954) counted the duct junctions in either the whole gland or in sample areas of the glands. Benson and Folley (1957) selected sections of the abdominal glands and projected them at a magnification of 40 diameters. The boundaries of the section and of secretory tissue within the section were outlined and the areas measured with a planimeter. The area of secretory tissue was expressed as a proportion of the total sectional area.

Macdonald and Reece (1960) suggested a modification of the previous methods based upon photographing the glands and cutting out the gland area. These photographs are then weighed and interpolated to actual areas.

Measurement of Deoxyribonucleic Acid (DNA)

A truly quantitative measure of mammary gland growth was developed from the concept of Boivin *et al.* (1948) and Mirsky and Ris (1949) that the somatic cell nuclei of any given species contained a constant amount of DNA. Davidson and Leslie (1950) suggested that the DNA content of a tissue would be an excellent measure of cell numbers.

Kirkham and Turner (1953-54) were the first to report studies of mammary gland growth in the rat using DNA as a quantitative estimate of cell number increase during sexual maturity, pregnancy, and lactation.

To prove that DNA was a valid quantitative index of mammary gland growth, Griffith and Turner (1957) determined the DNA content per cell in the rat and mouse. In the rat, kidney and spleen cells of virgin animals showed a mean of 6.1 picograms DNA per cell. In pregnant animals the mean mammary gland DNA/cell was 6 picograms. In the mouse the mean mammary gland DNA/cell was 5.1 picograms compared to 5.0 picograms per nucleus of the kidney and spleen. It was concluded that during pregnancy or experimentally induced gland growth the estimation of DNA would be an accurate measure of cell multiplication.

A study by Tucker and Reece (1962) concerns an alternative procedure for the extraction of nucleic acids from mammary glands. They also extended the study concerning the DNA content of the mammary gland cells of pregnant and lactating rats (Table 1).

Simpson and Schmidt (1969) reported the DNA per nucleus of the rat mammary glands on day 18 of pregnancy, day 10 of lactation, and day 7 of involution (Table 2).

It will be noted that the DNA per nucleus for pregnant and lactating glands were similar but the involuting glands showed a lower DNA which might be expected due to the gradual loss of nucleotides as the cells are lost.

Reporting a more extended study, Simpson and Schmidt (1969) describe separating individual glands and noting significant differences in DNA contents per nucleus among glands during pregnancy, lactation, and involution. They make the extreme statement that "sufficient evidence has been accumulated to

TABLE 1. MEAN DNA PER NUCLEUS OF RAT MAMMARY GLANDS

(Tucker and Reece, 1962)

Stage	DNA (Picograms/nucleus) Mean ± S.E
Pregnant 12 days	9.19 ± .44
Pregnant 20 days	8.47 ± .35
Lactation 1 day	8.33 ± .24
Lactation 4	8.57 ± .22
Lactation 8	8.66 ± .24
Lactation 14	8.49 ± .16
Lactation 21	8.53 ± .25

They reported that DNA per mammary cell nucleus was not significantly different in pregnant and lactating rats.

TABLE 2. MEAN DNA PER NUCLEUS OF RAT

Stage	DNA (picograms/nucleus) Mean ± S.E.
Pregnant 18 days	11.16 ± 0.17
Lactation 10 days	11.43 ± 0.16
Involution 7 days	9.54 ± 0.17

reject the use of DNA per mammary gland as an index of the number of cells in the gland." In the light of the extensive studies reported in this paper of normal and experimental growth during pregnancy, lactation, and involution their rejection of the method is open to serious question.

The question of polyploidy in the mammary epithelial cell nuclei has been studied by Persson (1960). His histochemical studies indicate that polyploidy does not take place.

The mammary glands of the rat are composed of epithelial and myoepithelial cells and are surrounded by connective tissue. The DNA estimation would include the nuclei of the epithelial cells and of the fat and connective tissues. While the epithelial cells increase with pregnancy, Harkness and Harkness (1956) reported that there was little increase in the collagen content of the mammary glands during pregnancy. This being true, control DNA values would represent the DNA of the epithelial and connective cells; the increase in DNA in pregnancy and following experimental growth would represent primarily increases in epithelial cells.

Nicoll and Tucker (1965) studied this problem in mice. By removing the mammary gland rudiment at three weeks of age, fatty pads without gland were compared with normal glands in the same mouse. Virgin mice when 15 to 20 weeks of age and lactating mice at day 10 were compared. It was observed that the DNA of the virgin gland-free fatty pads and those of the lactators were the same indicating that the cell population of the adipose stroma had not increased due to pregnancy and lactation.

Traurig (1967) reported that fibroblasts incorporated tritiated thymidine into DNA during the early lactational growth of the mouse gland and further that Asboe-Hansen (1958) reported on the effects of hormones on connective tissue. Paape and Tucker (1969) studied the changes in hydroxyproline (collagen-connective tissue) and hexosamine (ground substance) during lactation and post-lactational involution. Hydroxyproline of pregnant rats starting their dry period at 8, 12, 16, and 20 days of lactation decreased 8, 15, increased 4 and decreased 18 percent by day 4 of the dry period. In the non-pregnant animals during the same intervals they decreased 19, 19, 9, and 1 percent. In comparison to the loss of DNA, mammary hydroxyproline is more resistant to the process of involution.

Estimation of Mammary Gland Growth by DNA

The estimation of mammary gland growth by the determination of DNA chemically may be subject to some error involving the possible growth of connective tissue during pregnancy and lactation; it is the best method available. The six posterior glands are removed and fat extracted. The dry-fat-free tissue (DFFT) is determined. Then the DNA content of a 25 mg sample is determined. The DNA/mg sample indicates the cell size. If the cells are small, the DNA/mg will be high but if the cells are large the DNA/mg will be lower.

The product of the quantity of DNA/mg DFFT and the DFFT/100g body weight is estimated as the DNA/100 g body weight of the six glands (Griffith and Turner, 1961).

Rates of Mammary Gland Cell Division Measured by DNA

During the period of cell division there is a phase during which the cell doubles its DNA, and thymidine has been shown to be a specific precursor of DNA. By the introduction of labeled thymidine, the duration of the process of DNA synthesis can be measured. By this technique Bresciani (1964-1965) measured the duration of DNA synthesis in the mammary glands of mice. It was reported that three days after ovariectomy DNA synthesis was virtually stopped. In normal six-month-old mice the average duration was 20.7 hours with a range from 12.5 to 30.7 hours. (It is suggested that part of this variation may be due

to the state of the estrous cycle), ovariectomized mice were injected for three to four days with 1 ug estradiol benzoate (E B) plus 1 mg progesterone (P). DNA synthesis was reduced to 10.7 hours indicating a doubling of the rate of cell division.

Review of Anatomical and Biochemical Changes in Mammary Gland

In addition to the review of this problem in the rat, the readers are referred to a general review in regard to other experimental and domestic animals by Munford (1964). It is his opinion that indices of structure and measures of the biochemical changes are to be regarded, not as alternatives, but rather as complementary methods of assessing the state of the gland in studies of mammary development. Possibly his conclusions were based in part on a study of the relation of structural and biochemical changes in the mammary glands of rats and mice (Munford, 1963). He observed in the rat that histological estimates of total alveoli, nuclei, and glandular tissue were significantly correlated with total DNA but not with DNA concentration.

Experimental Procedure

In the experiments conducted in the Milk Secretion Laboratory at the University of Missouri, unless otherwise indicated, the rats used were of the Sprague-Dawley-Rolfsmeyer strain. The rats were fed Purina Lab Chow with an energy value of 4.41 Cal/g and 23.4% total protein and water *ad libitum.*

The rat has 12 mammary glands, six anterior and six abdominal-inguinal glands. It was observed that there were sheets of muscular tissue located between the pectoral glands which were difficult to dissect. Therefore, only the six abdominal-inguinal glands which are free of muscle were used to estimate the DNA content. In a study of pregnant rats there was no significant difference between the mean DNA of the six anterior glands (9.01 mg/100 g) and the abdominal-inguinal glands (8.46 mg/100 g). If one wishes to estimate total DNA increase in rats, the DNA can be doubled.

In estimating mammary DNA of the rat, the six abdominal-inguinal glands were removed, placed in a deep freeze for four days, extracted in 95 percent ethanol for three to five hours and ether for an additional three to five hours. The dry, fat-free tissue (DFFT) was weighed and ground to a fine powder in a Wiley mill.

The DNA content of a 25 mg aliquot sample was determined by the method of Webb and Levy (1955).

In the studies of Tucker and Reece, hooded Norway rats were used and in the study of Hahn and Turner (1967) on hypophysectomized rats, Sprague-Dawley rats were used.

Early Growth of the Mammary Gland

Myers (1917) observed extensive proliferation of the duct system of the rat during the fourth and fifth week after birth, and Cowie (1949) reported that the duct system grew at the same rate as body surface area (isometry) between birth and 21-22 days of age. Following this time (which is well in advance of puberty), the glands commence to increase at a rate significantly greater than for body surface area (allometry). Ovariectomy at day 22 abolished the allometric growth.

Sinha and Tucker (1966) determined the DNA of the mammary glands from days 10 to 100 at 10-day intervals (Table 3). In relating mammary DNA

TABLE 3. DNA OF THE MAMMARY GLANDS FROM 10 TO 100 DAYS
(Sinha and Tucker, 1966)

Age Days	Body Weight g	Gland Weight g	Total DNA mg	DNA/100g bw mg
10	22	0.985	0.33	1.50
20	42	1.054	0.53	1.62
30	77	2.053	1.25	1.79
40	125	2.879	2.24	2.01
50	140	3.274	2.81	1.47
60	173	4.466	2.55	1.29
70	180	4.947	2.33	1.23
80	196	6.127	2.41	1.80
90	198	4.389	3.57	1.80
100	208	5.218	3.52	1.69

to body surface area (body weight $^{2}/_{3}$) a single straight line fit the data, indicating an allometric type of growth. In addition to DNA estimate, a whole-mount of the right abdominal gland was prepared, magnified, and outlined and the area determined. The average area increased from 9.3 mm^2 at 10 days to 494.6 mm^2 at 80 days, a 52-fold increase. The correlation between area and DNA measurements was 0.93.

Effect of First Estrous Cycles on Mammary Gland Growth

Sinha and Tucker (1969) studied the increase in DNA of the mammary glands of rats during the first five estrous cycles as well as changes during stages of the cycles.

It was shown (Table 4) that there was an increase in DNA of 10 percent between the first and second cycle, 8 percent between the second and third, 3 percent between the third and fourth and less than 1 percent between the fourth and fifth estrous cycle.

TABLE 4. GROWTH OF THE MAMMARY GLAND DURING FIRST FIVE ESTROUS CYCLES AND DURING CYCLES
(Sinha and Tucker, 1969)

Estrous Cycle	Age	No. rats	Body Weight g	DNA/100g b.w. mg	Increase %
Number					
1	37	48	123	1.30	
2	46	48	150	1.43	10
3	50	48	161	1.54	8
4	54	48	176	1.58	3
5	60	48	176	1.59	1
Stage					
Proestrus	47	60	153	1.41	
Estrus	48	60	154	1.52	
Metestrus	50	60	158	1.52	
Diestrus	52	60	164	1.50	

On the basis of all five estrous cycles, DNA increased 8 percent between proestrus and estrus whereas changes between estrus and diestrus were not significant.

Normal Development of the Rat Mammary Gland

In the study of the normal and experimental development of the mammary glands of the rat using DNA as an index, studies are reported concerning the DNA of growing and mature virgin animals. The various groups of virgin animals show mean DNA varying from 2.6 mg/100g body weight to 3.62 mg. Most of the groups are about 3.0 mg or below (Table 5).

It will be observed further, that ovariectomy had little or no effect on the mean DNA of these groups. They range from 2.8 mg/100 g body weight to 3.3 mg. These observations are taken to indicate that the absence of the ovarian hormones does not cause an involution of the epithelial cells of the duct system.

However, after hypophysectomy there was a significant loss of DNA from 2.89 mg to 2.01 mg/100 g body weight, and after hypophysectomy, adrenalectomy, and ovariectomy a further decline to 1.84 mg was observed (Hahn and Turner, 1967).

The values for DNA in these studies have been used as a base line for studies of normal and experimental gland growth.

TABLE 5. DNA OF THE MAMMARY GLANDS OF VIRGIN, OVARIECTOMIZED HYPOPHYSECTOMIZED, ADRENALECTOMIZED FEMALE RATS

Type	No. of animals	Body Weight g	DFFT Mean mg	DNA ug/mg DFFT	Total DNA mg/100 g body weight mg	Reference
Virgin	10	281	303.2	28.8	3.11	(Wada & Turner, 1959)
Virgin	8		322.0	20.0	2.61	(Anderson & Turner, 1962)
Virgin Hooded Norway	12	172	204.3		2.77	(Tucker & Reece 1963)
Sprague-Dawley	22	246	381.1	18.46	2.95	(Hahn & Turner 1967)
Virgin	12	218	327.0	18.00	2.67	(Anderson & Turner,
Virgin	14	123	160.0	20.4	2.60	(Panda & Turner 1966)
Virgin	19	169	291.2	20.3	3.42	(Panda & Turner 1966)
Virgin	18	200	428.0	18.0	3.62	(Panda & Turner 1966)
Virgin	12	(200-250)		(Total)	3.81	(Desjardins et al, 1968)
Ovariectomized	10	279	356.1	25.9	3.32	(Wada & Turner 1959)
Ovariectomized	15	274	362.0	23.2	3.05	(Moon, Griffith & Turner, 1959)
Ovariectomized	8	282	682.0	11.6	2.81	(Damm, Miller & Moon)
Ovariectomized	5	296	448.5	16.2	2.60	(Moon, Turner 1961)
Ovariectomized	10	274	290	28.1	3.00	(Kumaresan & Turner, 1965)
Sprague-Dawley	22	290	445	17.9	2.89	(Hahn & Turner 1967)
Hypophysectomized (Sprague-Dawley)	25	211	213	19.8	2.01	(Hahn & Turner 1967)
Ovar-Hypo-Adrenal-ectomy	10	175	167	19.7	1.84	(Hahn & Turner 1967)

NORMAL GROWTH OF THE RAT MAMMARY GLAND

Pseudopregnancy

Normal cyclic rats have intervals varying from four to six days. If cyclic rats are mated with a sterile male or if the cervix is stimulated with a glass rod, pseudopregnancy is stimulated, resulting in extension of the life span of the corpora lutea to about 12 days. By whole mounts it has been observed that the mammary glands change from the duct system observed in virgin rats to a lobule-alveolar system characteristic of mid-pregnancy.

Pseudopregnancy with a more extended interval can also be induced by hysterectomy (18 days) and by the stimulation of uterine deciduomata (21 days). Since these latter types of pseudopregnancy approach or equal the duration of normal pregnancy, it might be expected that mammary growth equal to pregnancy would be induced.

Anderson and Turner (1968) compared the extent of gland growth in groups of rats induced to become pseudopregnant by these three methods (Table 6). The mean DNA of the rats on the twelfth day of normal pseudopregnancy was 3.49 mg/100 g body weight. This was 40 percent of the DNA found at day 12 of normal pregnancy and only 18 percent of the DNA increase to day 18 of normal pregnancy. This suggests that the corpora lutea of pseudopregnancy were functioning at about ⅖ of the level of those at day 12 of normal pregnancy and ⅕ of those at day 18.

Hysterectomy, which resulted in a six-day extension of pseudopregnancy was without effect in increasing DNA over normal pseudopregnancy.

The production of deciduomata pseudopregnancy resulted in a 9-day extension of the cycle. The mean DNA on day 18 was 4.05 mg/100 g body weight. This value is only 30 percent of that at day 18 of pregnancy.

The lowered growth of the mammary gland can be explained on the basis of the secretion of progesterone (P) during pseudopregnancy. The recent studies of Fajer and Barraclough (1967) and Hashimoto *et al.* (1968) indicate lowered levels of P in the ovarian venous blood of pseudopregnant rats. However, those with deciduomata showed the highest levels of P.

Desjardins *et al.* (1968) reported that the presence or absence of the decidual response during the first 12 days of pseudopregnancy did not significantly influence mammary DNA. Further decidual response to day 21 failed to increase DNA.

Effect of the Placenta on Mammary Gland Growth

Leonard (1945) reported that the placentas alone in the absence of the ovaries, pituitaries, and fetuses maintained the morphological integrity of the rat mammary glands comparable to that observed on the thirteenth day of preg-

TABLE 6. MAMMARY GLAND DNA IN PSEUDOPREGNANT RATS.[a]
(Anderson and Turner, 1968)

Group	Day	No. of rats	Body wt. (gm)	ug of DNA/ mg of DFFT	DFFT (mg)	DNA (mg)	mg of DNA/ 100 gm of bw
Virgin control	–	12	218	18.0±0.6[b]	327±17	5.82±0.26	2.67±0.16
Normal pseudopregnancy	3	12	224	21.3±1.1	358±28	7.38±0.32	3.30±0.14
	6	12	226	22.4±0.8	350±24	7.70±0.96	3.41±0.13
	9	12	231	23.0±0.5	311±18	7.42±0.38	3.22±0.14
	12	12	246	23.9±0.6	364±29	8.60±0.46	3.49±0.17
Pseudopregnancy after hysterectomy	9	12	240	26.0±1.0	302±21	7.66±0.39	3.20±0.17
	12	12	240	26.3±1.0	333±38	8.34±0.55	3.46±0.16
	15	12	233	26.1±0.6	325±25	8.50±0.69	3.63±0.24
	18	12	235	25.8±0.8	302±17	7.72±0.42	3.29±0.12
Pseudopregnancy with deciduomata	9	12	209	29.5±1.8	287±25	8.21±0.59	3.93±0.28
	12	12	218	24.7±0.9	341±29	8.23±0.51	3.82±0.28
	15	12	230	25.3±1.8	366±35	8.83±0.74	3.83±0.31
	18	12	228	27.8±1.4	344±24	9.22±0.34	4.05±0.13

[a]Abbrev.: DNA = deoxyribonucleic acid; and DFFT = dry fat-free tissue.
[b]Mean and SE.

nancy. Reece and Yard (1970) reported no effect on mammary gland DNA by differences in the number of fetuses or placental weight.

Placental Hormones of the Rat

Ray *et al.* (1955) reported that the 12-day rat placenta given to hypophysectomized-ovariectomized rats with estrone and progesterone induced mammary gland growth and lactation. Lactation was increased when hydrocortisone and growth hormones were added.

Pregnancy

The growth of the mammary gland of the rat during pregnancy as measured by DNA, has been studied by several investigators (Table 7). The mean total DNA/100 g bw at the end of pregnancy varied from 7.27 mg to a high of 8.47 mg. The weighed mean of 69 animals was 7.67 mg/100 g body weight (Fig. 1).

In the studies where rats were examined at intervals during pregnancy, it was observed in all cases that growth as measured by DNA increased throughout pregnancy rather than during the first half or ⅔ suggested by earlier observations by means of whole mounts.

Effect of Fetus Removal

Desjardins *et al.* (1968) removed the fetuses and fetal placentas of rats at various stages of pregnancy. The removal of the fetuses before mid-pregnancy,

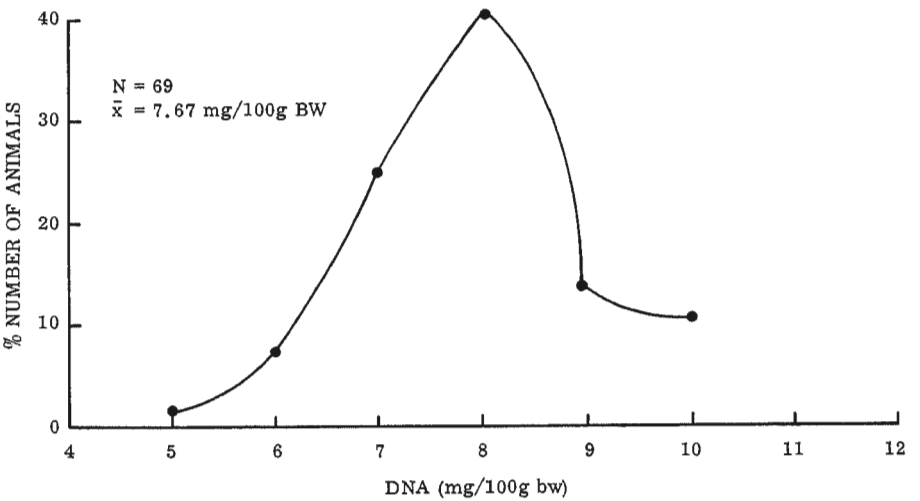

Figure 1

TABLE 7. DNA OF THE MAMMARY GLANDS OF THE RAT DURING PREGNANCY

Stage	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight	Reference
Pregnancy						
18-20 days	19	324	749	33.2	7.63	Griffith & Turner 1959
5	21	253	288	25.2	2.83	Griffith & Turner 1961
10	21	255	363	33.0	4.52	Griffith & Turner 1961
15	19	264	422	43.0	6.76	Griffith & Turner 1961
4	12	173	198		2.80	Tucker & Reece (1963)
8	12	181	218		3.33	Tucker & Reece (1963)
12	12	198	278		4.47	Tucker & Reece (1963)
14	12	202	330		5.69	Tucker & Reece (1963)
18	12	217	407		7.36	Tucker & Reece (1963)
20	12	218	441		7.89	Tucker & Reece (1963)
20	20	228	494	39.7	8.47	Kumaresan & Turner 1965
9	12	262	477	25.7	4.48	Anderson & Turner (1968)
12	12	262	426	28.9	4.70	Anderson & Turner (1968)
15	12	284	547	27.4	5.34	Anderson & Turner (1968)
18	12	271	578	33.9	7.27	Anderson & Turner (1968)
20	22	238	568	-	7.50	Kumaresan, Anderson & Turner (1967)
14	10	257	512		5.68	Ferreri & Griffith
21	8	272	604		7.86	Ferreri & Griffith 1969
12	12	(206-250)		(Total)	10.70	Desjardins et al. (1968)
21	12	(206-250)		(Total)	17.60	Desjardins et al. (1968)

TABLE 8. DNA OF THE MAMMARY GLANDS OF PREGNANT RATS AFTER FETUS AND PLACENTAS REMOVED

Stage of pregnancy days	Description	No. of animals	Body weight g	Total DNA g	Reference
21	Fetus removed on day 8	12	(200-250)	6.59	Desjardins et al. 1968
21	Fetus removed on day 12	12	(200-250)	15.06	Desjardins et al. 1968
21	Fetus removed on day 16	12	(200-250)	18.22	Desjardins et al. 1968

but not after, interfered with the normal increase in DNA. Excision of the fetal placentas caused mammary development to regress to non-pregnant control values. They suggest that maternal hormones are the primary stimulant of mammary gland growth during the first half of pregnancy but the fetal placenta commences to stimulate growth at mid-pregnancy and later.

Relation of the DNA of Immature Glands and Day 15 of Lactation

Hackett and Tucker (1969) studied the relation of mammary gland growth as measured by DNA of rats 21, 35, and 49 days of age (all prior to pregnancy) with the same rats on day 15 of lactation (Table 9-10).

The three right abdominal-inguinal glands were removed surgically on days 21, 35, and 49. The animals were bred beginning on day 63. On day 3 of lactation, all litters were adjusted to six pups to nurse the nine remaining glands. On day 15 of lactation, the pups were separated from mothers for 12 hours, then

TABLE 9. DNA OF 3 RIGHT ABDOMINAL-INGUINAL GLANDS

Successful	Age at Mastectomy (days)		
	21	35	49
Body weight g	42	113	161
Mammary gland weight, dry (mg)	20	92	177
Mammary gland DNA mg.	.34	1.35	2.11
Unsuccessful			
Body weight g	44	116	161
Mammary gland weight, dry (mg)	24	85	170
Mammary gland DNA, mg	.37	.80	1.68

TABLE 10. DNA OF GLANDS ON DAY 16 OF LACTATION

Successful	Age at Mastectomy (days)		
	21	35	49
Body weight g	284	308	301
Mammary weight, dry mg	790	761	766
Mammary DNA, mg	15.9	16.5	16.8
Litter wt. gain days 3-15 g	118	121	123
Milk yield g	6.1	6.1	6.0
Unsuccessful			
Body weight g	303	300	271
Mammary weight	791	778	642
Mammary DNA mg	13.2	12.9	14.2
Litter wt. gain days 3-15	88	79	83
Milk yield g	3.6	4.5	5.3

allowed to suckle only the three left glands with aid of oxytocin. The cumulative litter weight gains between days 3 and 15 were recorded as was the milk obtained at nursing (difference in weight of pups before and after nursing). Some mothers had insufficient milk to nurse their young adequately so foster pups were added as required to maintain a litter size of 6. These groups were called successful and unsuccessful.

Body and mammary gland weights of the two groups increased similarly between days 21 and 49. Between days 35 and 49 the DNA of the successful rats was 19 percent higher.

In all groups mammary DNA at the sixteenth day of lactation was positively and significantly correlated with litter weight gain. In comparing the DNA of immature rats with the DNA in lactation of the successful group, the correlation coefficients were 0.54 at 21 days, 0.98 at 35 days, and 0.71 at 49 days, suggesting that rats possessing a greater number of cells at immaturity will also have more cells at the peak of lactation. In the unsuccessful group only the 49-day group was positively and significantly correlated with litter weight gain.

Effect of Various Hormones on DNA of the Mammary Glands at End of Pregnancy

The weighted mean DNA/100 g body weight of 69 rats at the end of pregnancy was 7.67 mg. The effect of individual hormones injected during pregnancy has been determined.

Thyroxine (L-T_4)

When a group of 15 pregnant rats were injected with 3.5 ug/100 g bw/day, the DNA increased to 9.66 mg/100 g bw. Another group of 45 rats were injected

with L-T_4 at levels of 2.5 ug, 3.0 ug, and 3.5 ug. The mean DNA of this group was 9.07 mg. These values represent increases of 37 percent and 22 percent respectively (Griffith and Turner, 1961).

In another experiment 3.0 ug/100 g bw was injected into a group of 16 rats. The mean DNA of this group was 7.22 mg/100 g bw (Kumaresan and Turner, 1966). In this group the control DNA was 7.41 mg so the L-T_4 was without benefit.

Effect of Growth Hormone Separately and With Thyroxine on Gland Growth of Pregnant Rats.

The effect of bovine growth hormone upon the growth of the mammary gland of pregnant rats has been studied (Kumaresan and Turner, 1965). A group of 20 rats was given 2 mg/day. Since the DNA of the control group was 8.47 mg/100 g bw, and the experimental group showed a mean total DNA of 8.49 mg, it was concluded that bovine growth hormone was without benefit in these rats. However, the DNA was slightly higher than the weighted mean of all pregnant rats (7.67 mg). In a later experiment the control group showed a mean DNA of 7.41 mg while the 20 rats on bovine growth showed a mean DNA of 8.37 mg, an increase of 13 percent (Kumaresan and Turner, 1966).

When 2 mg of bovine growth hormone was administered with 3 ug of L-T_4 during pregnancy, the mean DNA was increased to 10.91 mg/100 g bw. This is an increase of 26 percent over the uninjected control group or the group given the growth hormone alone. It is 1.25 mg greater than that stimulated by L-T_4 alone.

When ovine growth hormone was given with L-T_4, the mean DNA was increased to 9.9 mg/100 g bw, a significant increase over the control group of 17 percent (Kumaresan and Turner, 1965).

When bovine growth hormone and L-T_4 were given to 23 pregnant rats the DNA increased to 10.14 mg, a 37 percent increase above the control group (7.41 mg) (Kumaresan and Turner, 1966).

Effect of Insulin on Mammary Gland Growth in Pregnant Animals.

When 3 u of insulin was administered to 19 pregnant animals the DNA showed only a 5 percent increase over controls. (Kumaresan and Turner, 1966).

Effect of Insulin and L-T_4

When 21 rats were injected with 3 u of insulin plus 3.0 ug L-T_4/100g bw during pregnancy, the DNA was increased to 8.8 mg, an increase of 19 percent above the controls (7.41 mg) (Kumaresan and Turner, 1966)

Effect of Insulin and Growth Hormone

The synergism of insulin and growth hormone was shown in a study with 17 rats injected daily with GH and insulin. The mean DNA was increased to 11.99 mg., an increase of 62 percent above the control group (7.41 mg) (Kumaresan and Turner, 1966).

Effect of Insulin, Growth Hormone and L-T_4

When these three hormones were administered to 14 pregnant rats, the mean DNA was increased to 10.48 mg, an increase of 41 percent above the controls (Kumaresan and Turner, 1966).

Effect of Glucocorticoids

The effect of graded levels of corticosterone upon the growth of the mammary glands of pregnant rats was determined by Kumaresan *et al.* (1967). Levels of hormone from 0.25 to 1.75 mg were given daily for 19 days. The optimal levels of corticosterone were in the range of 0.75 and 1.25 mg/day with a mean increase in DNA of 9.2 mg/100 g bw, or 23 percent above the controls (Table 11). Ferreri and Griffith (1969) injected hydrocortisone acetate at a level of 2 mg/day to rats from 9 to 13 and 16 to 20 days of pregnancy. In neither case was the mammary DNA changed.

Growth of the Mammary Gland During Lactation

In the past, mammary gland growth was thought to occur primarily during the first two-thirds of pregnancy (or during pseudopregnancy), followed by the gradual initiation of lactation during the final trimester. Reviews demonstrate that mammary gland growth continued throughout pregnancy.

It was then shown that the DNA of rats exhibited additional growth by the fifth day of lactation (Griffith and Turner, 1959) and more by the tenth and fourteenth days. In comparison with the DNA at the end of pregnancy, the increase amounted to 41.5 percent.

Since the first five days appeared to be the critical period of lactational growth, the mean DNA of the glands of rats on days 1 to 4 of lactation were determined. At the end of pregnancy, the DNA content averaged 7.63 mg/100 g bw. On day 1 of lactation it had increased to 8.46, on day 2 to 10.05 mg, and on day 3 to 10.78 mg. Thus the greater part of "lactational growth" occurred during a three-day period (Griffith and Turner, 1961).

Tucker and Reece (1963) reported that mammary gland development from day 20 of pregnancy to day 1 of lactation as measured by DNA increased 9.8 percent. From day 1 to day 4 of lactation the increase was 25.9 percent. Considering maximum development as 100 percent, it was reported that 24.8 percent of the total development occurred during lactation.

TABLE 11. EFFECT OFVARIOUS HORMONES ON THE DNA OF THE PREGNANT RAT

Hormone	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight	% Increase Over Controls	Reference
$L\text{-}T_4$							
3.5 ug/day	15	283	740	37.7	9.66		Griffith & Turner (1961)
2.5, 3.0 & 3.5 ug	45	283	736	35.9	9.07	22	Griffith & Turner (1961)
3 ug/100 g b.w.	16	225	540	16.2	7.20		Kumaresan & Turner (1965-1966)
Growth Hormone							
2 mg/day (Bovine)	22	245	550	37.9	8.49		Kumaresan & Turner (1965-1966)
/day (Bovine)	20	208	516	17.4	8.37	13	Kumaresan & Turner (1965-1966)
$L\text{-}T_4$ + G H							
2 mg G H (Bovine) + 3 ug $L\text{-}T_4$/100g b.w.	11	233	551	46.5	10.91		Kumaresan & Turner (1965-1966)
2 mg G H (Ovine) + 3 ug $L\text{-}T_4$/100 g b.w.	14	232	644	36.2	9.90		
G H + $L\text{-}T_4$	23	233	561	23.7	10.14	37	Kumaresan & Turner (1965-1966)
Insulin 3 u/day	19	211	563	16.3	7.76	5	Kumaresan & Turner (1965-1966)
Corticosterone 0.75mg /day	22	225	555		9.20		Anderson & Turner (1967)
1.25mg/day	21	226	565		9.20	23	Anderson & Turner (1967)
$L\text{-}T_4$ + Insulin	21	237	568	20.8	8.80	19	Kumaresan & Turner (1966)
G H + Insulin	17	233	723	27.8	11.99	62	Kumaresan & Turner (1966)
$L\text{-}T_4$ + G H Insulin	14	234	720	24.7	10.48	41	Kumaresan & Turner (1966)

Effect of Extended Lactation on DNA

By providing one to four-day old foster pups every 21 days, Tucker and Reece (1963) extended lactation for 41 to 61 days. At the ends of these periods, mammary gland DNA was determined. Based on final body weight the DNA on day 21 was 11.0 mg/100 g bw compared to 9.58 mg on day 41 and 9.48 mg on day 61. These differences were not significant.

Effect of Ovariectomy on DNA Development During Lactation

After parturition an estrous cycle occurs and a corpus luteum of lactation forms. To determine whether this gland had an influence upon lactational growth, Griffith and Turner (1959) ovariectomized a group of rats on day 2 of lactation. On day 14 of lactation the DNA was as high as in normal rats. This indicates that the corpus luteum of lactation was without effect upon lactational growth.

Tucker and Reece (1963a) ovariectomized rats one to four hours after parturition and sacrificed them on day 8 of lactation. Sham operated rats served as controls. The DNA/100 g bw was 10.97 mg for ovariectomized and 11.46 mg for the controls, a non-significant difference.

Tucker *et al.* (1967) determined the effect of ovariectomy on day 1 of lactation and nursing intensity on the DNA of rats killed on day 8 or 16. Ovariectomized rats contained at days 8 and 16 an average of 10.3 and 3.5 percent less mammary DNA than intact control rats. Glands of ovariectomized rats increased more than 56 percent in DNA as suckling increased from two to six pups.

Effect of Lactation and Pregnancy on DNA of the Mammary Glands

A post-partum estrus occurs in rats and if the animals are mated, lactation and pregnancy occur concurrently. Tucker and Reece (1964a) reported that the DNA/100 g body weight of the mammary glands of lactating-pregnant rats was greater than that in lactating rats on day 18 but less on day 24. No differences were noted on days 21 and 28. It was suggested that mammary development declined with advancing stages of concurrent pregnancy. It was shown further, that litters of 7 or more fetuses stimulated significantly less DNA than smaller litters, suggesting that fetuses have priority over lactating mammary tissue for available nutrients.

Paape and Tucker (1969) compared the DNA of rats which were lactating and pregnant with a second group of lactating non-pregnant animals. The greatest DNA in the lactating, pregnant rats was observed on day 16 with a decline on days 20 and 24. In the non-pregnant group DNA remained relatively constant.

TABLE 12. DNA OF THE MAMMARY GLANDS OF THE RAT DURING LACTATION

Stage	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight	References
Lactation						
5th	10	302	954	34.1	10.8	Griffith-Turner (1959)
10th	10	302		31.8	12.3	Griffith-Turner (1959)
14th	25	298	1240	26.3	10.9	Griffith-Turner (1959)
21	9	305	1210	28.3	11.3	Griffith-Turner (1959)
day 14						
2nd Lactation	18	290		22.3	12.8	Griffith-Turner (1959)
1st	11	245	584	35.6	8.5	Griffith-Turner 1961a
2nd	10	248	659	37.7	10.1	Griffith-Turner 1961a
3rd	8	269	789	37.1	10.8	Griffith-Turner 1961a
4th	8	263	735	37.5	10.5	Griffith-Turner 1961a
1st	12	204	565		8.7	Tucker & Reece 1963a
4th	12	212	699		10.9	Tucker & Reece 1963a
8th	12	217	876		11.5	Tucker & Reece 1963a
12th	12	238	1058		11.1	Tucker & Reece 1963a
14th	12	233	1029		10.7	Tucker & Reece 1963a
18th	12	236	1207		11.2	Tucker & Reece 1963a
21th	12	231	1163		11.0	Tucker & Reece 1963a
24th	12	232	1081		10.6	Tucker & Reece 1963a
28th	12	209	776		9.7	Tucker & Reece 1963a
41th	11	242	912		9.6	Tucker & Reece 1963b
61th	12	256	893		9.5	Tucker & Reece 1963b

TABLE 13. GROWTH OF MAMMARY GLANDS
LACTATING-PREGNANT RATS
(Tucker and Reece, 1964a)

Days of lactation	No. of rats	Mean body weight g	DFFT mg	Total DNA/100g bw mg
18	12	251	1268.4	11.88
21	12	239	1146.7	11.55
24	8	227	898.4	9.81
28	7	221	743.3	9.51
		Pregnant and lactating 19 - 20 days		
No. of fetuses				
Less than 6	7	235	1071.0	11.26
7 or more	18	238	851.6	9.78
None	23	225	1060.0	10.79

DNA of the Mammary Glands of Rats Nursing Increasing Numbers of Young

The DNA of the mammary glands on day 21 of lactation of rats nursing from two to 10 pups was determined by Tucker (1964). Total DNA increased as the number of pups increased. Where two and four pups nursed, some of the glands were not being nursed and were undergoing involution.

Moon (1965) ligated the galactophores of the right six mammary glands of rats on day 3 postpartum and litters were adjusted to contain three, six, nine, or 12 pups. Mammary glands on the left side were not ligated. Rats nursing six young but without galactophore ligation served as controls. Animals were sacrificed on day 14 postpartum and DNA content of both left and right abdominal-inguinal glands was determined. In galactophore-ligated rats, ligated glands were significantly smaller than contralateral non-ligated glands. DNA content of ligated glands of rats nursing 12 young was similar to that of corresponding control glands, whereas DNA content of glands of rats nursing three to nine young was less than that of control glands. In animals with galactophore ligation, non-ligated glands were significantly larger than corresponding glands of rats without galactophore ligation. It appears that the strength of the suckling stimulus is directly related to maintenance of the mammary parenchyma during lactation.

Tucker (1966) reported upon the influence of two, four, or six suckling pups on the DNA of the six abdominal-inguinal glands during lactation. No increase in DNA was observed in rats suckling two pups per six glands after day 4, whereas DNA of rats suckling four pups per six glands increased to day 12. DNA of rats suckling six pups per six glands increased 108 percent between days 1 and 16. DNA declined in all sucking intensities at days 20 and 24.

TABLE 14. DNA OF THE MAMMARY GLANDS OF THE LACTATING RAT PREGNANT OR NON-PREGNANT

(Paape and Tucker, 1969)

	Lactating, pregnant		Lactating, non-pregnant	
Day of lactation and pregnancy	Body weight g	Total DNA mg/100g b w	Body weight g	Total DNA mg/100g b w
8	252	11.98	248	12.10
12	245	12.53	258	12.64
16	245	13.31	256	12.73
20	250	11.72	250	11.92
24	235	9.57	256	12.62
Overall mean	246	11.79	254	12.40

Influence Of Number Of Fetuses And Placenta on DNA Content of Mammary Glands. (Reece and Yard, 1970).

No. of rats	Body weight g	Fetuses and Placenta		Total DNA mg/100 g body wt.
		Number	Total Weight g	
23	240	1-3	12.61±0.94	8.16±0.24
31	252	6-13	39.48±2.24	8.06±0.33

In a second experiment, progressive increases in suckling stimuli with maintenance of a constant pup-to-gland ratio did not promote increased DNA. Nucleic acid losses in teat-ligated gland were partially retarded but not prevented by increasing the suckling intensity from one to three pups between days 3 and 16 of lactation. Total DNA was highly correlated with litter weight gains.

In a study by Kumaresan *et al.* (1967), the DNA/100 g body weight of rats nursing from two to 12 pups was determined (Table 15).

TABLE 15. EFFECT OF LITTER SIZE ON DNA AT DAY 20 OF LACTATION

No. of pups/litter	No. of rats	Body weight g	Mean DFFT mg	DNA ug/mg DFFT	Total DNA/100g body weight
2	22	308	907.0	27.04	8.92
4	20	306	1130.3	31.21	10.28
6	20	293	1179.6	31.78	10.87
8	19	278	1176.2	31.43	11.44
10	20	286	1378.6	35.59	12.42
12	23	285	1455.4	38.70	13.56

It will be noted that the mean DNA/100 g body weight increased with increasing numbers of pups. The reduced DNA of the rats nursing two to four pups may be explained as due to involution due to the glands not being nursed. The increased DNA in the glands of the rats nursing 10 and 12 pups might be due to a stimulus for increased growth of cells during lactation or the high level of DNA may represent the maximum growth of the mammary gland with the intense lactation preventing involution from occurring up to the twentieth day.

Moon (1969) studied the effect of litter size (6, 9, and 12) upon the mammary DNA on day 14 of lactation (Table 16).

TABLE 16. EFFECT OF LITTER SIZE ON DNA ON DAY 14 OF LACTATION

No. in litter	No. of lactating rats	Body weight g	DFFT mg	DNA/mg DFFT(ug)	Total DNA (mg/100g)	Difference %
6	10	325	1296	20.04	8.02	-
9	12	296	1336	21.76	9.73	22
12	10	272	1238	24.50	11.11	39

The DNA of rats nursing nine or 12 young was 22 and 39 percent greater than that of rats nursing six. The DNA of those nursing 12 was significantly greater than that of dams nursing nine.

Effect of Intensive Nursing During Extended Lactation on DNA

Thatcher and Tucker (1968) ligated the 6 anterior nipples of rats so that the 6 pups would nurse the posterior 6 glands intensely. The litters were replaced each 4 days with 12-day-old foster litters. The mothers were killed on days 16, 20, 28 and 36 of lactation and the DNA determined (Table 17).

TABLE 17. EFFECT OF EXTENDED NURSING ON DNA

Day of lactation	Body weight g	Fresh gland wt. (g)	Total DNA mg	Total DNA (100g/body wt.) mg
16	242	12.0	36.2	14.96
20	253	12.9	37.2	14.70
28	259	10.6	32.0	12.36
36	273	11.2	27.0	9.89

It was shown that DNA did not change significantly between days 16 and 20 but declined linearly between days 20 and 36. It should be noted in this study that the DNA/100g bw on days 16 and 20 was quite similar to that observed when 12 pups were nursed.

Effect of Increasing Amounts of Milk in the Gland on DNA Content

Tucker and Reece (1964) compared the DNA content of the glands of lactating rats on days 13 and 14 when nursed and not nursed for periods from three to 12 hours. The object of the experiment was to determine the effect of increasing amounts of milk in the glands on the DNA estimation. It was shown that as the amount of milk present in the glands increased there was a linear increase in DNA up to 12 hours. It was suggested that the increase in DNA was due to increased numbers of leucocytes present in the milk.

Effect of Various Hormones on Mammary Gland DNA at End of Lactation

The effect of a number of hormones on the intensity of milk secretion to day 20 of lactation has been studied. The results of these studies will be reported in another research bulletin. At the end of lactation (day 20) the mean DNA of the mammary glands was determined to learn the effect of the hormone upon involution, the maintenance of the gland, or the possible additional growth of the gland, as a result of hormone treatment.

The Lactogenic Hormone

The lactogenic hormone was injected daily at levels of 1, 2, or 3 mg from days 7 to 19 of lactation. On day 20 after the dams were nursed, the DNA was determined (Kumaresan, Anderson and Turner, 1966) (Table 18).

TABLE 18. EFFECT OF LACTOGENIC HORMONE ON DAY 20 OF LACTATION

Hormone	No. of rats	Body weight g	Mean DFFT mg	DNA ug/mg DFFT	mg/100g bw mean	% Increase over control
Control	13	286	980	30.32	10.19	
1mg LGH	18	276	1101	25.52	10.27	0.79
2mg LGH	17	320	1313	27.40	11.22	10.11
3mg LGH	18	300	1245	26.54	11.15	9.42

While the groups on 2 and 3 mg lactogenic hormone showed about 10 percent greater mean DNA compared to the controls, the increases were not statistically significant.

Parathyroid Extract, Dihydrovitamin-D_2 II and Calciferol

Djojosoebagio and Turner (1964) injected parathyroid extract containing 30 USP units/100 g body weight daily to 44 rats from day 7 to day 20 of lacta-

tion. The mean DNA of the glands was 10.4 mg/100 g bw. The 27 control animals had a mean DNA of 9.3 mg. The increase was significant. Dihydrovitamin-D_2 II was injected daily at a level of 125 ug/100 g body weight to 24 rats from day 7 to day 20 of lactation. The mean DNA was 10.1 mg, a non significant increase.

Dihydrovitamin-D_2 II was injected daily at a level of 125 ug/100 g body weight to 24 rats from day 7 to day 20 of lactation. The mean DNA was 10.1 mg, a non-significant increase.

Calciferol (Vit. D_2) was injected daily at a level of 0.2 mg/100g bw to 33 rats from day 7 to day 20 of lactation. The mean DNA of the group was 9.2 mg which was slightly less than that of the controls.

Dihydrotachysterol

A group of 23 lactating rats was thyroparathyroidectomized on day 4 of lactation and administered 100 ug crystalline dihydrotachysterol and 3 ug L-T_4/100 g body weight per day. A control group of 13 animals was sham operated. On day 20 of lactation the sham-operated group had a mean DNA of 9.10 mg/100 g bw whereas the operated animals showed a decline to 8.33 mg.

Effect of Estrogen and Progesterone

Griffith and Turner (1962) injected both normal and ovariectomized lactating rats with 1 ug estradiol benzoate, 3 mg progesterone, and 1 ug E B + 3 mg P for 13 days. The results are summarized in Table 19.

TABLE 19. EFFECTS OF ESTROGEN AND PROGESTERONE

Treatment	No. of animals	Body weight g	Total DNA (mg/100g bw)
		Normal	
Normal	21	294	10.88
E B 1 ug.	22	291	9.09
1 ug EB+ 3 mg. P	15	305	9.88
P 3 mg.	19	303	10.96
		Ovariectomized	
Controls	14	284	9.99
E B 1 ug.	11	276	11.77
1 ug EB+ 3 mg. P	9	295	11.18
P 3 mg.	13	285	12.58

In the normal rats, the ovarian hormones had no significant effect on DNA on day 14. In the ovariectomized rats the DNA in each group was higher than that in the controls.

Insulin

The effect of 1, 2, and 3 units of insulin injected daily from day 7 to 19 of lactation was reported by Kumaresan and Turner (1965). The mean DNA of 24 control rats on day 20 was 9.52 mg, whereas the 17 rats on 3 units of insulin was 10.41 mg, a non-significant increase.

Effect of Adrenalectomy and Replacement Therapy

A group of 12 normal lactating rats on day 20 had a mean DNA of 11.1 mg/100 g bw. A group of 15 similar rats was adrenalectomized on day 4. Without treatment, they were all dead on day 20. A group of six given 12.5 ug aldosterone daily had mean DNA of 6.4 mg/100 g bw. A second group of five given 25 ug/day had a mean DNA of 10.7 mg. A group of 12 given 1 mg corticosterone had a mean DNA of 9.5 mg. A group of four given 12.5 ug aldosterone and 1 mg corticosterone had a mean DNA of 10.3 mg. When 25 ug aldosterone was given with 1 mg of corticosterone the mean DNA was 9.7 mg (Anderson and Turner, 1963).

A group of 15 rats was adreno-ovariectomized. When they received 2 mg corticosterone daily they contained a mean DNA of 11.4 mg, whereas a group of eight given 25 ug aldosterone and 1 mg corticosterone had a mean DNA of 9.7 mg.

The best maintenance of the glands was obtained by 2 mg corticosterone, 25 ug aldosterone, or 12.5 ug aldosterone and 1 mg corticosterone.

In a further study, Anderson and Turner (1963) injected 500 ug cortisol plus 250 ug desoxycorticosterone acetate (DCA) to 10 animals. The mean DNA was 10.1 mg. When 1 percent NaCl was added to the drinking water and 250 ug cortisol were injected the mean DNA was 9.2 mg.

Experimental Stimulation of "Lactational Growth"

From the fact that "lactational growth" occurred in ovariectomized rats it became clear that this growth was not stimulated by hormones of ovarian origin. At the initiation of lactation, the lactogenic hormone has been shown to be discharged by the stimulus of nursing. It is possible that growth hormone, TSH, ACTH and other anterior pituitary hormones may be released at nursing time.

In initiating experiments to determine if anterior pituitary hormones or hormones from their target glands would be effective in stimulating "lactational growth," it was decided to stimulate the equivalent of the pregnancy development with estrogen and progesterone, then inject one or more hormones to be tested for 3 days, sacrifice the animals on day 24, and then determine the DNA at that time.

Lactogenic Hormone. The rats in this experiment were injected for 19 days with 1 ug estradiol benzoate (EB) and 3 mg progesterone (P). The mean total DNA of the group was 6.81 mg. A second group was injected at the same level for 24 days. Their mean DNA was 6.37 mg, indicating no increase by three days of additional treatment. When 2 mg lactogenic hormone/day was given for 3 days to animals treated for 19 days with E B + P, the mean DNA was 6.41 mg/100 g bw, indicating that lactogenic hormone was without benefit on lactational growth (Griffith and Turner, 1963).

In repeating the experiment, the rats were injected with 2 ug E B + 6 mg P for 19 and 24 days. The mean total DNA in these groups was 6.06 and 6.22 mg, respectively. The injection of 2 mg lactogenic hormone/day for three days produced glands with a mean DNA of 6.63 mg. Again, lactogenic hormone was without effect (Griffith and Turner, 1963).

Growth Hormone. Groups of rats were injected with 2 ug E B + 6 mg P for 19 days. Then one group was given 1.5 mg of growth hormone (GH) for 3 days and a second group 2 mg GH/day for 3 days. The first group showed a mean DNA of 9.05 mg, a significant increase over controls but the second group showed DNA of 6.05 mg/100 g bw which was not different from the control values (Griffith and Turner, 1963).

Hydrocortisone Acetate. A group of rats was given 1 ug E B + 3 mg P for 19 days, then given hydrocortisone acetate (HCA) for three days. The mean DNA of the group was 7.18 mg/100 g bw, a non-significant increase above the control group. In a second group, the EB was increased to 2 ug + 6 mg P for 19 days. The animals were then given 250 ug HCA for three days. The mean total DNA was 8.37 mg, a significant increase over controls (Griffith and Turner, 1963).

Lactogenic and Hydrocortisone Acetate. A group of rats was injected for 19 days with 1 ug E B + 3 mg P, then injected with 2 mg lactogenic hormone and 250 ug HCA for three days. The mean DNA of the group was 7.25 mg/100 g bw, not significantly greater than that of the control group (Griffith and Turner, 1963).

Lactogenic Hormone and Growth Hormone. A group of rats was injected for 19 days with 2 ug E B + 6 mg P daily, then injected for three days with 2 mg/day of lactogenic hormone and 1.5 mg/day of growth hormone. The mean DNA of the group was 8.62 mg/100 g bw, a significant increase over the controls (Griffith and Turner, 1963).

Growth Hormone and Hydrocortisone Acetate. A group of rats was injected with 2 ug E B + 6 mg P daily for 19 days then 2 mg of growth hormone and 500 ug hydrocortisone acetate was given daily for three days. The mean DNA of the group was 9.15 mg/100 g bw, a significant increase over the

mean of the control group. This value is 47 percent greater than the control group value at day 24 (Griffith and Turner, 1963).

Thyroxine and Other Hormones

In a further study by Srivastava and Turner (unpub), the effect of L-T_4 on lactational growth was studied. The rats were injected with 2 ug EB + 6 mg P for 19 days. The mean DNA of the control group was 6.95 mg/100 bw. L-T_4 at a level of 3 ug/100 g bw daily for 5 days stimulated the DNA to 7.87, a 13.2 percent increase. When 1.5 mg of bovine GH and 1.25 mg of corticosterone were added the DNA increased to 9.34 mg, a 34.4 percent increase. When 2 mg lactogenic hormone was added, the DNA was 9.3 mg, a 33.8 percent increase. Since the lactogenic hormone had no beneficial effect, it is suggested that L-T_4, growth hormone, and corticosterone are primarily involved in lactational growth.

An alternative experimental technique was tried by Sinha and Turner (unpublished). In this case lactating animals were used and the homones were injected for eight days post-partum. On day 10 of lactation, the DNA of the control group was 9.08 mg/100 g bw. When 2 mg GH, 1 mg corticosterone, 3 u insulin, 4 mg bovine lactogenic, and 3 ug L-T_4/100 g bw were given, the DNA was increased to 13.24 mg, 45.8 percent above the controls. When GH and corticosterone were injected, the DNA increased to 11.41 mg, 25.7 percent above the controls. When insulin, L-T_4, and lactogen were injected the DNA was not increased above that in the controls. It appears that GH and corticosterone play a major role in lactational growth but other hormones may synergize with these hormones.

Involution of the Rat Mammary Gland. It has been recognized for a long time that the cessation of the nursing stimulus (weaning) results in a rapid loss of the lobule-alveolar gland structure (Myers and Myers, 1921; Kuramitsu and Loeb, 1921; and Maeder, 1922).

Selye (1934) and Williams (1945) suggested that the withdrawal of the suckling stimulus, rather than the accumulation of milk within the gland was primarily responsible for the rapid involution that took place. By means of the DNA method, the rate of normal involution of lactating rats has been studied (Table 20). When the young were separated from their dams on day 1 of lactation, DNA decreased 42 percent by day 5, and 85 percent by day 10, which then remained constant up to 20 days. This latter value is similar to that of virgin or ovariectomized animals (Griffith and Turner, 1961).

Tucker and Reece (1963) also determined the rate of involution on days 1, 3, 12, and 21 after the young were removed. The DNA/100 g bw one day after weaning did not differ significantly from that of glands on day 21 of lactation. DNA decreased significantly on day 3 and on day 21 was similar to that of glands from sexually mature virgin rats.

TABLE 20. DNA OF THE MAMMARY GLANDS OF THE RAT DURING INVOLUTION

Stage Involution	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body wt.	References
5th day	27	258	494.0	32.2	6.10	Griffith & Turner (1961)
10th day	19	266	347.0	30.1	3.76	Griffith & Turner (1961)
14th day	9	277	389.0	30.2	4.02	Griffith & Turner (1961)
20th day	11	279	471.0	21.4	3.47	Griffith & Turner (1961)
1	12	238	1869.0		11.56	Tucker & Reece (1963)
3	12	224	1188.4		9.13	Tucker & Reece (1963)
12	12	214	278.5		3.57	Tucker & Reece (1963)
21	12	216	258.1		2.87	Tucker & Reece (1963)

Effect of Pregnancy on Post-lactation Involution

Paape and Tucker (1969) determined the effect of pregnancy on the DNA of the mammary glands of rats lactating concurrently for eight, 12, 16 or 20 days. These data were compared to groups which were not pregnant. After weaning at eight days, the non-pregnant animals declined in DNA, compared to the rats weaned at parturition. By 16 days they showed DNA/100g bw of only 2.33 mg. Those weaned at later periods showed corresponding declines. (Table 21.). The

TABLE 21. EFFECT OF PREGNANCY ON INVOLUTION

(Paape and Tucker, 1969)

Days of lactation when litters removed	Post-lactation, pregnant				Post-lactation, nonpregnant	
	Days dry	Days pregnant	Body weight g	Total DNA mg/100g body weight	Body weight g	Total DNA mg/100g body weight
8	4	12	238	6.47	233	5.19
	8	16	252	6.19	236	3.81
	12	20	264	6.78	239	3.26
	16	24	266	7.48	240	2.33
12	4	16	258	8.91	236	5.72
	8	20	271	6.79	239	4.18
	12	24	257	7.59	243	3.79
16	4	20	251	9.36	238	5.84
	8	24	263	8.56	260	4.00
20	4	24	249	7.91	239	5.94
Over-all mean			257	7.59	240	4.42

rats pregnant during lactation for various intervals showed a much reduced involution or none at all. Note that the pregnant rats that began their dry periods on day 8 or 12 of lactation increased their minimal values a total of 29 and 6 percent during the remainder of the dry period. These results suggest that the hormonal condition after the 12th day of concurrent pregnancy was not only sufficient to retard mammary involution but to stimulate net mammary cell mitosis during the 16- and 12-day dry period.

Prevention of Involution. The influence of estrogen and progesterone was first studied in an investigation to determine what hormones might prevent the loss of the lobule-alveolar structure upon the cessation of the nursing stimulus. Estrogen alone was found to increase the rate of involution during the first five days. DNA decreased 71 percent and was 50 percent below the normal control rats which were weaned at the same time. Growth effects of estrogen were evi-

dent by 10 days and by 20 days DNA was 79 percent above control levels (Griffith and Turner, 1961).

When estrogen and progesterone were administered, it did not prevent initial phases of involution, but renewed growth then occurred, bringing DNA up 82 percent over control values at 20 days.

Many years ago, it was reported that lactogenic hormone reduced the rate of involution following weaning. We have studied the effect of 1 and 2 mg per day for 10 days. One mg maintained DNA 15 percent above controls and 2 mg resulted in DNA 48 percent greater than the control level. It also maintained secretory activity in the remaining cells (Griffith and Turner, 1962).

Hydrocortisone acetate at a level of 500 ug/day inhibited involution slightly (13%). One hundred and 1000 ug were without benefit. However, all levels stimulated maintenance of milk secretion.

Recently, claims of the beneficial effect of oxytocin in the prevention of involution were made. It was suggested that oxytocin was effective in causing the release of lactogen hormone. In our studies injection of oxytocin at the rate of 2 USP units three times daily, either subcutaneously or intravenously, had no beneficial effect upon involution by DNA. It did, however, maintain some secretory activity in remaining cells. Since lactogen retarded involution, these data suggest that oxytocin plays no role as a lactogen-releasing factor (Turner and Griffith, 1962).

Effect of Teat Ligation on DNA of Lactating Rats

Tucker and Reece (1963) reported a study to determine whether or not the suckling stimulus would prevent mammary involution in teats ligated to prevent milk removal. Hooded-Norway rats were used. The total amounts of DNA/100 g body weight of ligated and non-ligated glands were compared.

In animals with teats ligated on the fourth day of lactation, DNA/100 g bw had decreased 42.3 percent after 8 days and 63.5 percent after 17 days. When ligation was started on days 12 and 21, DNA was decreased 44.8 percent. It is clear from these observations that the suckling stimulus did not prevent cellular loss of the glands when milk was not removed.

Experimental Growth of the Ovariectomized Rat Mammary Gland

In the early studies of experimental mammary gland growth in the rat, it was shown that estrogen would stimulate the extension of the gland duct system in ovariectomized animals. It was then shown that estrogen and progesterone would stimulate the growth of the lobule-alveolar system. However, it was difficult to evaluate the extent of lobule-alveolar growth by whole mounts or to compare the extent of such growth with that induced by pregnancy.

TABLE 22. HORMONAL PREVENTION OF INVOLUTION OF THE MAMMARY GLAND OF THE RAT

Treatment	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g B W	Reference
1 ug EB						
5 days	21	259	386	30.1	4.48	Griffith & Turner (1961)
10 days	10	264	389	32.4	4.95	Griffith & Turner (1961)
14 days	11	271	438	36.9	5.88	Griffith & Turner (1961)
20 days	15	291	539	32.1	5.84	Griffith & Turner (1961)
1 ug EB+3mg P						
5 days	10	273	482	33.4	5.64	Griffith & Turner (1961)
10 days	10	270	566	35.3	7.46	Griffith & Turner (1961)
14 days	10	284	517	33.6	6.18	Griffith & Turner (1961)
20 days	19	284	725	27.6	6.91	Griffith & Turner (1961)
2 USP units Oxytocin 3x/day Intra-peritoneally						
10 days	20	258	290	29.3	3.25	Griffith & Turner (1962)
Subcutaneously						
10 days	14	264	330	27.6	3.46	Griffith & Turner (1962)
Lactogenic 1 mg						
10 days	19	285	439	28.2	4.43	Griffith & Turner (1962)
Lactogenic 2 mg						
10 days	21	278	529	29.5	5.58	Griffith & Turner (1962)
HCA 100 ug						
10 days	9	264	327	30.1	3.67	Griffith & Turner (1962)
HCA 500 ug						
10 days	18	238	374	29.6	4.61	Griffith & Turner (1962)
HCA 1 mg						
10 days	14	217	389	27.6	4.85	Griffith & Turner (1962)

EB = Estradiol Benzoate
P = Progesterone
HCA = Hydrocortisone acetate

TABLE 23. COMPARISON OF DNA OF LIGATED AND NON-LIGATED MAMMARY GLANDS

Day of Lactation	Teats ligated sacrificed	No. of animals	Body weight g	DFFT mean mg		Total DNA/100 g body weight mg	
				Lig	Non	Lig	Non
4	12	12	231	328	577	3.54	6.13
4	21	12	230	247	656	2.20	6.03
12	21	12	220	328	619	3.26	5.91

By means of the DNA method, quantitative study of growth became possible. Estrogen administered to sexually mature ovariectomized rats showed little or no increase in DNA, probably because the duct system had already been stimulated to full growth (Table 24). In immature rats, estrogen increased the mean DNA (Panda and Turner, 1966).

To determine the most favorable ratio of estrogen to progesterone, Moon, Griffith, and Turner (1959) injected 1 ug E B with increasing levels of P. The injection of 1 ug E B with either 2 or 3 mg P stimulated an increase in DNA comparable to the mean DNA of rats pregnant 18 to 20 days. Increasing the P level to 10 mg/day had little further beneficial effect on mammary gland growth.

Effect of Doubling the Estrogen and Progesterone

In the earlier studies, 1 ug EB and 3 mg P appeared to be optimal for mammary gland growth of ovariectomized rats as observed in Table 24. In some later experiments where other hormones were injected with the ovarian hormones, it was observed that 2 ug EB and 6 mg P were more effective in synergism with these hormones. Thus in the case of L-T_4, Moon and Turner (1960) found it ineffective at the lower level but at the higher level a significant increase in DNA was observed.

For this reason, in many experiments where other hormones were injected with the ovarian hormones, the higher levels of EB and P were used. In the various experiments where 1 ug EB plus 3 mg P were administered the mean DNA of the groups varied to some extent (Table 24). In the large group of 111 rats, the mean DNA was 7.83 mg/100 g bw. In contrast the groups on 2 ug EB plus 6 mg P average about 7.0 mg (Table 25). It is clear that the higher levels of EB and P did not stimulate higher levels of DNA.

Effect of Restricted Feed Consumption on DNA of Mammary Gland

To determine the effect of the restriction of feed consumption on mammary gland growth in rats, Srivastava and Turner (1966) determined the individual normal feed consumption of ovariectomized rats. They were divided into two

TABLE 24. DNA OF THE MAMMARY GLANDS OF OVARIECTOMIZED RATS STIMULATED WITH ESTROGEN AND ESTROGEN AND PROGESTERONE

Hormone	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight	Reference
Estradiol Benzoate (EB)	5	246	485	22.8	4.48	Moon, Griffith & Turner (1959)
1 ug/day	12	304	823	13.0	3.52	Damm, Miller & Turner (1961)
1 ug/day (20 days)	16	122	175	24.2	2.81	Panda & Turner (1966)
1 ug/day (40 days)	19	155	208	26.1	3.45	Panda & Turner (1966)
1 ug/day (60 days)	19	160	225	21.2	3.00	Panda & Turner (1966)
1 ug/day	20	250	340	25.4	3.30	Griffith, Williams & Turner (1963)
1 ug/day (7 days)	10	279	329			
2 ug/day (19 days)	13	268	463	24.4	4.11	Moon & Turner (1960)
2 ug/day (19 days)	10	260	468	20.2	3.68	Moon (1961)
1 ug EB+1mg (P)						
Progesterone	5	255	535	27.3	5.71	Moon, Griffith & Turner (1959)
1 ug EB+2mg P	20	291	549	39.3	7.41	Moon, Griffith & Turner (1959)
1 ug EB+3mg P	19	269	590	35.7	7.72	Moon, Griffith & Turner (1959)
1 ug EB+2-10mg P	111	278	612	36.1	7.83	Moon, Griffith & Turner (1959)
1 ug EB+3mg P	10	318	1499	15.4	7.01	Damm, Miller & Turner (1961)
1 ug EB+3mg P (20 days)	15	221	430	37.5	7.30	Damm, Miller & Turner (1961)
1 ug EB+3mg P (30 days)	12	231	408	44.7	7.90	Damm, Miller & Turner (1961)
1 ug EB+3mg P (40 days)	10	246	396	52.8	8.50	Damm, Miller & Turner (1961)
1 ug EB+3mg P (50 days)	15	252	442	46.7	8.20	Damm, Miller & Turner (1961)
1 ug EB+3mg P	21	278	606	31.5	6.81	Griffith, Williams & Turner (1963)

TABLE 25. DNA OF THE MAMMARY GLANDS OF OVARIECTOMIZED RATS STIMULATED WITH HIGHER LEVELS OF ESTROGEN AND PROGESTERONE

Hormone	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight	Reference
2 ug EB+6mg P	12	272	593	37.0	8.1	von Berswordt-Wallrabe & Turner (1960)
2 ug EB+6mg P	22	292	606	29.3	6.1	Griffith, Williams & Turner (1963)
2 ug EB+6mg P	28	254	476	42.1	7.84	Kumaresan & Turner (1965)
2 ug EB+6mg P	78	264	512	35.4	6.95	Srivastava & Turner (1966)
2 ug EB+6mg P	16	264	534	29.1	5.73	Moon (1962)
2 ug EB+6mg P	28	248	459	13.5	5.50	Kumaresan & Turner (1967)
2 ug EB+6mg P	16	236	432	30.9	5.47	Kumaresan & Turner (1965)
2 ug EB+6mgP	46	253	555	18.3	7.24	Hahn & Turner (unpub)

groups of approximately equal body weight and feed consumption per day. Mammary gland growth was then stimulated by the daily injection of 2 ug EB and 6 mg P for 19 days. The mean DNA of 78 rats injected with 2 ugEB+ mg P was 6.95 mg/100g bw. The rats fed 75 percent of their normal consumption had DNA of 6.69 mg, a reduction of 3.89 percent and those fed 50 percent of their normal consumption had DNA of 5.70 mg/100g bw, a reduction of 17.84 percent, significant at the 1 percent level. It is probably that if feed restriction had been started earlier, the effect on gland growth would have been greater.

Effect of Orally Administered Progesterone-Like Compounds

A number of progesterone-like compounds have been produced which are orally effective. Several of these compounds have been administered orally to ovariectomized rats with estrogen to determine their capacity to stimulate mammary gland growth (Griffith *et al.,* 1963). The following compounds were used: 17αhydroxy-6 methylpregn-4 en-3 3-20-dione one 17 acetate (Provera-Upjohn Co.), 17αethinyl-17-hydroxy 19-Norandrost-4-en-3-one (Norlutin-Parl, Davis & Co.), 17αethinyl-19 nor-androst-4 en 3-one 17-acetate (Norlutate-Park, Davis & Co.), 6 chloro-6 dehydro-17-acetoxy-progesterone (Lutoral-Syntex Corp).

Norlutin or Norlutate given orally plus EB by injection failed to stimulate DNA to a greater extent than EB alone. When these compounds were fed alone they showed lower DNA than EB alone (Table 26). Lutoral fed at levels of 1 to 2.98 mg/day with EB stimulated DNA to levels similar to EB + P by injection. The feeding of Provera at levels of 1 to 5.2 mg/day with EB also stimulated DNA growth comparable to EB and P by injection.

Effect of Progesterone Metabolites On Mammary Gland Growth

It has been shown that pregnenolone is the precursor of progesterone and that in turn, progesterone is converted to 17α-hydroxyprogesterone to androstenedione and finally to testosterone.

Since progesterone and EB stimulated mammary gland growth, Damm *et al.* (1961) determined the growth promoting effect of the steroids involved in the biosynthetic transformations of pregnenolone to testosterone.

Since 1 ug EB plus 3 mg of P stimulated DNA, these compounds were each injected at the 3 mg level (Table 27). These compounds increased the DNA over that produced by EB alone as follows: Pregnenolone 29.0 percent, 17α-hydroxyprogesterone 26.5 percent, androstenedione 35.8 percent, and testosterone 33.0 percent.

Effect of 3-Methylcholanthrene

It has been shown that 3-methylcholanthrene in normal rats will develop mammary carcinomas, but they develop much less rapidly in ovariectomized rats.

TABLE 26. DNA OF THE MAMMARY GLANDS OF OVARIECTOMIZED RATS STIMULATED WITH ESTROGEN AND PROGESTERONE LIKE COMPOUNDS ADMINISTERED ORALLY

Hormone	No. of animals	Body weight g	DFFTT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight	Reference
Oral Progesterone						
EB 1 ug + Norlutin, 0.28 mg	10	216	392	23.5	3.04	Griffith, Williams & Turner (1963)
EB 1 ug + Norlutate 2.7 mg	8	244	315	27.8	3.90	Griffith, Williams & Turner (1963)
EB 1 ug + Lutoral 1.0 mg	10	255	478	35.3	6.60	Griffith, Williams & Turner (1963)
EB 1 ug + Provera 1.0 mg	10	277	598	33.7	7.10	Griffith, Williams & Turner (1963)
Injection						
EB 1 ug + Norlutin enanthate 6 mg	12	236	522	38.7	7.24	Griffith, Williams & Turner (1963)
EB 2 ug + Norlutin enanthate 6 mg	10	251	517	28.1	7.10	Griffith, Williams & Turner (1963)

TABLE 27. DNA OF THE MAMMARY GLANDS OF OVARIECTOMIZED RATS STIMULATED WITH ESTROGEN AND PROGESTERONE METABOLITES

Hormone	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g b w	Reference
1 ug EB + 3 mg pregnenolone	9	267	824	14.7	4.54	Damm, Miller & Turner (1961)
1 ug EB + 3 mg 17 OH progesterone	10	316	1089	12.9	4.45	Damm, Miller & Turner (1961)
1 ug EB + 3 mg androstene-dione	10	289	998	13.8	4.77	Damm, Miller & Turner (1961)
1 ug EB + 3 mg testosterone propionate	9	282	748	17.6	4.67	Damm, Miller & Turner (1961)

TABLE 28. DNA OF THE MAMMARY GLANDS OF OVARIECTOMIZED RATS TREATED WITH 3-METHYLCHOLANTHRENE

Treatment	No. of animals	Body weight g	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g B W,	Reference
3-methylcholanthrene (50 days)	12	270	243.0	36.6	3.30	Damm & Turner (1961)
EB 1 ug + 3 mg P (50 days) (with above)	12	266	362.0	49.2	6.70	Damm & Turner (1961)
EB 2 ug + 6 mg P (with above)	12	272	375.0	45.0	6.20	Damm & Turner (1961)

To determine its effect on mammary gland growth, 10 mg was administered orally twice a week for seven weeks to ovariectomixed rats. This dose is reported to induce carcinoma in 50 percent of normal rats. A group of 12 animals showed a mean DNA of 3.3 mg/100g bw after 50 days without any signs of tumor development (Damm and Turner, 1961). When 1 ug EB plus 3 mg P or twice that amount was given for 50 days with the above compound, DNA was reduced about 25 percent on either level without tumor development (Table 28).

Effect of 20 B-hydroxy-pregnene-4-ene-3-one on Mammary Gland Growth

Until recently it was thought that progesterone was the only naturally occurring hormone with progestational properties. However, two related compounds have been isolated. They are 4 pregnene-20α-ol-3-one and 4 pregene-20 B-ol-3-one. To determine the effectiveness of 20 B-ol in comparison with P, it was injected into ovariectomized rats with 1 ug EB at levels of 3, 6, and 9 mg for 19 days by Kumaresan and Turner (1968). In comparison with EB alone, 3 mg 20 B-ol increased the DNA 28 percent, 6 mg increased it 28 percent and 9 mg increased it 35 percent. In comparison to EB + P, the 3, 6, and 9 mg of 20 B-ol resulted in 43, 35, and 32 percent less DNA. It is estimated that 20 B-ol is less than one-ninth as active as P in stimulating mammary gland growth in the rat.

Effect of Relaxin on Mammary Gland Growth

It has been suggested that estrogen and progesterone stimulate endogenous secretion of relaxin and that relaxin might stimulate mammary gland growth rather than progesterone. Wada and Turner (1959) reported that intact and ovariectomized rats injected with 1 ug EB and 15 and 45 guinea pig units of relaxin stimulated significant growth of the lobule-alveolar system in about 10 days as indicated by DNA and morphological observations.

Effect of Pituitary Tumors and Lactogen on Mammary Gland Growth

Ghen *et al.* (1967) implanted pituitary tumors (M t T W_5) in 8-week old ovariectomized, adrenalectomized Wistar rats for 66 to 104 days. The tumors were reported to secrete lactogen and GH. Intense stimulation of mammary lobule-alveolar growth was observed whereas the controls showed only duct growth.

Sinha and Tucker (1968) injected mature virgin female Sprague-Dawley rats with 1 or 10 mg of lactogen (ovine) daily for 10 days and in other groups of rats 2, 5, or 10 AP from mature female rats were transplanted under the left kidney capsule for 14 days. Daily injection of 1 or 10 mg lactogen increased mammary DNA/100 g body weight 100 and 225 percent; two, 5 or 10 AP transplants increased mammary DNA 150 to 212 percent.

The Synergistic Effect of Various Hormones in Ovariectomized Rats Injected with Ovarian Hormones

The synergistic effect of various hormones in pregnant rats has been reported. Comparable studies have been conducted in ovariectomized rats injected with estrogen 1 or 2 ug (EB) and progesterone 3 or 6 mg (P) for 19 days.

Thyroxine

The effect of thyroxine (L-T_4) upon the growth of the rat mammary gland of ovariectomized animals administered the ovarian hormones was studied by Moon and Turner (1960). When 1 ug EB plus 3 or 6 mg P was injected with 3 or 6 ug L-T_4 for 19 days, the mean DNA was not increased significantly above that of the controls. However, when 2 ug EB + 6 mg P was injected with 3 or 6 ug L-T_4, the DNA was increased a significant 26 percent above the controls. It was suggested that L-T_4 might be a limiting factor in mammary gland growth when EB and P are adequate, but when the thyroid secretion rate is optimal, EB and P secretion may limit growth.

Moon (1962) gave drinking water containing 0.025 percent tapazole to block L-T_4 secretion. He then injected increasing levels of L-T_4 plus 2 ug EB plus 6 mg P to stimulate gland growth. The rats receiving 0.5 ug L-T_4/100 g bw had a mean DNA value which did not differ from the animals on ovarian hormones. With 1, 1.5, or 2 ug L-T_4 the DNA was elevated to a significant degree.

In a later study by Kumaresan and Turner (1967) the injection of L-T_4 at a level of 3 ug/100 g bw with EB and P resulted in a non-significant increase of 7 percent over the controls.

Growth Hormone

Moon (1961) injected 1 mg GH/day for 19 days in ovariectomized rats. It resulted in an insignificant increase in DNA. When 2 ug EB and 1 mg GH were administered, the DNA was elevated above that of either hormone alone.

Kumaresan and Turner (1965) injected 2 ug EB plus 6 mg P into groups with 2 mg bovine GH or 2 mg ovine GH daily for 19 days. The mean DNA was 8.52 mg and 8.20 mg/100 g bw respectively (Table 29) compared to the 7.84 mg of the control group.

Thyroxine and Growth Hormone

Moon (1961) injected 2 ug EB plus 6 mg of P, plus 3 ug of L-T_4/100 g bw with increasing levels of GH (from 0.5 to 2.0 mg). The DNA was increased significantly above that of the EB, P, and L-T_4 alone (Table 30).

Kumaresan and Turner (1965) injected the ovarian hormones and 3 ug L-T_4 with 2 mg bovine GH. The mean DNA increased to 10.0 mg/100 g bw, a

TABLE 29. EFFECT OF VARIOUS HORMONES ON DNA OVARIECTOMIZED RATS

Hormone	No. of rats	Body wt. g	DFFT mg	DNA/mg DFFT ug	DNA ug/100g b w	Reference
$L\text{-}T_4$						
2ugEB+6mg P+3ug $L\text{-}T_4$	35	253	663	38.5	9.87	Moon, Turner (1960)
2ugEB+6mg P+6ug $L\text{-}T_4$	14	257	592	44.5	9.95	Moon, Turner (1960)
2ugEB+6mg P+3ug $L\text{-}T_4$	10	264	414	27.4	5.33	Moon (1961)
2ugEB+6mg P+3ug $L\text{-}T_4$	21	248	434	14.3	5.97	Kumaresan, Turner (1967)
G H + $L\text{-}T_4$						
2ugEB+6mg P+3ug $L\text{-}T_4$						
plus 0.5 mg GH	10	248	573	25.9	6.76	Moon (1961)
plus 1.0 mg GH	10	305	685	25.5	7.12	Moon (1961)
plus 1.5 mg GH	10	268	622	26.6	7.70	Moon (1961)
plus 2.0 mg GH	10	264	567	29.9	7.76	Moon (1961)
plus 2mg bovine GH	17	309	608	43.9	10.00	Kumaresan, Turner (1965)
G H						
2ugEB+6mg P+						
2mg bovine GH	10	294	625	37.1	8.52	Kumaresan, Turner (1965)
2mg ovine GH	10	319	552	40.7	8.20	Kumaresan, Turner (1965)
Insulin & Alloxan						
2ugEB+6mg P +						
1 unit insulin	21	252	508	29.4	5.90	Kumaresan, Turner (1965)
2 unit insulin	21	258	569	29.3	6.41	Kumaresan, Turner (1965)
3 unit insulin	24	263	627	30.2	7.22	Kumaresan, Turner (1965)
alloxan	21	243	413	27.3	4.50	Kumaresan, Turner (1965)
alloxan+3units	27	255	675	28.9	7.45	Kumaresan, Turner (1965)
2ugEB+6mg P +						
3 units insulin	18	243	493	15.7	6.89	Kumaresan, Turner (1967)
3 units insulin+3ug $L\text{-}T_4$	18	254	487	14.8	6.15	Kumaresan, Turner (1967)
3 units insulin+1mg GH	12	299	586	19.3	7.62	Kumaresan, Turner (1967)
3 units insulin+$L\text{-}T_4$+GH	11	297	600	21.5	8.41	Kumaresan, Turner (1967)
Corticosterone						
0.75 mg	14	255	655	21.28	8.38	Hahn & Turner (unpub.)
1.00 mg	16	278	666	21.70	8.29	Hahn & Turner (unpub.)

TABLE 30. COMPARISON OF THE EFFECTS OF VARIOUS HORMONES ON THE DNA DURING PREGNANCY AND IN OVARIECTOMIZED RATS STIMULATED WITH THE OVARIAN HORMONES

	Pregnant			Ovariectomized		
Hormones	No. of rats	DNA/100g b.w.	Increase %	No. of rats	DNA/100g b.w.	Increase %
Normal	69	7.67		124	7.06	
L-T_4	15	9.66	25.9	35	9.87	39.8
L-T_4	45	9.07	18.3	14	9.95	40.9
L-T_4	16	7.20	6.1	21	5.97	15.4
				10	5.33	24.5
GH	22	8.49	10.7	10	8.52	20.7
	20	8.37	9.1	10	8.20	16.1
Insulin	19	7.76	1.3	24	7.22	2.3
				18	6.89	2.4
Corticosterone	22	9.20	19.9	14	8.38	18.7
Corticosterone	21	9.20	19.9	16	8.29	17.4
L-T_4 + GH	11	10.91	42.2	10	7.76	9.9
	14	9.90	29.1	17	10.00	41.6
	23	10.14	32.2			
L-T_4 + Insulin	21	8.80	14.7	18	6.15	12.9
GH + Insulin	17	11.99	56.3	12	7.62	7.9
L-T_4, GH + Insulin	14	10.48	36.6	11	8.41	19.1

highly significant increase over the control group injected with EB and P. The DNA level of 10.0 mg is the highest level so far attained in ovariectomized rats.

Insulin and Alloxan

Kumaresan and Turner (1965) injected ovariectomized rats with 2 ug EB plus 6 mg P and with either 1, 2, or 3 units of insulin daily for 19 days. The DNA/100 g bw was increased 8 percent, a non-significant increase with 1 unit, 17 percent a significant increase with 2 units, and 32 percent a highly significant increase with 3 units (Table 29).

Other groups were injected with alloxan at a level of 15 mg/100g bw to induce a chronic state of diabetes. One group was then injected with 2 ug EB plus 6 mg P whereas to a second group 3 units of insulin was given. The rats on the ovarian hormones alone had a mean DNA of 4.5 mg, a reduction of 18 percent, whereas the group with insulin had a mean DNA of 7.45 mg, an increase of 66 percent, in comparison with the group without insulin. The important role of insulin was shown by its deficiency and by replacement therapy.

In a further study with 3 units of insulin Kumaresan and Turner (1967) reported a 24 percent increase in DNA above the controls.

Insulin Plus L-T_4 and G H

When the ovarian hormones were injected with insulin and L-T_4, the mean DNA was increased 10 percent above the controls. When 1 mg bovine GH and 3 units of insulin were given together, the DNA was increased 37 percent above the controls. When L-T_4, GH and insulin were given, the DNA was increased 51 percent (Kumaresan and Turner, 1967).

Thyroparathyroidectomy and Replacement Therapy

The value of replacement therapy in thyroparathyroidectomized and ovariectomized rats was studied by von Berswordt-Wallrabe and Turner (1960) in regard to growth of the mammary glands with EB and P. Instead of parathyroid extract, dihydrotachysterol (AT 10) and L-T_4 were used.

Daily administration of 1 ug EB + 3 mg P to mature ovary-thyro-parathyroidectomized rats for 19 days stimulated DNA comparable to ovariectomized rats receiving same treatment. Replacement therapy with 10 and 20 ug AT 10/100g bw/day, respectively, in combination with 1 ug EB + 3 mg P increased total DNA significantly. No benefit was observed by increasing EB to 2 ug and P to 6 mg. Combined administration of AT 10 at above levels and 1.5 and 3 ug L-T_4/100, bw, depressed DFFT significantly, resulting in reduced total DNA.

Synergism of Various Hormones in Pregnant and Ovariectomized Rats

The mean DNA/100 g bw of a large group of rats (69) at the end of pregnancy was 7.67 mg. In comparison, a group of 124 ovariectomized rats injected with EB + P had a mean of 7.06 mg. Based on these averages, it is seen that EB + P in ovariectomized rats is slightly less effective than normal pregnancy in stimulating mammary gland growth.

Considerable variation has been observed in the DNA of individual rats in both groups. It is suggested that the cause of the variation in individual rats may be due in part to the variation in the normal secretion rate of one or more hormones which may synergize with the ovarian hormones in stimulating gland growth.

In Table 30 a comparison is presented concerning the synergism of various hormones alone and in combination with both groups of rats. While there is some variation it will be observed that L-T_4, GH, and corticosterone increased DNA. Insulin alone was ineffective.

In combination, L-T_4 and GH were effective in both types of rats. L-T_4 and insulin were slightly effective in pregnant rats. GH and insulin and L-T_4, GH, and insulin were effective in pregnant rats.

Effect of Hypophysectomy

Since 1936 it has been recognized that in hypophysectomized rats, the ovarian hormones are without effect in stimulating lobule-alveolar growth of the

mammary gland. Since that observation, the presence of a factor or factors in the anterior pituitary, a *mammogen,* was postulated as involved in mammary gland growth. These studies were based upon visual observations of the glands. Hahn and Turner (1966) determined the effect of hypophysectomy on the DNA of the glands. In a group of 25 rats the DNA 33 days after hypophysectomy was 2.01 mg/100g bw compared to 3.05 mg in similar ovariectomized rats. This observation suggests that a pituitary factor or factors are involved in the maintenance of the duct system at the level observed in ovariectomized rats.

When 2 ug EB and 6 mg P were injected for 19 days, the mean DNA of 18 rats was 2.79 mg. Thus the ovarian hormones failed to stimulate the DNA even up to the ovariectomized value.

Effect of Various Hormones in Hypophysectomized Rats

To determine what pituitary or other hormone or combination of hormones would stimulate DNA in hypophysectomized rats, Hahn, Anderson, and Turner (unpub) administered a number of hormones separately and in combination.

Lactogenic Hormone. The injection of 1 mg of ovine lactogenic hormone resulted in a DNA content of 3.26 mg/100g bw, a significant increase of 84 percent over the hypophysectomized control group but not significantly different from the virgin or ovariectomized control groups (Table 31).

Insulin. The injection of 1 unit of insulin/day resulted in a mean DNA value of 2.87 mg, which was 54 percent above the hypophysectomy level and equal to that of the normal control groups. This suggested that insulin stimulated the ducts in the absence of the A. P.

Growth Hormone. The injection of 1 mg/day of bovine GH had no effect on DNA. The value of 1.95 was 3 percent below the hypophysectomy level. It was concluded that GH was not involved in the maintenance of DNA at the control level.

Combination of Hormones. A combination of 3 ug of L-T_4/100 g bw, 1 mg corticosterone, 1 mg bovine GH, and 1 unit of insulin was injected. The mean DNA was 2.75 mg, 74 percent above the hypophysectomy level but less than the control level.

However, when 1 mg of ovine lactogenic hormone was substituted for insulin, the mean DNA was increased to 3.95 mg. This is an increase of 145 percent above the hypophysectomy level and also above the control level. It is also above the level stimulated by lactogen alone.

Value of the Ovarian Hormones

In normal rats, 2 ug EB plus 6 mg P stimulates DNA values comparable to that at the end of pregnancy. The value of these hormones in combination with several A. P. hormones was determined next.

TABLE 31. EFFECT OF VARIOUS HORMONES ON MAMMARY GLAND GROWTH IN HYPOPHYSECTOMIZED FEMALE RATS

Group	Type	Treatment	No.	Final b.w. g	Total DFFT mg	Total DNA mg	% change over hypox control	Total DNA/ 100 g b.w. mg	% change over hypox control
				Mean±S.E.					
1	normal	Control	22	246±3	381±19	7.34±0.32	75	2.98	48
2	ovarx*	Control	22	290±5	445±28	7.97±0.45	90	2.89	44
3	hypox	Control	25	211±3	213±17	4.20±0.28	--	2.01	--
4	hypox	LtH+	8	238±3	288±21	7.73±0.36	84	3.26	62
5	hypox	Insulin	7	223±4	300±21	6.46±0.40	54	2.87	43
6	hypox	GH	9	276±3	316±25	5.35±0.37	27	1.95	-3
7	hypox	T_4+C+GH+I	10	257±5	402±31	7.29±0.51	74	2.75	37
8	hypox	T_4+C+GH+LtH	8	261±5	497±35	10.27±0.74	145	3.95	97
9	hypox	EB+P	18	190±2	254±22	5.36±0.31	28	2.79	39
10	hypox	EB+P+T_4+C+LtH	13	189±8	315±25	8.83±0.48	110	4.49	123
11	hypox	EB+P+T_4+C+GH	22	227±3	290±20	7.60±0.39	81	3.32	65
12	hypox	EB+P+T_4+C+GH+LtH	14	232±2	417±33	11.97±0.61	185	5.01	149
13	hypox	EB+T_4+C+GH+LtH	14	205±3	349±28	8.51±0.70	103	4.08	103

*ovarx = ovariectomized; hypox = hypophysectomized

+Hormones were injected daily for 19 days

The combination of EB + P with L-T_4, corticosterone, and lactogenic hormone increased DNA to 4.49 mg, 123 percent above the hypophysectomy level. This value suggests that some lobule growth may have developed.

When in this combination GH was substituted for the lactogenic hormone, the DNA level was reduced to 3.32 mg.

When the ovarian hormones were combined with all four hormones, the DNA was increased to 5.01 mg/100 g bw. This was the highest level observed and suggested that growth equal to that of 12 or 14 days of pregnancy had occurred. Since the hypophysectomized rats started at a DNA level lower than normal controls, it is possible that a longer period of treatment might have stimulated DNA to a level equal to that obtained experimentally in ovariectomized rats.

Effect of Absences of Progesterone

Ovariectomized rats show duct growth when stimulated with estrogen and lobule-alveolar growth when P is added. It has been suggested that P may stimulate the increased secretion of the pituitary hormones which stimulate lobule growth. If L-T_4, corticosterone, GH, and lactogen represent the possible pituitary combination of hormones, then P could be omitted without effect. However, when P was omitted the mean DNA dropped to 4.08 mg. It was concluded that P has an effect on lobule growth separate from any effect it may have on the secretion of the pituitary hormones.

Growth of the Mammary Gland of the Male Rat

The mammary apparatus of the male rat differs from that of the female in the absence of nipple development (Myers, 1917). Mammary gland duct development occurs as in the female. Turner and Schultze (1931) reported that immature male rats undergo considerable development of the glands after birth. In mature males, marked lobule-alveolar proliferation was found after 70-80 days of age, although some showed limited proliferation.

Castration of males did not affect the continued growth of the duct system and it neither inhibited nor hastened the characteristic lobule proliferation. The estrogenic hormone stimulated duct development in castrates twice as great as in normal males, but no lobule development was stimulated.

Cowie (1949) studied the growth of the glands of the male hooded Norway rats by outlining the extent of development with a body weight range from 7.3 to 344 g. In normal males, gland growth showed a very slight positive allometry. Castration at 22 days of age had no significant effect on the relative growth rate of the ducts but prevented the lobule-alveolar development which began at 40 to 60 days of age. Adrenalectomy of the male caused a slight but not significant increase in the relative growth rate. Ahren and Etienne (1957) also reported that the lobules disappeared after castration.

Effect of Estrogen and Progesterone

Srivastava and Turner (1966) injected 2 ug EB/day for 20 days into gonadectomized rats, followed immediately by the injection of 2 ug EB plus 6 mg P for 20 days. This treatment significantly increased DNA to 5.65 mg/100 g bw in comparison with the DNA of gonadectomized control animals or those injected with EB alone (Table 31).

Panda and Turner (1966) repeated this treatment with 15 gonadectomized male rats. In this group the mean DNA was increased significantly to 6.09 mg. Since the level of DNA stimulated by EB + P in the male rat is similar to the level of DNA stimulated in the female rat, it indicates that the male glands are equal to the female glands in responding to the ovarian hormones.

Neumann and Elger (1966) injected cyproterone acetate in pregnant rats from days 13 to 22. This treatment stimulated the growth of the nipples in the male rat fetuses. When adult rats (weight 300-350g) of this type were castrated and injected with 2 ug EB plus 6 mg P for 19 days the mammary glands were developed and lactation was stimulated.

In a later report Neuman and Elger (1967) produced feminized male rats, castrated them when 150 days old, and injected them for 22 days with 10 ug E B plus 30 mg P. Castrated females and males were given the same treatment to serve as controls.

On examination they exhibited development of the mammary glands. The normal females showed the greatest gland weight (9g), the feminized males (7.5 g) were slightly smaller but the normal males (2 g) showed much less development. Histologically, the glands showed development similar to normal females shortly before parturition. Thus the feminized rats showed more glandular tissue than normal male rats following EB plus P.

Development of Nipples in Male Rats

Neumann and Elger (1966) reported that treatment of pregnant Sprague-Dawley rats with an anti-androgen, cyproterone acetate (1, 2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3, 20-dione-17αacetate) from the thirteenth day of pregnancy resulted in the formation of nipples comparable to the normal female.

At six weeks of age, the nipples resembled those of immature females. The mammary duct extended to the tip of the nipple. Later, there was further significant growth of the nipples until they reached a length of 0.5 to 2mm.

Stimulation of Lactation in Feminized Male Rats

Neumann *et al.* (1966) stimulated the growth of the mammary glands of feminized male rats with nipples by the injection of EB and P. After 19 days of this treatment, it was stopped and the rats were injected for eight days with 1

mg cortisol acetate, 1 ug EB plus 9 mg of ovine lactogenic hormone containing 20 iu/mg.

Histological examination of the mammary apparatus showed that the animals had typical nipples, normally canalized and directly connected with the underlying glands.

The glands were the same as those in normal females in lactation. The alveoli were distended and milk was present in the ducts and sinuses. The epithelial cells of the alveoli were typical of intensive lactation.

Effect of Testosterone on Mammary Involution in the Male

In the previous studies it was shown that the ovarian hormones (EB and P) will stimulate growth of the male mammary gland comparable to the female. It has been shown that following lactation or when the young are removed at parturition there is a rapid involution of the gland as shown by a rapid loss of DNA.

To determine whether testosterone would prevent the involution of the glands stimulated by EB and P, Panda and Turner (1966) injected a group of 14 animals for 20 days with EB and P to stimulate growth, then injected 3 mg testosterone daily for 20 days. Testosterone was ineffective in preventing involution of the glands of these animals as indicated by the mean DNA of 3.59 mg/100g bw, which is the same as for the groups prior to EB and P treatment (Table 32).

Effect of Testosterone on the Lobule-Alveolar System

Jacobsohn (1962) reported that the lobular system of male rats disappears after castration and reappears when androgens are injected. These observations have led to the assumption that mammary growth is promoted by androgenic hormone alone. However, it is possible that estrogens derived from the testes, adrenal glands, or even from androgens in gonadectomized rats may play a role. For this reason male rats were hypophysectomized to eliminate sources of estrogen.

Growth of the Mammary Glands of Hypophysectomized Male Rats

Jacobsohn (1962a) reported that estrogens stimulate growth of the male rat with an intact pituitary. However, after hypophysectomy, estrogen was without effect on the atrophic gland. When such rats were treated with insulin alone or together with cortisone, estrogen was effective. When insulin was given with testosterone, the growth was limited and abnormal.

When hypophysectomized males were injected with PMS (to produce androgens) and estrogen, alveolar growth occurred. In gonadectomized rats testosterone was equally effective.

In further study, Jacobsohn (1962b) reported that without estrogen, the glands of hypophysectomized males injected with PMS showed an abnormal response, irrespective of simultaneous treatment with thyroxine, cortisone, and insulin.

In similar rats treated with thyroxine, estrogen, and PMS or testosterone the response was uniformly abnormal (absence of end buds). In rats treated with estrogen, thyroxine, cortisone, and insulin, alveolar lobules were present.

Growth of the Mammary Glands of Adrenalectomized Male Rats

Jacobsohn and Norgren (1965) reported that male rats gonadectomized and adrenalectomized showed reduced response to testosterone propionate (TP) in comparison to controls. With few exceptions, alveolar lobules failed to develop. When daily injections of 0.125 mg of cortisone acetate (Ca) were added, the number of glands showing alveoli increased slightly. Estrone (E) at a level of 0.05 ug/day failed to modify the response.

Combined treatment of 0.125 mg of Ca, 0.05 ug/day of E, and 0.1 or 0.2 mg. of TP every other day resulted in alveolar lobules in a great majority of glands. It is suggested that the mammary response to androgen is dependent on concomitant action of estrogen and adrenal cortex steriods.

Experimental Growth of Male Mammary Gland

Panda and Turner (1966) compared the normal growth of the mammary glands of male rats with that induced by 1 ug/day of EB from 40 to 80 days by the DNA method. The mean DNA/100g bw of the normal male at 40 days was 2.25 mg. At 60 days it was 2.90 mg, an increase of 28.8 percent and at 80 days it was 2.87 mg (Table 32).

The rats injected with EB from 20 to 40 days had a mean DNA/100 g bw of 1.92 mg, 37 mg less than the controls. After 40 days of EB (age 60 days), the mean DNA was 2.33 mg still 0.57 mg less than the controls. After 60 days of EB (age 80 days), the DNA was 3.0 mg, the same as the controls. This level of DNA is equal to that present in mature ovariectomized or normal females (Table 5).

Srivastava and Turner (1966) determined the DNA of 55 normal male rats weighing 292 g. The mean DNA of the group was 3.99 mg/100 g bw. A similar group of 19 males weighing 341 g were gonadectomized for 30 days. Their DNA was 3.85 mg, indicating that gonadectomy did not cause involution of the glands.

EB at a level of 2 ug/day for 20 days in 20 gonadectomized male rats induced slight, but not significant, mammary gland growth as indicated by a mean DNA of 4.38 mg (Table 32).

TABLE 32. DNA OF THE MAMMARY GLANDS OF NORMAL MALE RATS

Type	Number of animals	Body weight	DFFT mean mg	DNA ug/mg DFFT	Total DNA mg/100g body weight mg	Reference
Normal male	20	157	166.9	21.33	2.25	Panda & Turner (1966)
Normal male	17	211	333.4	20.19	2.90	Panda & Turner (1966)
Normal male	11	281	580.2	14.67	2.87	Panda & Turner (1966)
Normal male	55	292	687.1	18.12	3.99	Srivastava & Turner (1966)
Normal male	11	281	580.2	14.7	2.87	Panda & Turner (1967)
Gonadectomized						
(30 days)	19	340	679.2	19.84	3.85	Panda & Turner (1967)
(20 days)	15	275	549.1	17.91	3.59	Panda & Turner (1966)
1 ug EB/day						
20 days	20	144	114.8	20.8	1.92	Panda & Turner (1966)
40 days	15	176	222.2	18.9	2.33	Panda & Turner (1966)
60 days	19	197	226.6	26.3	3.00	Panda & Turner (1966)
2 ug EB/day						
20 days	20	254.7	549.9	20.4	4.38	Srivastava & Turner (1966)
2 ug EB/day 20 days then 2 ug EB + 6 mg P/day						
(20 days)	14	270	822.4	18.6	5.65	Srivastava & Turner (1966)
2 ug EB + 6 mg P/day						
(20 days)	15	265	809.5	20.7	6.09	Panda & Turner (1966)

SUMMARY

Beginning in 1930, a study of the normal growth of the mammary gland of the rat was undertaken in this laboratory. Since the glands are found in flat sheets of connective tissue, whole mounts of the glands could be prepared, fixed, stained, and mounted for observation. The growth of the duct system following sexual maturity was observed. In pseudo- and normal pregnancy, the ducts were observed to develop the lobule-alveolar system. It was believed at the time that lobule-alveolar growth was complete at about two-thirds of pregnancy, followed by the initiation of lactation. During lactation, with the glands engorged with milk, the structures of the glands were obscured.

Following weaning and the cessation of lactation, it was possible to again observe the gradual loss of the lobule-alveolar system and the return to a duct system.

In ovariectomized rats, it was shown that the estrogens would stimulate the growth of the duct system and the combination of estrogen and progesterone would stimulate the growth of the lobule-alveolar system.

These studies gave us a qualitative picture of normal and experimental mammary gland growth. They failed to give a quantitative measure of mammary gland growth. It was not possible to determine the extent of lobule-alveolar growth in individual animals at the end of pregnancy or psuedo-pregnancy. It was not possible to determine the extent of growth at the end of pregnancy compared to the growth stimulated by estrogen and progesterone. For this reason an attempt was made to find a quantitative method of study. With the discovery of deoxyribonucleic acid (DNA) as an integral part of the cell nucleus and its chemical determination as well as the suggestion that each nucleus contained a constant amount of DNA, it seemed possible to quantitatively measure mammary gland growth.

The present bulletin summarizes the research which has been conducted at the Missouri Experiment Station during the past 15 years as well as studies by Dr. Ralph P. Reece, one of my former students, at the New Jersey Experiment Station. One of his students, H. A. Tucker, began similar studies at New Jersey and has continued his extensive studies at the Michigan Experiment Station.

As a result of these studies, it was observed that the total growth of the mammary glands of rats varied greatly. It was shown that growth of the glands extended not only during the first two-thirds of pregnancy but throughout pregnancy. Even more surprising was the discovery that extensive growth continued during early lactation as well. Since ovariectomized-lactating rats showed the same degree of "lactational growth" it was concluded that the ovarian hormones were not involved in this phase of growth. It was suggested that either anterior pituitary hormones or the hormones of their target glands were involved.

The quantitative method made possible for the first time the study of the role of other hormones (in addition to estrogen and progesterone) upon mam-

mary gland growth. It is clear from these observations that the greatest growth of the mammary glands occurs when optimal amounts of these other hormones are being secreted or injected to supplement the deficiencies. These observations were supplemented by the effect of the removal of individual endocrine glands.

It was recognized early that the removal of the anterior pituitary (AP) interfered drastically with mammary gland growth. Estrogen and progesterone were without effect upon the growth of the lobule-alveolar system. These observations were confirmed by the DNA method. In fact, it was shown that there was even further loss of DNA in such animals compared to normal virgins or ovariectomized groups. This early observation led to the concept that the AP secreted a "mammogenic" hormone. In the current studies no evidence of a separate "mammogenic" hormone has appeared. It is believed, rather, that the combination of pituitary hormones and hormones of their target glands synergize with the ovarian hormones to influence mammary gland growth. In other words, all the endocrine glands and most of the hormones synergize to influence mammary gland growth. Hormones which do not influence gland growth include oxytocin and vasopressin from the posterior lobe and the melanocyte stimulating hormone (MSH).

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