STUDIES ON THE MAMMARY GLAND

I

OBSERVATION ON THE EFFECT OF CERTAIN ENDOCRINE MATERIALS UPON THE MAMMARY GLAND OF THE ALBINO RAT.

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PREFACE

This paper is the first report of three years study on the mammary gland and its changes. During the first year and one-half the immediate problem was not undertaken. The rat colony was being developed and a complete study of the normal anatomy from birth through pregnancy and lactation on through to involution was made. The experimental work presented in this paper was done during the past one and one-half years.

This work would not have been possible except for the very able guidance and counsel of Dr. Frank R. Menne, under whom I have had the great pleasure of working for the past four years. I am also indebted to Dr. Menne for his very learned criticism and correction of this paper, to Polly Laird, the secretary of this department, for her very kind and careful stenographic assistance, to the technicians of this department for their careful technical work and to Mr. Charles Norris of the photography department for his excellent and careful photo-micrographs.

INTRODUCTION

The abnormal hyperplasias of the mammary gland, both lobular and cystic, have held the interest of Clinicians and Pathologists for many years. In the human breast the changes produced by the menstrual cycle, pregnancy, lactation, normal and abnormal involution and progressive cumulative changes, have resulted in such variegated abnormalities that many confusing terms have been applied, such as: Chronic Cystic Mastitis, Benign Cystic Disease, Senile Parenchymatous Hypertrophy (Bloodgood), Schimmelbusch's Disease, Fibroadenomatosis Cystica (Semb) and Cystiphorous Desquamative Epithelial Hyperplasia (Cheatle and Cutler). Many of these terms indicate an inflammatory process, but we know that many leucocytes are normally present in the functioning mammary gland. Therefore many of these conditions must be different stages in the normal or abnormal changes caused by a hormone or hormones. The physiology of the normal development, lactation and involution of the mammary gland has been quite well established by previous investigators. At the same time little investigation has been carried out on the abnormal function or pathological physiology of the mammary gland. Very little is known about the cumulative changes caused by the recurring estrus cycles in normal animals. Nothing is known about the changes occurring in the mammary gland of an animal with an imbalanced or poorly functioning endocrine system. We know very little about the changes occurring in the mammary gland during and after the menopause. Why do some glands atrophy completely while others begin an abnormal type of

hyperplasia? Why does the alveolar system respond in an abnormal manner in the presence of a primary ovarian hypofunction? Why does an hyperfunction of the ovarian tissue cause a cystic condition in the mammary gland? Are these responses entirely due to the concerned hormones of the pituitary gland and ovary or to some other unknown factor? Do some of these benign types of breast hyperplasias become malignant as Cheatle and Cutler (1931) say or is its relation to malignancy purely indicental as Lewis and Deschickter (1934) maintain? These constitute some of the inquiries that as yet await a solution. It was with some of these in mind that the present study was undertaken.

The literature abounds with isolated studies and considerations of abnormal anatomical and functional changes observed in the study of human breasts. Since however, most of these consist of observations based upon the breasts removed at surgery or autopsy, the pathologist can only interpret the alterations that are then static while the whole process is dynamic, accurate conclusions cannot be drawn. Because of the lack of control factors in a study of the functional and pathological abnormalities in the human breast, the investigator must proceed to the study of the breasts of animals under proper control as to age, diet, general health, breeding and the estrus cycle. Accordingly the Albino rat was selected because of the size and number of breasts, short estrus cycle, easy control of generations and the short life cycle.

In order to fully understand the basic changes in the breast of Albino rats altered by experimental conditions it seems advisable to review the literature dealing with the normal anatomy and physiology of the mammary gland.

Natural Character and Habitat of the Albino Rat in Breeding and Nursing.

In the Albino rat (Mus Norvegicus albinus) there is considerable variation in the age at which they become sexually mature. Greenman and Dukrig (1923) record one instance where a female cast a litter at 57 days of age and another instance as early as 55 days of age. Long and Evans (1922) observed that in two rats the average time for the opening of the vagina is on the 72nd day of life, and the average time for the first ovulation is on the 77th day of life. To obtain an average number of pups in a litter the mating should be delayed until both sexes are about 110 to 120 days of age. From 110 days to 350 days of age the female Albino rat is in her most vigorous reproductive period, that is, considering that she is not allowed to mate until 110 days of age. After 350 days of age when she has given birth to 5 or 6 litters, the reproductive function declines rapidly until the female is about 450 days of age when breeding ceases.

As the estrus cycle occurs about every 5 days in the rat, a successful mating usually takes place 3 or 4 days after caging the pair together. The continuous breeding of one male and one female, being the most successful. It is inadvisable to remove the male from the cage when the female becomes pregnant or after her litter is born. He will not destroy the young if conditions are normal. As the female ovulates soon after parturition, the presence of the male accelerates the process of reproduction. The period of gestation is about 22 days, with slight variations. According to the experience of Greenman and Duhing (1923) the gestation period is not prolonged by the presence of a suckling litter when proper nutritional and hygienic conditions are

maintained at a high level.

It is necessary to remove one litter from the cage just before the succeeding litter is born, as the older litter would continue to nurse while the newborn litter would stand little chance of getting proper nourishment. The Albino rat female, like many other mammals, eats the placenta. There is experimental evidence by Hammett (1918) that placenta fed to a lactating mother stimulates the growth of nursing young.

In continuous breeding with one female and one male in each cage, a litter may be expected about every 25 days during the first 4 or 5 months of breeding activity.

The number of young in a litter varies greatly. Under favorable conditions the average may reach 7 or 8 or even more. Greenman and Durhing (1923) record a litter of 18. Their average number of pups in first litters was 9.97 and in the fifth litters the average number was 6.

The Normal Development of the Mammary Gland in the Albino Rat.

Mammary streak and line - Henneberg (1900) found the first indication of the mammary streak in an eleven-day-old embryo in the form of a few large cuboidal cells at the border of the trunk or body zone. The streak or line extends from the shoulder region caudad to the inguinal region. It gradually forms a two cell layered streak in the thirteen-day-old embryo. The final differentiation of the mammary streak is called the mammary line. In the fourteen-day-old embryos the inguinal anlagen reach this stage. The gland anlagen from the milk streak in the pectoral region. In the 14½ to 15-day-old embryos the inguinal anlagen reach this stage. The gland anlagen in 15½-day old embryos have moved ventrad and attained a definite position. At this time the milk streak has disappeared.

Mammary bud stages - Myers (1917) described the mammary bud stage in a 15-day 9 hour rat embryo as a small, visible eminence associated with each developing gland area. The spheroidal mass of epithelial cells forming the gland anlage is surrounded by the basement membrane of the Malpighian layer. This mass of cells is attached to the epidermis by a short, constricted neck. The inguinal glands at this stage are slightly behind the others in their development.

Primary sprout - The beginning of the primary sprout in an 18-day-old female is described by Myers (1917). At the time the primary sprout begins to develop a mammary pit appears on the surface of the skin over each developing gland area. In cross section the Malpighian layer is depressed to form a shallow, funnel-shaped outline. The mouth of the funnel is directed towards

the surface and the outlet extends into the corium and becomes continuous with the anlage of the primary sprout. One primary sprout grows from the proximal end of the bud. The superficial portion of the gland anlage is undergoing vacuolization, cornification and desquamation; thus forming the pit superficial to the primary sprout.

The primary sprout of the first thoracic gland anlage is directed cephalad; the second inguinal primary sprout is directed caudad. The free edge of each gland anlage is directed to the area which the future gland will occupy. In the male, at this stage, there is an eminence marking the location of the gland externally instead of the mammary pit. Also, the primary duct of the male shows more marked expansion at its free end than that of the female.

Secondary sprout development during fetal life - The earliest appearance of secondary sprouting was observed in one of the abdominal glands of an 18-day-old female, but normally secondary sprouting was observed only in 19-day-old fetuses. At this time secondary ducts are present in all glands and most of the secondary ducts have tertiary sprouts. In the male of this age there is considerable variation, in some glands the primary sprouts are terminal, in others tertiary sprouts are terminal.

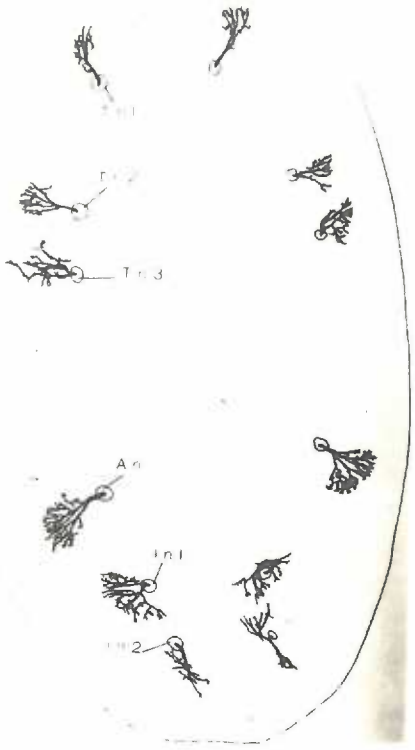
Development of the nipple during fetal life - The first differentiation destined to give rise to the future nipple appears as an eminence in the hillock stage in a 15-day-old fetus. In the 18-day-old female fetus a sheath which encircles the future nipple area is present. This sheath invaginates around the nipple area; the cells become cornified and gradually slough off, thus forming a circular pit around the central nipple area.

This is called the epithelial hood by Myers. At 20 days the anlage of the epithelial hood has grown deeper into the corium and is encroaching upon the tela subcutanea. Cornified cells occupy the space between the inner and outer surfaces of the hood. In the 20-day-old fetus there is no indication of an epithelial hood.

Canalization of the sprouts:- Myers (1917) noted that in the thoracic and abdominal glands of an 18-day-old female fetus there was present a slight indication of a lumen in one of the ducts. There is a variation in the time of canalization, for some 20-day-old fetuses still have sprouts which show no trace of a lumen. The canalization of the terminal ducts has proceeded further than in the other ducts. At 19 days the lumina in the male glands are further developed than in the female glands. Canalization takes place as a result of irregular, centrally located cells being separated from each other, thus producing an indefinite lumen.

Up to the time of birth (20 days) no lobules have developed; neither have the masses of fat which are so conspicuous in the postnatal stages. The cephalic glands are further developed than those more caudally located. The female gland areas are very conspicuous at 20 days, but in the male there are no external indications of the mammary glands.

General nature and arrangement of the glands:- Normally there are six pairs of glands in the Albino rat, although Henneberg (1900), Frank and Unger (1911) and Donaldson (1924) observed that the number varies in a few cases from 10 to 14. Myers (1915) observed only one supernumerary gland in an examination



7 Drawn from a cleared preparation (internal view) of an albino rat one week old (weight 8.5 grams) to show distribution and relations of ducts of left abdominal gland (*A*); left first inguinal gland (*B*); and left second inguinal gland (*C*). × 5. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct; *e.b.*, end-bud; *c.*, collateral duct.

8 Same as figure 7, but from an albino rat two weeks old (weight 15.5 grams). × 5.

9 Same as figure 7, but from an albino rat three weeks old (weight 30 grams). × 5.

Drawn from a cleared preparation of a two weeks' albino rat (internal view) to show the general arrangement of the nipples and the branching of the mammary ducts. × 4. *T.n.1*, *T.n.2*, *T.n.3*, 1st, 2d and 3d thoracic nipples; *A.n.1*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples.

From Myers.

of 100 rats. Schiockele (1899) observed 11 teats in 6.66 per cent of the rats observed; 12 teats in 80 per cent of the animals; and 13 teats in 13.33 per cent of the animals. Donaldson reports that Statsenburg has noted cases where only 6 of the 12 nipples were present. He has been able to carry a deficiency through four generations. The defect is in the system rather than in a particular pair or group of glands. In this case the deficiency was most commonly represented by the absence of the upper pectoral glands. Of the six pairs of normal glands there are three pairs of thoracic glands, one pair of abdominal glands, and two pair of inguinal glands. The glands are located so that two V-shaped areas are formed. The three pairs of thoracic glands form a V with the point directed cephalad, the two most anterior pair lying nearer each other than the last pair of this group. The abdominal pair and the two inguinal pairs also form a V with the point directed caudad, the most caudad of the inguinal glands lying nearer the median line than the most anteriorly located abdominal glands.

Myers showed the general location of the glands and their direction and extension from the nipple (Figure).

The ducts of all glands except the last pair of inguinal glands aredirected on the side of the nipple away from the median line.

Development from birth to puberty:- The period from birth (averaging 21.8 days after conception) until the time of first ovulation (averaging 77 days - Long and Evans 1922) is characterized by an extension and increase in duct growth.

Development of the ducts:- In the newborn the gland area in the female is evidenced by a lighter colored area (Myers 1918).

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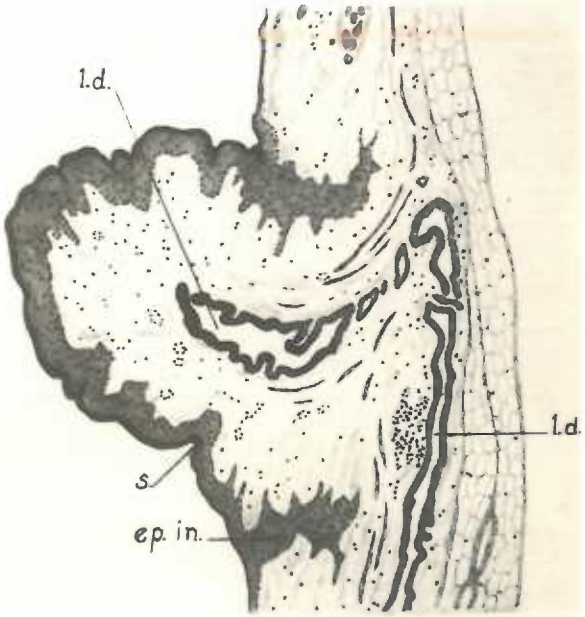

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Lettering for figures 14 to 17 as follows: *ep.in.*, epithelial ingrowth of the nipple; *h.f.*, hair follicle; *l.d.*, lactiferous (primary) duct; *m.*, muscle; *n.a.*, nipple area; *s*, sulcus at base of nipple.

14 Drawn from a section through the nipple area of the second inguinal gland of a newborn albino rat. × 67.

15 Drawn from a section through the nipple area of the second inguinal gland of an albino rat one week old. × 67.

16 From a section through the nipple area of the second inguinal gland of an albino rat two weeks old. × 67.

17 From a section through the nipple of the second inguinal gland of an albino rat nine weeks old. × 67.

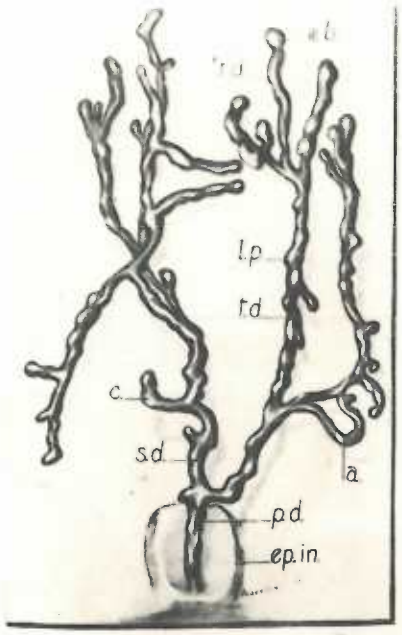
From Myers.

There are no external indications of the mammary gland in the male. The ducts lie in a single plane at this stage except where there are obstructions as in the last inguinal gland where the ducts have grown in three or four planes, each of which are parallel to the surface. The secondary ducts are more extensive than the primary ducts and break up into tertiary ducts. These tertiary ducts at birth have one to three terminal branches. The ends of most terminal branches have a small, bud-like enlargement which was once thought to be a true alveolus, but this was later found to be incorrect.

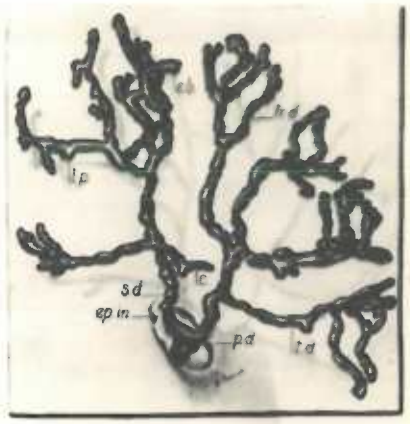
Myers observed that there was considerable variation in the size of the glands in the same rats, and even between glands of the same pair. Also there is variation between different rats of the same age and weight.

As the age advances to 10 weeks there is an increase in the number and length of ducts, the period from the fifth week to the ninth and tenth weeks is characterized by increased growth of the glands. During the fifth week the ducts increase rapidly in length and many new branches are formed from the distal ducts. The growth of the abdominal and inguinal pairs overlap on each side. However, a wide interval still exists between the first and second thoracic gland on each side. The mammary glands of the male at five weeks have failed to keep pace with the rapidly developing glands of the female at this age.

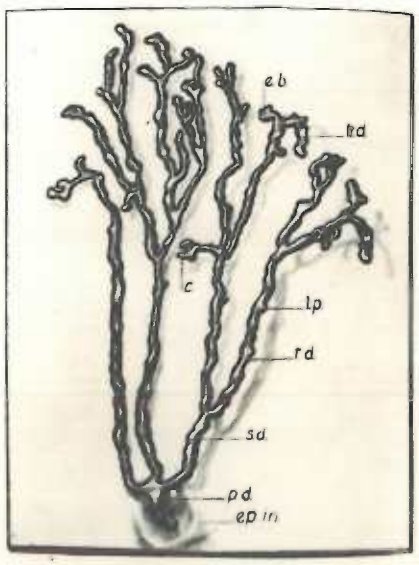
From the fifth to the ninth and tenth weeks of postnatal life the female glands gradually, but slowly, increase in size. At eight weeks the ducts of the different glands have so overlapped that four gland masses are apparent. The abdominal and first and second pairs of inguinal glands form a continuous mass



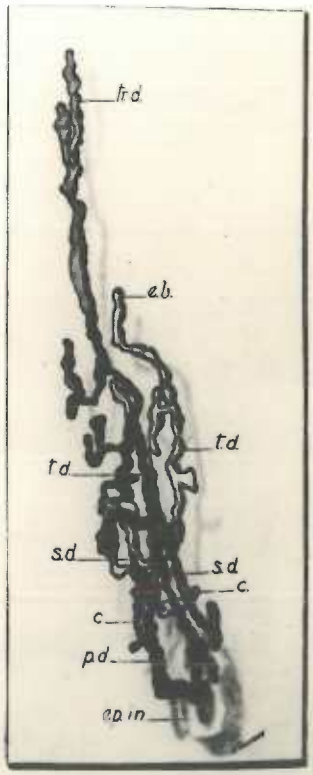
Internal view of a wax model reconstructed from the left second thoracic gland of a newborn albino rat. × 40. *a*, anastomosis; *c*, collateral duct; *ep.in.*, epithelial ingrowth of nipple; *e.b.*, end-bud; *l.p.*, lateral process; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct.



Internal view of a wax model reconstructed from the left first inguinal gland of a newborn albino rat. × 40.



Internal view of a wax model reconstructed from the right third thoracic gland of a newborn albino rat. × 40.



Internal view of a wax model reconstructed from the left second inguinal gland of a newborn albino rat. × 40.

From Myers.

on each side in the caudal region, while the three thoracic glands form a mass on each side in the anterior region.

During the ninth and tenth weeks there is a tremendous increase in gland development. The first thoracic pair come to meet and overlap in the midline and the last inguinal pair, both cephalad and caudad to the vagina, meet in the midline. Inanition retards growth and results in stunted growth even when the rats are brought back to normal.

The male at this age (Myers 1917) has much less extensive growth.

Nipple development:- At birth the epithelial hood is continuous around the nipple (Myers 1916). During the second week after birth rapid development of the nipple takes place and at this time resembles a true nipple. Up to the nine-week stage studied there is a slow increase in the size of the nipple. Rein (1881) states that in a three weeks old white rat the teat is surrounded on all sides by an indentation of the skin. The edges of the groove have about two-thirds the height of the teat. In the male no nipples were observed at any age studied. However, Schickele (1899) found in one young male, twelve nipple anlagen.

Canalization of the sprouts:- At birth a small irregular slit-like lumen is present in the primary sprout (Myers 1916), which disappears in the intradermal epidermal portion. The lumen of the primary sprout is continuous with the lumen of the secondary sprouts. The lumen opens on the surface of the nipple at two weeks of age. There is a gradual increase in the size of the lumen up to ten weeks of age. No true alveoli were found up to ten weeks of age by Myers.

Development during recurring estrus cycles:- A study by

10 Drawn from a cleared preparation (internal view) of an albino rat four weeks old (weight 53 grams) to show distribution and relations of ducts of left abdominal gland (*A*); left first inguinal gland (*B*), and left second inguinal gland (*C*). × 5. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct *e.b.*, end-bud; *L*, lymph-node.

11 Same as figure 10, but from an albino rat five weeks old (weight 54 grams × 5.

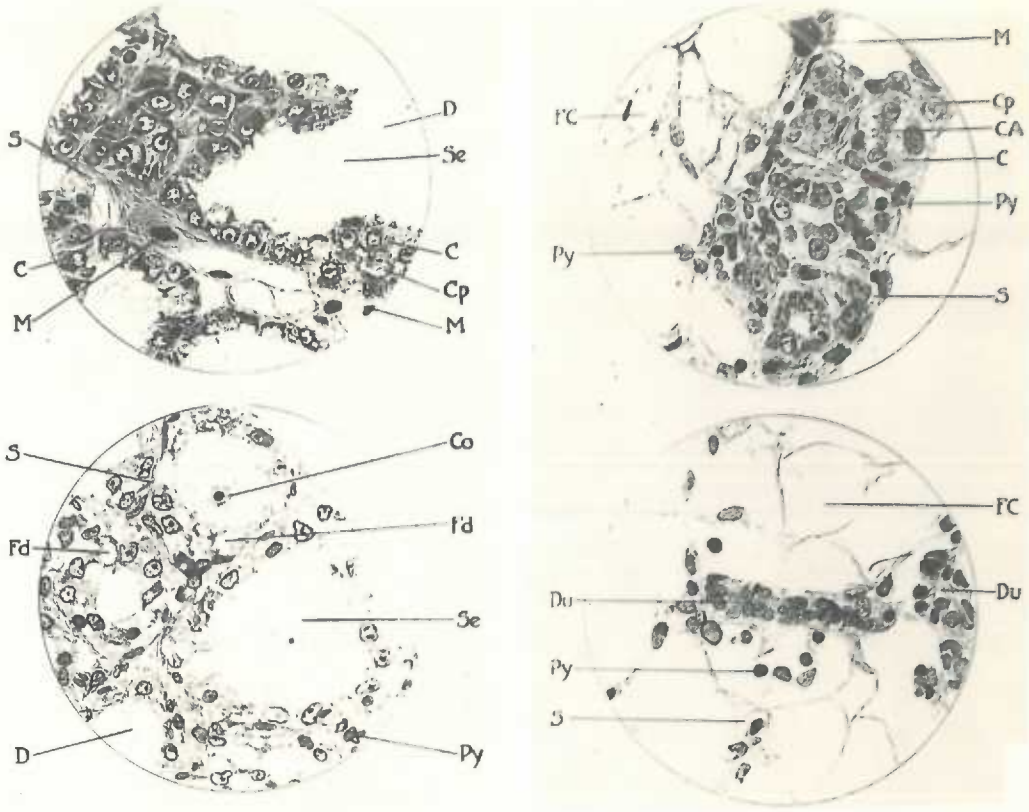
12 Drawn from a cleared preparation (internal view) of an albino rat seven weeks old (weight 75 grams) to show distribution and relations of ducts of left abdominal gland, first inguinal gland, and second inguinal gland. × 5. *A n*, abdominal nipple; *I n 1*, *I n 2*, 1st and 2d inguinal nipples; *L*, lymph-node; *p d*, primary duct; *s d*, secondary duct; *t d*, tertiary duct; *c*, collateral duct; *tr d*, terminal duct; *e b*, end-bud.

13 Same as figure 12, but from an albino rat nine weeks old. (Weight 104 grams.) × 5.

From Myers.

Sutter (1921) shows that there is a relation between the appearance of the mammary gland and the stage of estrus. During proestrum the mammary tree exhibits long, slender branches which have a few almost naked twigs projecting from them. During estrum the small buds, or mammary twigs, have sprouted out to varying degrees and new ones have appeared. The branches and twigs are covered with numerous projecting buds. By the time ovulation has occurred and young corpora lutea have been formed, further evidence of increasing complexity of the secondary branches can be seen. Near the next proestrum stage regression occurs.

Development during pregnancy:- Development during pregnancy is characterized by the formation of lobules and a tremendous increase in the amount of glandular tissue. The nipples have increased in size and reach their maximum length by the thirteenth day of pregnancy (Roberts 1921). This increase in length is associated with complete degeneration of the epithelial hood. The beginning of lobule formation is present in the gland of a female pregnant ten days. Roberts describes the alveoli in the thirteenth day pregnant rat as spherical. These alveoli gradually increase in size up to the time of parturition. He concludes that from the fact that there is an absence of cell division and no increase in number of cells about the alveoli the gland has reached its maximum growth so far as the number of cells is concerned by the thirteenth day of pregnancy. Weatherford (1929) who has made a cytological study of the mammary gland of the rat, confirms this and states that in the latter half of pregnancy cellular proliferation is rather rare. The growth of the gland from the thirteenth day is due to the increase in the size of capillaries and increase in the size of the alveoli. In the latter



Portion of transverse section of the mammary gland of a rat (M 3.2) in the fifteenth day of lactation. Section 5 μ thick; paraffin section stained with cresyl violet. × 720. The acini are widely distended with secretion, *Sc*, in which are suspended fat-droplets, *D*, of various sizes. The cytoplasm, *C*, of the cells contains deeply staining granules and secretory granules. The nuclei are spherical and contain clumps of chromatin. The connective-tissue stroma, *S*, is reduced to fine septa. Several mast cells, *M*, are shown. *Cp* designates several blood corpuscles in a small capillary. Figure 2 and 6 are of similar stages, but from different rats.

Portion of transverse section of the mammary gland of a rat (M 11.1) three days after weaning. Paraffin section 5 μ thick, stained with cresyl violet. × 720. Definite signs of atrophy are evidenced by the presence of larger fat-droplets, *Fd*, and irregular and pycnotic nuclei, *Py*. A colostrum corpuscle, *Co*, is shown in one of the alveoli. The cytoplasm is more irregularly vacuolated and stains less deeply. Figure 3 is a low-power view of a similar stage.

Portion of transverse section of the mammary gland of a rat (M 14.1) eight days after weaning. Paraffin section 5 μ thick, stained with cresyl violet. × 720. Remains of collapsed alveoli, *C. A.*, filled with degenerated, vacuolated cytoplasm. Pycnotic nuclei, *Py*, are quite numerous. The fat-cells, *F.C.*, have replaced the glandular parenchyma. A mast cell, *M*, is present. Figure 5 is the corresponding low-power view.

Portion of transverse section of the mammary gland of a rat (M 19.1) twenty-eight days after weaning. Paraffin section 5 μ thick, stained with cresyl violet. × 720. Of the parenchyma there remains only ducts, *Du*, composed chiefly of closely packed, deeply staining cells in a syncytial mass of cytoplasm. A few atrophic cells, with pycnotic nuclei, *Py*, are found in the neighborhood of the ducts. The fat cells, *F.C.*, of the stroma have almost entirely replaced the parenchyma. Figure 1 corresponds well to a low-power view of a section at this stage.

half of pregnancy there is an increase in size of the lumen of the alveolus and increase in the size of the cells of the alveolus up to the time of parturition. At the eighteenth day of pregnancy the capillaries supplying the blood to the alveoli become greatly dilated and extremely so at twenty days.

Vacuolisation of the cytoplasm of the alveolar cells, which is indicative of secretory activity, increases from the thirteenth day of pregnancy and believed by Roberts to be at its height at the nineteenth day of pregnancy. There is some secretion in the ducts and lumina of the gland during pregnancy and an abundant secretion is evident from the nineteenth day up to delivery.

In the pseudo-pregnant rat the condition of the mammary gland at the end of the pseudo-pregnant period is similar to that of a pregnant animal at the corresponding period of pregnancy (Turner 1932).

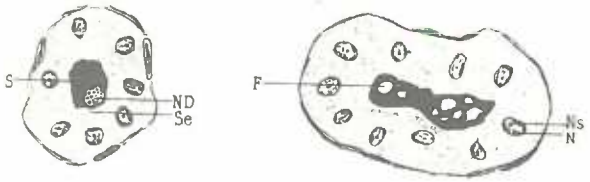
Changes during lactation and involution:- The macroscopic aspect of the mammary gland thruout lactation remains unchanged and is the same as at the end of pregnancy (Meader 1922). Glandular parenchyma in the form of closely packed lobules is in great preponderance during lactation. The ducts are distended and the alveoli are filled with secretion. The blood vessels and capillaries are greatly dilated.

Kuramitsu and Loeb (1921) state that twelve hours after parturition the alveoli are not usually distended nor have the epithelial cells reached their full size. Secretion is not fully established until seven days after parturition.

Sure (1930) observed that there is little milk flow during the first 24 hours of lactation, but that the young invariably

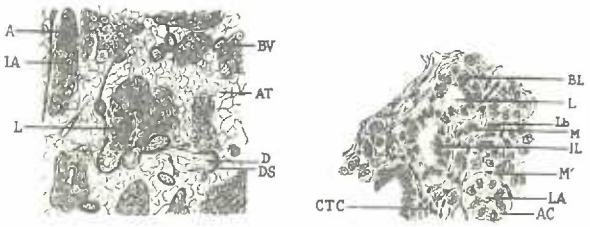
Alveolus from gland of rat thirteen days pregnant. Connective tissue sheath, *CT*, is relatively loose. Endothelial cell nuclei, *E*, show no pressure effects. Mitotic figure, *M*, shown in one of the cells. Zenker's fixation. Weigert's iron-hematoxylin, counterstained with Van Gieson's stain; 4 microns.

Acini from gland of rat, eighteen days and twelve hours pregnant. *IAC I*, interalveolar connective tissue is seen to be stretched around the acinus, giving an appearance of a membrana propria. A mass of secretion, *S*, is seen in the lumen of the central acinus. Secretion granules, *S*, are seen being extruded from one of the acinar cells. A distended capillary, *C*, and a mast cell, *MC*, are also shown. Zenker's fixation. Cresyl violet stain; 4 microns.



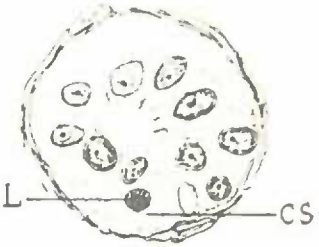
Acinus from the gland of a rat eighteen days and twelve hours pregnant. Cytoplasm much more vacuolated than in Figure 107. *S*, secretion mass; *ND*, nuclear (?) débris; *Se*, secretion being extruded from cell. Zenker's fixation; Cresyl violet stain; 6 microns. × 750.

Acinus from the gland of a rat nineteen days pregnant. Secretion mass with fat vacuoles, *F*, seen in lumen. This secretion shows characteristic arrangement of chromatin in nucleus, *N*. A well defined nucleolus, *Ns*, is shown. Zenker's fixation. Cresyl violet stain. 6 microns. × 750.

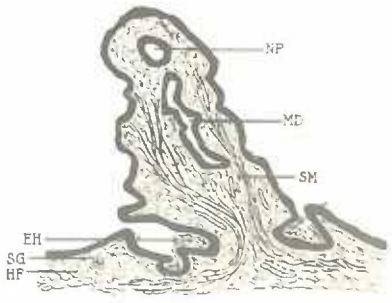


Drawing (low power) of a section of one of the abdominal mammary glands of a rat eighteen days and twelve hours pregnant. Panniculus carnosus muscle not shown. *BV*, blood vessel; *AT*, adipose tissue; *D*, duct; *DS*, duct sheath; *L*, lobule; *IA*, interalveolar connective tissue; *A*, alveolus. Zenker's fixation; Mallory's connective tissue stain; 5 microns.

Section through duct and neighboring acini. Rat fifteen days pregnant. Mast cells and the rapid thinning of the fibrous sheath of the duct are shown, as this sheath passes around the lobule at *LB*. *BL*, basal layer of duct epithelium; *L*, lumen of duct. *M*, mast cell; *M'*, mast cell between two alveoli; *IL*, inner layer of duct epithelium. No vacuolization shown at this stage. *LA*, lumen of alveolus; *AC*, vacuolated alveolar cytoplasm; *CTC*, cellular elements of connective tissue. Zenker's fixation; Cresyl violet stain; 4 microns.



Acinus from the gland of a rat thirteen days pregnant. Many fine vacuoles in cytoplasm of the cells are shown. The section is tangential so some of the cells appear to have more than one nucleus. A lymphocytic-like nucleus is shown at *L*. Zenker's fixation; Cresyl violet stain; 6 microns. × 750.



Drawing of nipple. Rat sixteen days and twelve hours pregnant. The epithelial bud has entirely disappeared, leaving a groove, *EH*, in its place. The groove contains some granular debris, *D*. Well developed smooth muscle, *SM*, around the main duct, *MD*, of the nipple. The smooth muscle does not extend up to the nipple proper, *NP*. A hair follicle, *HF*, and a sebaceous gland, *SG*, are shown on the left. Zenker's fixation. Weigert's iron hematoxylin counterstained with Van Gieson's stain; 7 microns. This section should be compared with Figure 11 in Meyer paper, which shows only a slight depression at the head.

From Roberts.

gain significantly on the second day of lactation, and that occasionally by the end of the third day and very often by the end of the fourth day of lactation, the litters will as much as double their initial weight.

No marked involution of the gland was observed by Meader (1922) to occur until the eighth day after weaning, when the stroma once more exceeds the parenchyma in amount. At the ninth day the appearance of the gland approaches that of the adult virgin. Three months after weaning the gland is apparently similar in structure to that of the virginal resting gland. The disappearance of the alveoli is accomplished by simple atrophy of the cells with shrinkage in the size of the cells and of the alveoli. Alveoli in various stages of involution may persist for several months after lactation, but changes due to theestrus cycle (corpora lutea) must be kept in mind.

Kuramitsu and Loeb observed the first sign of decided retrogression in the rat five weeks after parturition. Each lobule consists of a much reduced number of alveoli which have a smaller lumen than formerly.

That there is an accumulation of milk up to the forty-eight hour stage after weaning was noted by Myers (1921). At the end of five days following weaning, the masses of glandular tissue have decreased to less than half their size at the forty-eight hour stage. At the end of two or three weeks the glands very closely simulate those of the adult virgin animal.

The inhibition of estrus during lactation:- In the rat ovulation occurs shortly after parturition. The corpora lutea forming after this ovulation have been called the corpora lutea of lactation. These corpora have been shown to differ to some

extent from the corpora of ovulation, pseudo-pregnancy, and pregnancy (Long and Evans 1922).

Loeb and Kuramitsu (1921) and Long and Evans (1922) noted the inhibition of estrus by lactation in the rat in the presence of corpora lutea of lactation. Within a short time after the removal of the young changes appear in the corpora. However, the cause of estrus cannot again operate as quickly after a period of lactation, and especially the lactation of a large litter as it can under the normal conditions characterised by the usual successions of ovulation.

REVIEW OF THE LITERATURE

The Effect of Hormones on the Mammary Glands of Animals.

Effect of the Oestrus-producing Hormone on the Mammary Gland.

The first to apply experimental methods to the study of hormonal effects on the growth of the mammary gland were Lane-Claypon and Starling (1906). They injected aqueous extracts of placentae, ovaries and fetuses as well as combinations of these tissues into normal virgin female rabbits. It is probably that their extracts had little effect as the figures from the glands of these experimental animals show the growth of the duct system similar to that observed after reaching puberty. Foa (1908) Riedle and Kongstem (1910), Aschner and Gregoriu (1911), Frank and Unger (1911), Schickele (1912) and Ascher (1913) all performed similar experimental work, but all neglected to use castrated animals so it is quite possible that the development of the mammary gland in many cases was simply the normal estrus growth. Following this other investigators began to study the effect of different extracts on both castrate and normal animals.

Iscovesco (1912) was the first to report that lipoid extracts of the ovary, corpora lutea, and placentae, when injected into mature, isolated and therefore not cyclical rabbits, produced marked hyperplasia and congestion of the uterus and vulva, as well as the breasts. In 1913 Fellner, using an ether-alcohol-acetone method of extracting the corpora lutea and placenta, was successful in producing growth of the duct system of castrate and normal male and female guinea pigs and rabbits. However, milk secretion was not obtained. Hermann (1913-1915) reported growth of themammary glands in both castrate and normal virgin female rabbits with lipoid extracts. Similarly,

Frank and Rosenbloom (1915) observed slight growth of the gland of castrate rabbits and rats with placental lipoid extracts. The changes noted by the above investigators were undoubtedly due to the estrus-producing hormone.

Later Allen and Doisey (1923) introduced the rat unit test of the estrus-producing hormone and also determined the tissues in which this hormone was present in greatest concentration. While determining the action of the ovarian follicular hormone, Allen et al (1924) observed that during artificially induced sexual cycles concomitant changes occurred in the mammary glands. By repeated injections of liquor folliculi from cow's ovaries, Vintemberger (1925) produced an extended growth of the ducts of the mammary gland in normal virgin female rabbits as well as in young castrate males and females. He states that Tsu-Zong-Yung (1924) had produced mammary gland growth in three month old rabbits (immature) with eight injections of liquor folliculi over a period of 20 days. Allen (1927) determined the effect of the estrus-producing hormone of the ovary and placenta on the growth of the mammary gland of the monkey. The left mammary gland of a spayed monkey was removed as a control. The ducts of this gland were small and shrunken, exhibiting only a few small alveoli. The right gland was removed at the end of a series of injections of the estrus-producing hormone. The whole amount showed marked growth and branching of the ducts with some increase in the number of alveoli. Laqueur, DeJongh, and Tousk (1927) report an increase in the size of the mammary glands of rats, both males and females, with subcutaneous and intraperitoneal injections of menoformon (estrus-producing hormone). Control animals showed much less gland growth. The injected animals were not castrate.

Subsequent to this a number of other similar investigations

were made by Champy and Keller (1927), Haterius (1928), Loeb and Kountz (1928), Laqueur, Barchardt, Dirgemense and DeJongh (1928) and Steinach, Dahrn, Schaller, Hohlueg and Foure (1928) all report extensive growth of the mammary gland of male and female guinea pigs, both normal and castrate, following injections of the estrus-producing hormone. Turner and Frank (1930) found that a daily injection of 20 rat units of the estrus-producing hormone for 30 days into male castrate rabbits and females castrated previous to puberty, produced a growth in the duct system of the glands equal to that produced during continued estrus in the normal female. Continuing these studies in the albino rat, Turner and Schultze (1931) found that small amounts (1 rat unit) of the estrus-hormone, administered to castrate immature male and female rats, resulted in extension of the duct system similar to the growth occurring during the period from birth to puberty. When relatively large amounts (5 to 15 rat units) of the extract were administered, limited lobule proliferation resulted. This proliferation was greater than that found in the normal adult virgin rat but less than that found in advanced pregnancy.

From the experimental evidence reported above it appears that the initial growth of the mammary gland complex, including the teat and duct system, is stimulated by the estrus-producing hormones formed in the ovary at the approach of and during puberty. The continued stimulation of the glands by these hormones eventually causes, in mature virgin animals, the growth of an extensive duct system with slight lobule formation in some species (Turner 1932). The estrus-producing hormones (theelin and theelol, Doisey) are ineffective in producing growth of lobules in the mouse, slightly effective in the rabbit and rat and greatly effective in the guinea pig.

While slow acinar and lobule growth appears to be induced, in some animals, by large amounts of the estrus-producing hormones injected over long periods, the rapid and extensive hyperplasia of the lobules of the mammary gland which occurs during the first half of pregnancy is believed by Turner (1927) to require an additional stimulus.

Corpus Lutea and Development of Mammary Gland.

The theory of the functional relation between the corpora lutea of pregnancy and the growth of the mammary gland during the first half of pregnancy or pseudo-pregnancy in rabbits was advanced by Ancel and Bouen (1911), and Hammond and Marshall (1914); in the marsupial Dasyurus by O'Donoghue (1912), and in the guinea pig by Loeb and Hesselberg (1917). During pregnancy or pseudo-pregnancy with the presence of active corpora lutea in the ovary, rapid lobule hyperplasia always followed. Loeb and Hesselberg (1917) prepared an aqueous extract of dry bovine lutein, in order to determine the possible presence of a hormone causing the proliferation of the mammary gland of a guinea pig. It was found that the extract produced no changes in the mammary gland in animals in which the corpora lutea had been removed. Bercan, Champy and Keller (1927) injected lipoid extracts of the corpus luteum which were believed to have physiological actions differing from theelin. However, little or no effect was observed in the glands of guinea pigs following the administration of such extracts.

Extracts of the corpus luteum have been prepared by Corner and Allen (1929) (called "progestin"); Hisaw, Fevold and Meyers (1930) (called "corporin"); and others, which were found to produce changes in the uterus similar to those during early pregnancy.

Corner (1930) in a series of experiments in which "progestin" was injected into spayed rabbits concluded that the corpus luteum acting alone in the absence of pregnancy does not induce proliferation or lactation of the mammary glands. Turner and Frank (1931) and Turner and Schultz (1931) confirmed the above findings of Corner. In contrast to these results Nelson and Pfiffner (1930-31) reported the production of marked hypertrophy of the glands and nipples of immature male and female castrate guinea pigs with daily injections of a lipoid extract of sow's corpora lutea (believed to be practically free of theelin). Turner and Frank (1931) by giving simultaneous injections of the estrus-producing hormone and "progestin" found that the mammary glands of male castrate rabbits (both ducts and lobules) developed in a manner similar to the normal development during the first half of pregnancy. However, even with injections for 30 days, lactation was not produced. Extending the experiments to albino rat, Turner and Schultze (1931) determined the effect of the simultaneous injection of the estrus-producing hormone and lutein extracts, using immature castrate males and females in which only the ducts were present. They observed that the administration of theelin in sufficient amounts with either "progestin" or "corporin" was capable of producing further duct growth and lobule formation. The estrus-producing hormone in amounts of 2.5 r.u. or less, plus "corporin" in amounts up to 0.5 cc. seemed incapable of producing any visible change in the gland. But when larger amounts of the estrus-producing hormone (5 r.u.) replaced the smaller amounts, proliferation of the lobules resulted even when the amount of "corporin" remained constant. The differences in the response of the corpora lutea extracts reported by Corner and Turner and associates, in comparison with those reported by Nelson and

Pfiffner, are difficult to harmonize unless it is assumed that the lutein extract used by the latter contained theelin.

Relation of Anterior Pituitary to Lactation and Mammary Development.

The initial demonstration of the existence of a gonad-stimulating hormone or hormones in the anterior lobe of the hypophysis by Zondek and Aschoim (1927) and Smith and Engle (1927) led to the assumption of an indirect pituitary mammary gland relationship. That is, the mammary glands might be affected by the ovaries, which are stimulated by the gonad-stimulating principles of the pituitary. Parkes (1929) observed complete mammary growth and a slight lactation in a rabbit injected with an anterior pituitary extract for the purpose of maintaining functional corpora lutea in the ovaries for a period equal to normal pregnancy. Stricker and Grüter (1928 and 1929) first reported the direct lactation stimulating effect of anterior pituitary extracts. Female rabbits were ovarectomized on the tenth day of pseudo-pregnancy and lactation was initiated following the administration of the pituitary extracts. A similar though somewhat delayed response was obtained in rabbits whose mammary glands had undergone considerable involution. Lactation was not induced in immature female rabbits. Grüter (1928) mentioned that marked ovarian stimulation was observed in two normal, 5 to 6 weeks old rabbits, following four or five daily injections of anterior pituitary extract. During the time of injection no changes were observed to occur in the mammary glands. Grüter therefore concluded that the mammary glands must be at least partially developed under the influence of the corpora lutea before the lactation stimulating effect of the pituitary could be observed. Corner (1930) however

obtained lactation in mature ovariectomized virgin female rabbits following pituitary extract treatment. Two of the rabbits which he used had been isolated at 4 months of age and he concluded were undoubtedly virgins. Control mammary glands removed at the beginning of the period of injection showed but slight development, while glands removed after an extended period of pituitary extract treatment showed a development comparable to the condition observed during late pregnancy. It was thus concluded that mammary hyperplasia as well as secretory activity was initiated by the pituitary extracts. These extracts were found to contain no appreciable amounts of theelin or progestin.

Similar extracts to those used by Corner, were used also by Asdell (1931) who used spayed virgin female rabbits which were brought into full mammary development with lactation. He also found that the extracts would not produce mammary development in the immature male or female rabbit. It was therefore concluded that some sensitizing substance, presumably other than corpus luteum was required before the extract was active in stimulating mammary development and lactation.

Later Turner and Gardner (1931) using mature, non-lactating ovariectomized female rabbits were able to bring them into full mammary activity by daily injections of pituitary extracts for periods up to ten days. They were successful even in rabbits having glands considerably involuted. Observations made on whole mounts of such control and experimental glands led them to conclude that growth of the gland was not produced by the extract injections but that they stimulated into activity the already existing secretory epithelium.

Nelson and Pfiffer (1931) observed milk secretion in rabbits

following the administration of pituitary extracts when the injections were made following a previous treatment with the corpus luteum extracts. Normal female rabbits responded similarly. Examination of the ovaries of these treated animals showed large masses of lutein tissue. Young normal and castrate male rabbits, whose glands had been developed by ovarian hormone treatments, were activated so that milk could be expressed from the teats. Normal and castrated males whose glands had not been previously developed did not respond to the pituitary extract. They concluded that ovarian hormones were essential for the initial development of the mammary glands but that the profound growth of the gland during pregnancy was due to anterior lobe hormones or a substance physiologically similar. This conclusion was further supported by the non-effect of pituitary extracts on the glands of immature rabbits as reported by Turner, Gardner and Schultze (1932).

In view of the galactopoetic nature of the active material of the pituitary extracts the term "galactin" has been applied to it (Turner and Gardner 1932). Galactin appears to exist in the extracts of the pituitary independently of the growth and gonad-stimulating hormones. Further observation on the relation of pituitary hormone to breast activity were made by Riddle, Bates and Dykshorn (1932 and 1932a) who observed that a sheep pituitary extract capable of initiating crop-gland activity in doves and pigeons was capable of initiating lactation in the rabbit. These extracts were ineffective in producing mammary hyperplasia. Their lactation-stimulating material has been called prolactin.

Lactation has also been initiated in young virgin rabbits by Catchpole and Lyons (1933). They state that these virgin female rabbits possess mature mammary glands but no milk.

Gardner and Turner (1933) do not agree on this point and state that completely grown glands showing both duct and lobule proliferation have never been observed in virgin female rabbits. Catchpole and Lyons (1933) likewise report obtaining lactation in male rabbits with pituitary extract following a "sensitization" of the mammary glands with corporin.

Anterior Pituitary Sex Hormone and Relation to Mammary Gland.

Mammary gland hyperplasia was observed in adult virgin rats by Evans and Simpson (1929) after a month of daily subcutaneous injections of an alkaline extract of the pituitary, rich in growth-stimulating hormone and comparatively poor in gonad-stimulating hormone. More rapid mammary hyperplasia took place following the administration of gonad-stimulating hormone from bovine or rat pituitary implants or from placenta or pregnancy urine extracts. The mammary glands of immature animals responded rapidly to gonad-stimulating implants or extracts (Evans and Simpson 1929).

Evans and Simpson (1931) reported further observations on the rat. They state that growth of the mammary tree was caused by rat and bovine pituitary implants, aqueous acid extracts of placenta, and by alcohol precipitate of the urine of pregnancy. The latter two products were practically free from folliculin and growth hormone. Males and spayed females failed to respond to injections of alkaline pituitary extracts and pituitary implants. To determine the early influence of the corpus luteum of pregnancy, groups of rats were bred and then spayed from 18

hours to three and one-half days after copulation. Rat hypophyses were then implanted on alternate days followed by autopsy at 10 days. Mammary ducts alone were present, showing further the necessity of the ovary. Turner and Schultze (1931) likewise observed the absence of any effect of pituitary implants or extracts on mammary glands of male and of castrate female rats. But the mammary glands of normal immature females developed rapidly when pituitary implants were administered. However, lactation resulted when the implants were continued over a longer period or when simultaneous injections of corporin, theelin and galactin were given (Schultz and Turner 1933). This was not concurred in by Nelson and Pfiffner (1931) who found that implants of pituitaries were followed by no changes in the mammary glands of castrate animals though normal animals responded rapidly. But they found that castrate rats given simultaneous treatment with corpus luteum and pituitary extract developed fully mature and functional glands. They concluded that "in the rat some ovarian factor must act synergistically with the anterior lobe hormone in order to induce extensive mammary gland growth".

Selye, Collip and Thomson (1933) found that complete mammary growth could be produced by the treatment of normal rats with an extract of pregnancy urine. While lactation, however, resulted only following the removal of the ovaries. In a second paper the same writers report that hypophysectomy of pregnant rats did not prevent the onset of lactation following parturition, but lactation was found to persist for only a very short time in these animals. Hypophysectomy performed during lactation resulted in immediate cessation of secreting activity.

The development of the mammary glands of mice was observed

following the injection of gonad-stimulating hormones (Bradbury 1932). The injection of the gonad-stimulating substance of pregnancy urine was not followed by as rapid a development of the glands as was observed following pituitary administration though the ovaries contained abundant lutein tissue. Pituitary extracts were observed to induce the formation of alveoli and lactation following ovariectomy providing the glands were sufficiently developed at the time of operation. In the mouse hysterectomy as early as the eleventh day of pregnancy was followed by lactation.

Pseudo-pregnancy and its Relation to the Mammary Gland.

Several investigators have found that a condition of pseudo-pregnancy may be provoked in the spontaneously ovulating rat or mouse after copulating with a vasectomized male (Long and Evans 1922, Wang, 1923; Slonaker, 1929; Parkes, 1929). The stimulus of copulation causes a prolongation of the life of the corpora lutea in the ovaries, the next oestrus period being deferred eleven to fifteen days. The phenomena resembling those of early pregnancy that manifest themselves during pseudo-pregnancy are, a prolonged leucocytic vaginal smear, mulcification of the vaginal epithelium, reduction in spontaneous activity, and some mammary gland development.

That mechanical simulation of the cervical canals of rats will provoke the pseudo-pregnant condition was shown by Long and Evans (1922), while Sheleenyak (1931a) demonstrated that even a higher percentage of positive pseudo-pregnancies resulted from electrical stimulation of the cervical canals. Myers, Leonard and Hisaw (1929) showed that a nervous mechanism is in some way involved, for mechanical stimulation of the cervical canals of

rats under general or local anaesthesia was usually not followed by pseudo-pregnancy. Friedman (1929) working with rabbits found that any nervous mechanism involved did not act directly on the ovaries for he obtained ovulation and subsequent corpus luteum formation in grafts after coitus. Just what mechanism is here involved has not as yet been demonstrated but the interpretation of most investigators has been that the anterior lobe of the pituitary is in some way nervously stimulated to increased action with a resulting elaboration of the hormone or hormones which bring about the follicular stimulating and luteinizing action of the ovary (Fee and Parks, 1929; Deanesly, Fee and Parks, 1930; Smith and White, 1931). Since Loeb (1907) first described the decidual reaction whereby traumatization of the uterine mucosa at certain periods resulted in growth of decidual cells known as placentomata, the dependence of the presence of functional corpora lutea has been clearly demonstrated by numerous investigators (Frank, 1911; Corner and Warren, 1919; Long and Evans, 1922; Teil, 1926; Broula, 1928; Weichert, 1928; Goldstein and Tatelbaum, 1929; Parkes, 1929; Courrier, 1930; Nelson and Pfiffner, 1930; Shelesnyak, 1931, a,b; 1933 a,b). In the rat, placentomata cannot be produced during normally recurring oestrus cycles (Long and Evans 1922; Weicher, 1928).

Pseudo-pregnancy and Hyperthyroidism in Relation to Mammary Gland.

Weichert and Boyd (1933) were able to produce typical pseudo-pregnancy with functionally active corpora lutea in female rats fed with desicated thyroid. The pseudo-pregnancies occurring in every case in which experimental hyperthyroidism was induced,

were spontaneous and not caused by mechanical stimulation of the cervical canals when taking the daily vaginal smears. The fact that some of the experimental animals had one or sometimes two normal cycles intervene between two pseudo-pregnant periods indicates that some hormonal action rather than a mechanical stimulus was responsible for this pseudo-pregnant condition.

It has been suggested (Weichert, 1930) that the hyperthyroid condition stimulates the anterior lobe of the pituitary body to elaborate its hormones. The prolonged gestation period of pregnant hyperthyroid rats (Weichert, 1930) and their inability in most cases to deliver, is quite comparable to the condition reported by Teil (1926) and D'Amour and D'Amour (1933) in pregnant rats given injections of anterior lobe extracts.

Evans and Simpson (1930) and VanHorn (1931) found that in rats fed thyroid, the pituitary content of gonad stimulating hormone was increased. The hypophysis of thyroidectomized female rats gave less of a gonad stimulation when given as implants than did pituitaries of normal littermate sisters (Evans and Simpson, 1930). However, Smith and Engle (1930) and VanHorn (1931) report no decrease in potency of the pituitary after thyroidectomy in rats.

It is also possible to explain this hyperthyroid phenomena by excluding the anterior lobe of the pituitary gland. It has been shown by Reiss and Perony (1928) and confirmed by Van Horn (1931, 1933) that thyroid feeding raises the threshold response of castrate rats to injected oestrin. Furthermore Van Horn has suggested that in the hyperthyroid animal the failure of the minimal effective dose of oestrin to act on the vagina and also the

increase in potency of the anterior lobe of the hyperthyroid animal can be explained by a physiological castration that occurs, due to the elimination of oestrin under the increased metabolism resulting from thyroid feeding. Leonard, Hisaw and Fevold (1932) have demonstrated that the effect of either the corpus luteum hormone or oestrus hormone in a castrated animal depends on their relative concentrations in the blood stream. Weichert (1933) suggests that the heightened metabolism incident to thyroid feeding may lower the oestrin concentration to such a degree that the function of the corpora lutea may be expressed.

The work of Smith and Engle (1927) and Zondek and Aschheim (1927, 1928) which demonstrate most lucidly the capacity of fresh anterior pituitary transplants to evoke precocious maturity in immature rats and mice showed that the hypophysis has the power to stimulate the ovaries to elaborate their hormones. Engle (1929) and Evans and Simpson (1929a) found that removal of the gonads results in a greater sex-stimulating capacity of the hypophysis which increases in weight after castration. This was also confirmed by Fechera (1905) and Hatai (1913).

Meyer, Leonard, Hisaw and Martin (1930) injected normal female rats with oestrin for 30 to 70 days and found that their hypophyses had to a considerable degree lost their power to stimulate the gonads of immature female rats as compared to normal controls. The same investigators (1932) obtained similar results when injections of oestrin were given to castrated females. Smith and Engle (1929) reported that the anterior pituitary glands of the guinea pigs during the oestrus period were weaker in gonad-stimulating power than in the dioestrus period. Moore and Price

(1932) in a series of experiments in which both male and female rats were injected with oestrin and testicular hormones, alone and in combination, as well as in combination with gonadatropic hypophyseal hormones, have demonstrated a gonad-hypophysis interrelationship. They maintain that gonads function only when they are forcibly stimulated by certain secretions that are normally provided by hypophyseal activity. The activity of the hypophysis, on the other hand, is to some extent controlled by gonadal secretions, for when these gonad hormones are present in effective amounts, hypophyseal activity is lowered.

The work of Weichert and Boyd (1933) in producing typical pseudo-pregnancy in hyperthyroid rats may also be interpreted in the light of these researches. The rapid elimination of oestrin under conditions of experimental hyperthyroidism, if this indeed be the case, should result in the removal of the inhibiting action on the hypophysis. This would result in the elaboration of the hypophyseal hormones to an increased extent and cause the formation of the large corpora lutea which suppress ovulation and oestrus up to a certain point and which are of such importance in evoking the decidual response in the uterus traumatized during pseudo-pregnancy. Thus hyperthyroidism probably results in a stimulation of the pituitary gland but its effect is indirect, working through oestrin elimination rather than directly on the hypophysis as has been previously suggested.

That the growth hormone of the hypophysis is likewise suppressed by the injection of oestrin has been shown by Spencer, Gustavson and D'Amour (1931) who noted a significant retardation of the growth curve of young male and female rats injected with

oestrin. Wade and Doisey (1931) noted a reduction in the growth increment in young male rats given crystalline theelin and theelol. If the gonadotropic and growth hormones of the hypophysis are suppressed by an over-abundance of oestrin, it is not inconceivable that "galactin", the so-called lactation-inducing hormone of the pituitary gland, might also be suppressed as has been suggested by Nelson (1932).

Riddle, Bates and Dykshorn (1933) have shown that thyroid hypertrophy following pituitary administration is a specific response to the gonad-stimulating hormone or to another pituitary substance having similar solubilities. The work of Greep (1933) indicates that thyroid hyperplasia is produced either by the luteinizing principle of the hypophysis or by another substance closely associated with it in preparation.

Weichert and Boyd (1934) feeding 0.5 gm. of dessicated thyroid daily to pregnant rats found that some stimulation was observed on the fifth day of pregnancy and an extraordinary increase of mammary tissue occurs between the seventh and ninth days. They found that the fat cells had been almost entirely obliterated, in the nine-day stage, and that a great number of lobules had found also that vacuolization of the alveoli had already begun and in some the lumina of the alveoli have increased in size. No indication of secretion was found in this stage. By the eleventh day their thyroid fed animals showed alveoli with secretion already formed, and a high degree of vacuolisation, with very few fat cells present while in the normal control eleven-day pregnant rat fat cells were very numerous. They (Weichert and Boyd) suggest that under conditions of heightened metabolism resulting from thyroid feeding, the normal oestrin level may be

lowered thus removing the inhibitory effect of the oestrin on the hypophysis, at least to a degree, allowing it to elaborate its hormones to a greater extent than normal.

MATERIALS AND TECHNIQUE.

Experimental Animals:- The Albino rat was selected for the work reported here, because of the ease with which a large number of them could be cared for, and their rather short estrus cycle and gestation period. The colony was started about two years ago from three pairs of pure bred Albino rats. The animals were bred one pair to a cage, the young weaned three weeks following delivery and isolated as to sex at the age of four weeks. Accurate records of the age and parentage of all experimental animals have been kept. Females were not bred until six months of age.

The stages in the estrus period were followed by the vaginal smear method of Evans. The diet for the experimental animals consisted of a dry mixed food to which water was added, fresh whole milk daily, cod liver oil, lettuce, carrots and fresh orange juice, also fresh meat once a week. The dry food consisted of the following ingredients: cracked corn, cracked wheat, alfalfa meal, linseed oil meal, brewers yeast, casein, dry whole egg

powder, sodium chloride, calcium carbonate and magnesium sulphate in proportions recommended by Dr. I. Manville of the physiology department.

The animals were killed by ether, the hair removed with a barium sulphid depilatory and the complete skin with subcutaneous fat removed immediately.

Operative technique:- Ovariectomies were performed by dorsal incision under intraperitoneal Nembutal anaesthesia. A stock solution was made by diluting 1.0 cc. of the standard Nembutal solution with 6.0 cc. of normal salt solution, using 0.05 cc. of this solution per 10.0 grams of body weight. After removal of the ovaries bleeding was stopped by ligature, the muscle and peritoneum sutured and the skin incision closed with Michel's skin clips.

In attempting to produce an artificial stasis of the mammary gland, the nipple was tightly ligated with fine silk thread. We found that this effectively stopped milk leaving the gland, but in many cases the nipple atrophied and dropped off. With no nipple the suckling stimuli was removed. In order to leave the nipple intact we decided to cut the galactophores subcutaneously. This was done under local anaesthesia, through a small incision 1.5 cm. medial to the nipple. By subcutaneous blunt dissection to the area under the nipple the milk ducts were effectively cut. This small incision was closed with a single suture. The efficiency of this operation was checked by placing a newborn rat (one that had never nursed) upon this breast. In no case did its stomach visibly fill with milk as it would have on a normal breast.

Histological technique:- The whole skins were placed out

flat on blotting paper immediately upon removal and placed in Formo-Zenker's solution for twenty - four hours. The skin was then washed in running water for twenty-four hours and as much muscle and connective tissue as possible removed. Sections for histology were then cut, dehydrated, cleared and embedded in paraffin. Sections were cut six to ten mu. and stained in hematoxylin and eosin. Ovaries, vagina and uterus were likewise fixed and sectioned upon removal.

Methods of administration of endocrine compounds:- The estrus producing hormone of the ovarian follicle, the anterior pituitary-like sex hormone and the lipoidal extract of corpus luteum were all injected subcutaneously. Injections were made daily unless otherwise stated. The dessicated thyroid was emulsified in a small amount of water and placed directly in the animal's mouth by dropper.

Source of endocrine compounds:- Antuitrin "S", Antuitrin, Theelin and Lipo-lutein were purchased with the exception of 6.0 cc. of the Lipo-lutein which was donated by Parke, Davis and Company. The Antuitrin "S" was restandardized on immature rats, as it was found to loose in strength with time. The dessicated thyroid (Armour and Company) was purchased.

EXPERIMENTAL WORK.

1. Mammary Glands of Mature Virgin Female Rats at Various Stages of the Estrus Cycle:- Female rats six months of age were used in this study. They were followed closely by the vaginal smear method, to be sure that each animal had a normal and regular estrus cycle. Two animals being sacrificed during each of the four stages of the estrus cycle as follows:-

Stage 0 (Evans) or the Pro-estrus (Heaps):- The vaginal smears were free of leucocytes, being composed of a large number of nucleated epithelial cells. Sections of the vaginal mucosa were high (9 to 12 cells) with beginning cornification of the stratum corneum. The ovaries showed developing follicles and atrophy of the corpora lutea. The sections of the mammary gland revealed many small branching ducts (Plate 7, Fig. 7 and 8).

Stage I or Estrus:- The vaginal smears were free of leucocytes and contained a large number of large cornified, transparent, non-nucleated, scale-like cells. Histological studies of the vaginal mucosa revealed marked cornification with a superficial stratum corneum. The ovarian follicles at this stage had reached their maximum size. Sections of the mammary gland during this stage disclosed more small ducts and epithelial buds than were found in the sections from the animal in Stage 0 (Plate 7, Fig. 2 and 3).

Stage II and III or Metaestrus:- The vaginal smears consisted of large masses of cornified epithelial cells with many leucocytes. Sections of the vaginal mucosa revealed it to be low (4 - 8 cells) with complete loss of the stratum corneum.

it is during this stage that ovulation occurs. Sections of the mammary glands in this stage revealed a seeming decrease both in number and size of the ducts (Plate 7, Fig. 4 and 5).

Stage IV or Diocstrus:- The vaginal smears consisted almost exclusively of leucocytes, with some nucleated epithelial cells. Sections of the vaginal mucosa revealed it to be thin (6 to 9 cells) with only a slight squamous transformation of its upper cells. Sections of the ovary showed growing follicles and well developed corpora lutea. Sections of the mammary gland revealed about the same number of ducts as were found in the previous stage but with a lower type of epithelium (Plate 8, Fig. 2 and 3).

Comparing the mammary gland sections taken from the animals during estrus and dioestrus, a marked difference is noted. During estrus there are many more small branching ducts, many of which have small epithelial buds projecting from their walls. At the time of estrus the epithelial cells lining the ducts are approximately twice the size of those found during dioestrus.

2. Mammary Glands of Mature Ovariectomized Rats:- Three female rats, six months of age were spayed when in estrus, (In order that all glands may be at the same stage of development at the time of the operation) and sacrificed on the 14th, 21st and 28th days after operation. Sections of mammary glands from these animals revealed no further development other than found in the normal animals during estrus. The predominant picture being similar to that found during the dioestrus period in normal females. (Plate 9, Fig. 2). These findings are in accord with those of Turner and Schultz (1931) with the exception that their studies were on whole mounts of the glands while these are on microscopic sections.

3. Mammary Glands of Mature Virgin Rats Given Theelin Injections:- Two, six month old, virgin female rats were injected subcutaneously with a daily dose of 5 rat units, one animals for 14 days and the other for 28 days. Sections from the mammary gland of these animals revealed considerable growth of the duct system, with more marked branching and budding. The development was more advanced than that found during the normal estrus cycle. These findings are also in accord with those of Turner and Schultz (1931) (Plate 8, Fig. 4).

4. Mammary Glands of Mature Ovariectomized Rats Given Theelin Injections:- Two adult virgin female rats were ovariectomized during estrus and 7 days after the operation daily injections of 5 rat units of Theelin were begun. The glands were removed and sectioned at the end of 14 days in one rat and 28 days in the other rat. The duct system was found to be about equal to that found in the normal animal in estrus. There was a slight degree of epithelial hyperplasia, but this did not equal that found in the intact animals given Theelin. The ducts were much more prominent in these animals than in the untreated castrates (Plate 9, Fig. 4).

5. Mammary Glands of Mature Virgin Rats Given Antuitrin "S" Injections:- Two animals six months of age were each given 5 rat units of Antuitrin "S" daily, one for 10 days and one for 20 days. Injections were begun while the animals were in estrus. In 10 days there was evident marked proliferation of the duct system with well defined lobules showing a slight amount of vacuolization (Plate 8, Fig. 5). This was even more marked in the breast of the animal injected for 20 days. Although the

development was not as far advanced as that found for a corresponding period of pregnancy. These findings agree with those of Evans and Simpson (1931) and Selye, Collip and Thomson (1933).

6. Mammary Glands of Mature Ovariectomized Rats Given Antuitrin "S":- Two female rats were ovariectomized during estrus, and 7 days after the operation, daily injections of 5 rat units of Antuitrin "S" were begun. One animal was sacrificed at the end of 7 days and the other at the end of 14 days. The duct branching was not found to be developed beyond that found in the normal virgin animal. The ducts were more prominent than those of a control animal ovariectomized for 28 days. (Plate 9, Fib. 6). These findings are in accord with those of Evans and Simpson (1931).

7. Mammary Glands of Mature Ovariectomized Rats Given Lipo-Lutin:- Two females were ovariectomized during estrus and 7 days after the operation, daily injections of 0.5 cc. of Lipo-Lutin were begun (a lipoid extract of the corpora lutea containing Progestin). One was injected for 7 days and the other for 14 days. Sections studied revealed no changes differing from the normal virginal animals (Plate 8, Fig. 7). This is in accord with Turner and Frank (1931) and Turner and Schultz (1931).

8. Mammary Glands of Mature Ovariectomized Rats Given Injections of Theelin and Lipo-Lutin:- Two animals were ovariectomized and 7 days after the operation daily injections of 5 rat units of Theelin and 0.5 cc. of Lipo-Lutin were begun. These were continued for 7 days in one and 14 days in the other. Sections of the animal injected for 7 days revealed early lobule

formation while the development of the 14 day gland resembled that of a corresponding stage of pregnancy. A lobule formation was marked and slight vacuolization was present (Plate 8, Fig. 6).

9. Mammary Glands of Mature Virgin Rats in a Period of Pseudo-Pregnancy Caused by Feeding Thyroid:- Ten female rats were fed 0.5 gm. dessicated thyroid gland daily. They were each examined by daily vaginal smears for the onset of pseudo-pregnancy. Glands being sectioned at different periods in the pseudo-pregnancy period. Sections removed at 2 day intervals in the pseudo-pregnancy revealed a definite and gradual increase in the branching of the ducts with diffuse epithelial bud proliferation. In no case did the process go on to the formation of definite lobules, although vacuolization occurred in the cells forming the epithelial buds and smaller ducts (Plate 8, Fig. 8, gland from animal 9 days pseudo-pregnant).

10. Mammary Glands of Mature Rats Given Injections of Antuitrin "S" during the Pseudo-Pregnant Period Induced by Thyroid Feeding:- Five mature rats were fed 0.5 gm. dessicated thyroid daily. The feedings being started when the animals were in estrus. On the fifth day of the resulting pseudo-pregnant period injections of Antuitrin "S" were begun with daily doses of 5 rat units each. Animals were sacrificed at two day periods following the start of injections, or at 7 days, 9 days, 11 days, 13 days and 15 days of the pseudo-pregnant periods. Sections of the 7 and 9 day animals showed changes similar in degree to those of animals not receiving Antuitrin "S" in Part 9. (Plate 9, Fig. 1 & 8). Sections from animals that received more injections showed a more marked proliferative change in the epithelium of the ducts and buds than did the uninjected pseudo-pregnant animals. In the

15 day animal after a total of 50 rat units of Antuitrin "S" there were well developed lobules with secretion in the lumen and definite vacuolization (Plate 10, Fig. 8).

11. Mammary Glands of Mature Ovariectomized Rats Fed Thyroid Gland:- Three ovariectomized female rats were fed 0.5 gm. dessicated thyroid gland daily. The feedings being started six days after the operation. Sections were taken from glands at 7, 14 and 28 day intervals after onset of thyroid feeding. The sections did not differ significantly from those of a normal castrate animal, except for the fact that the fat cells were greatly reduced insize and number (Plate 9, Fig. 3).

12. Mammary Glands of Mature Ovariectomized Rats Given Theelin Injections and Fed Thyroid Gland at the Same Time:- Two female rats were fed 0.5 gm. dessicated thyroid gland and given an injection of 5 rat units of theelin daily. One animal was given this for 2 weeks and the other for 4 weeks. Sections of these glands did not differ greatly from the sections of a castrate getting theelin alone. (Plate 10, Fig. 1 and 2).

13. Mammary Glands of Mature Rats Injected with Theelin during the Pseudo-Pregnant Period Induced by Thyroid Feeding:- Three female rats were fed 0.5 gm. dessicated thyroid until pseudo-pregnancy was induced. Then at the 7th, 9th and 11th days of pseudo-pregnancy, twice daily injections of 2.5 rat units of theelin were injected. After 48 hours (total 10 rat units) two animals were sacrificed. At this time the vaginal smear of both animals showed the changes characteristic of estrus. The branching of the ducts and epithelial bud proliferation in all three was much more advanced than that seen in an intact animal that had

received theelin injections. Compared with uninjected pseudo-pregnant animals of the same period however, one could see the marked regression that had taken place. No lobules were observed. In some areas desquamation of epithelial cells almost obliterate the lumen (Plate 9, Fig. 5). The third animal was sacrificed in 72 hours (24 hours after the last injection). Further regression was found in this section (Plate 9, Fig. 7).

14. Mammary Glands of Mature Rats During Lactation:- Female rats pregnant for the first time were allowed to deliver their young and nurse them. The lactating females being sacrifices at 1 day, 7 days, 14 days and 21 days after partuition, and 2 days, 7 days and 14 days after weaning (Plate 10, Fig. 3,4,5, Plate 11, Fig. 1). The sections revealed almost no change during the period of lactation. The epithelium of the ducts and alveoli was found to be of a single layer throughout lactation. The amount of secretion in the alveoli varied in different parts of the same gland. Mitosis was rarely observed in the glandular epithelium during normal lactation. The mammary gland showed very little change during the 48 hours after weaning. Seven days after weaning no secretory activity is apparent, there is a marked atrophy of the duct system and alveoli with an increase in the strona. Many alveoli collapse as their epithelial cells degenerate. Two weeks after weaning the gland appears similar to that of the resting gland of the adult virgin. There is considerable variation in the involutional process in different animals and even in different parts of the same gland.

15. Mammary Glands of Mature Lactating Rats Injected with Theelin:- Six pregnant females were allowed to deliver their young. On the

first day post-partum daily injections of 2.5 rat units of theelin were started. By the end of the first week the young had ceased to gain weight as they normally should. By the end of the second week all young were under weight. As soon as their eyes opened they began to drink cow's milk from a dish. By the 16th day post-partum the young ceased to gain in weight unless fed cow's milk, although they still nursed the dry breasts of the mothers. Two animals were sacrificed at the end of the first week of theelin injections. The glandular tissue was found to be greatly decreased in amount, with few alveoli and a decrease in the number of branching ducts. A slight amount of secretion was present in the alveoli. The fat cells were large and abundant in the stroma (Plate 11, Fig. 6). The lumen of the larger ducts contained many desquamated epithelial cells. The lining epithelium being 2 and 3 layers thick in places with frequent mitotic figures (Plate 10, Fig. 6). Sections from the animals injected for 2 weeks showed a further atrophy of the alveolar and duct system, with only a very few ducts containing secretion (Plate 11, Fig. 7). The larger ducts showed a more advanced degree of degeneration, with 2 and 3 cell layers in the walls and an increase in fat and fibrous connective tissue (Plate 10, Fig. 7).

16. Mammary Glands of Mature Lactating Rats Injected with Antuitrin "S":- Lactating female rats were started with daily injections of 5 rat units of Antuitrin "S", on the day of delivery. Sections of the glands from animals injected for one week revealed no significant changes from the normal lactating gland. After two weeks injection there was still no change in the glandular structure (Plate 11, Fig. 5 and 8).

17. Mammary Glands of Mature Lactating Rats in which the Main Galactophores Have Been Cut:- Six normal pregnant females were operated on 2 days before delivery, and the galactophores to the right third thoracic gland cut. These animals were sacrificed at the end of the 1st, 2nd, 3rd, 4th and 5th weeks, after delivery. At the end of the first week sections reveal greatly dilated ducts and acini with atrophy of the secretory epithelium (Plate 12, Fig. 2). At the end of two weeks part of the gland was similar to the picture at one week, while in other parts the acini had apparently ruptured allowing their contained secretion out into the inter-lobular spaces (Plate 12, Fig. 4). By the end of the third week the acini were nearly all empty and of small size, with many degenerating epithelial cells forming their walls (Plate 12, Fig. 3). At the end of the fourth week when normally involution is almost complete many acini still contained secretion and leucocytes are present in large numbers both inter- and intra-acinar (Plate 11, Fig. 2). Five weeks after parturition some of the large ducts still contained secretion with numerous leucocytes scattered through it. The bulk of the glandular tissue had involuted, with a marked increase in fibrous connective tissue. A few leucocytes are still present (Plate 11, Fig. 3).

18. Mammary Glands of Mature Lactating Rats in Which the Main Galactophores Have Been Cut and Theelin Injected:- The galactophores were cut before birth of the young and daily injections of 5 rat units of theelin started at the birth of the young. Sections of glands removed at one week reveal more of a regression in size of the alveoli compared with uninjected tissue. The epithelium lining the ducts and alveoli however, is 2 to 4 cells in height

with numerous mitotic figures. Compare this (Plate 11, Fig. 4 and Plate 12, Fig. 3) with the unoperated glands in (Plate 11, Fig. 6). The sections after two weeks of injection show more regression of the secretory portion but with many ducts completely filled and dilated by dense growths of cells with dark nuclei and many mitotic figures (Plate 12, Fig. 7, compare with Plate 11, Fig. 7). By the end of the third week of injection much more regression has occurred. Few alveoli are present and the ducts seldom have a patent lumen, being solid cords of cells for the most part (Plate 12, Fig. 6).

For the sake of uniformity in all cases the third left thoracic mammary glands were studied. The gland on which the galactophores were cut was the third right thoracic gland in all cases.

DISCUSSION.

Sutter (1921) working on whole mounts of mammary glands found distinct differences in the mammary structure during different periods of the estrus cycle. Other investigators have not mentioned differences discernible microscopically. I found that during the stage of estrus there is a marked increase in the number of small branching ducts, many of which have small buds formed of epithelial cells projecting from their walls. The epithelial cells at this time are about twice the size of those found during the diœstrus interval. With the onset of diœstrus these small epithelial buds regress until no longer visible. This continual process of epithelial hyperplasia during œstrus, with regression during diœstrus, taking place every five days in the rat, can so easily be upset by an imbalance between the hormones of the ovary on one hand and those of the pituitary gland on the other. In the rat, at least, the development of the mammary gland cannot progress beyond the duct stage unless the ovaries are intact, or properly proportioned doses of the ovarian hormones supplied the operated animals. That the ovarian follicular hormone (the œstrus-producing hormone, theelin) is not the only ovarian element of importance is shown by the fact that when this hormone alone is administered to the ovariectomized animal mammary development beyond the branching duct stage does not occur. Injection of theelin alone into mature animals causes an increased growth of the duct system with no lobule or alveolar formation. This demonstrates that theelin stimulates development of the duct system alone. When mature rats were given anterior pituitary-

like sex hormone (Antuitrin "S") injections a well defined lobule formation was found with slight vacuolization. When injected into ovariectomized animals no development of the duct or lobule system was found beyond that of an untreated ovariectomized animal. This proves that the anterior pituitary-like sex hormone has no direct stimulative power on the mammary gland but works by way of the ovary and through another substance than the follicular hormone, because lobule formation was present. Injections of the lipoid extract of the corpus luteum (Progestin, the luteal hormone of the ovary) alone into ovariectomized animals produced no change in the mammary gland but injection of the ovarian follicular hormone (theelin) concomitantly with the luteal hormone of the ovary (lipo-lutin) produced a growth of both the duct and of the lobule system of the gland similar to that found in a corresponding stage of pregnancy in the normal animal. This shows that the duct system of the mammary gland must first be stimulated to growth by the ovarian follicular hormone (theelin) before the corpora luteal hormone (progestin) can stimulate lobule and alveolar formation. These findings prove that the mammary gland is acted upon by at least two different hormones before it even reaches the secretory stage, where a third hormone begins to act. The anterior pituitary-like hormone (Antuitrin "S")which supposedly contains Prolan A and Prolan B (Zondek) acts only indirectly on the mammary gland. The Prolan A supposedly stimulating the development of the ovarian follicle with formation of the follicular hormone (theelin) which stimulates proliferation of the duct system alone. After ovulation the Prolan B fraction is supposed to stimulate corpora lutea development with resulting

formation of the corpus luteum hormone Progestin and some Theelin, which causes lobule and alveolar development in the mammary gland. Thus in the normal rat the breast is constantly pregressing and than regressing in regular cycles.

The feeding of dessicated thyroid will produce a pseudo-pregnant period, during which the duct system of the mammary gland will proliferate to a marked degree with vacuolization of the duct epithelial cells and some secretion, but no typical lobule or alveolar formation as is seen in a normal pregnancy. This is an atypical process as normally vacuolization and secretion does not occur when the gland is only in the duct stage. The hyperthyroid condition may cause an early increased excretion of the ovarian follicular hormone (theelin) before that hormone has had time to cause the development of a mature duct system. This allows the sex hormone of the anterior pituitary gland to function unapposed, causing early and marked function of the corpora lutea with excess production of the progestational hormone. This hormone in turn causing vacuolization of the duct epithelium and secretion but not lobule and alveolar formation as the ovarian follicular hormone (theelin) has had insufficient time or is in too low a concentration to stimulate development of the duct system to that point where the epithelium can undertake lobule formation.

In the mammary gland of hyperthyroid pseudo-pregnant animals given 50 rat units of the anterior pituitary-like sex hormone (Antuitrin "S") definite lobule and alveolar formation is present with abundant secretion. This does not fit in well with the above given theory unless one assumes that the excess amount of

Antuitrin "S" caused marked corpora lutea formation which in turn formed more of the follicular hormone (theelin) than usual along with its luteal hormone (progestin). The amount of theelin formed would thus mature the duct system so that the epithelium could then go on and form alveoli. The feeding of thyroid gland alone to ovariectomized rats, causes no change in the mammary gland other than reduction in amount of fat present. This proves that a hyperthyroid condition alone in the absence of ovarian function will not cause mammary hyperplasia. When the ovarian follicular hormone (theelin) was given to the hyperthyroid ovariectomized rats no hyperplasia was noted. But when both the follicular hormone (theelin) and the corpus luteal hormone (lipo-lutin) were given to the hyperthyroid animals definite hyperplasia of the duct system and lobule and alveolar formation was revealed, although no vacuolisation of the duct epithelium was noted. Possibly the proportions were not the same as those occurring in the intact hyperthyroid animal. Further work is being done on this point. Injection of the ovarian follicular hormone (theelin) into hyperthyroid pseudo-pregnant animals caused an immediate estrus with resulting regression of the mammary gland structure. Proving that an excess amount of the follicular hormone is not compatible with a pseudo-pregnant condition. Injection of the ovarian follicular hormone (theelin) into normal lactating rats caused a rapid regression in function of the mammary gland with atrophy of the alveolar structure leaving large ducts having markedly hyperplastic epithelium which revealed frequent mitotic figures. Some areas of the duct epithelium have almost the appearance of

a malignant process. This abnormal hyperplasia of the duct epithelium with cessation of function and atrophy of the alveoli may be due to diminution of the secretion of the anterior pituitary sex hormone caused by the large amount of ovarian follicular hormone, which is antagonistic to the function of the anterior pituitary gland. The cessation of anterior pituitary action would result in atrophy of the corpora lutea leaving a large unapposed amount of the ovarian follicular hormone (theelin) which would in turn cause a marked stimulation of the duct system with hyperplasia of the epithelium. When the anterior pituitary-like sex hormone (Antuitrin "S") was injected into lactating animals no significant mammary changes were noted. This is compatible with the theory that the anterior pituitary-like sex hormone (Antuitrin "S") stimulates the formation of corpora lutea allowing a persistent corpora luteal hormone (progestin) stimulation of the breast. An animal was maintained in continual lactation nursing young for eight weeks when given small doses of the anterior pituitary-like sex hormone (Antuitrin "S") possibly because the corpora lutea were maintained at a functioning level. When the galactophores were cut producing an artificial stasis the operated gland was found to continue milk secretion as long as the other normal glands were nursed. However, when all nipples were removed, thus preventing nursing, milk secretion stopped in a few days. This shows that there must also be a nervous stimulae from the nipple causing or maintaining lactation. Selye (1934) believes that there is a definite nervous stimuli to the anterior pituitary gland, from the nipple, causing it to continue the formation of both the sex hormone, which

keeps the corpora lutea body at a functioning level, and of the lactation hormone. Injections of the ovarian follicular hormone (theelin) into animals having the galactophores cut produced the same picture as that found in the normal lactating gland with follicular hormone (theelin) administration. This does not completely agree with the work of Adair and Bagg (1925) who believe that chronic breast stasis will eventually cause a malignant epithelial hyperplasia. However I have not carried these studies over a sufficient period of time to note the cummulative effects. Further studies are being carried on at present, with the animals being treated both continually and intermittantly.

Adair (1934) points out the fact that, women who have had one or more children and have not nursed them properly show a greater incidence fo both benign and malignant breast tumors, than do women who have nursed their children normally. He believes that the abnormal hyperplasia is due to some substance or substances formed in the stagnate breast secretions. I believe that this fact can be explained as due to an endocrine imbalance rather than some irritative substance formed in the stagnate breast secretions. When a woman fails to nurse the normal nervous stimuli from the nipple to the anterior pituitary gland is not initiated. Lacking this stimuli the pituitary gland fails to form an adequate amount of the sex hormone (Prolan B) to keep the corpus luteal body in a functioning stage. With cessation of the action of the corpus lutea the ovarian follicles begin to develope with resulting formation of the follicular hormone (theelin). Since the corpus luteal

hormone (progestin) normally apposes the formation of the follicular hormone, this balance is upset and the follicular hormone (theelin) is allowed to act unapposed for a time. Therefore without the luteal hormone (progestin) the lobular and alveolar portion of the breast will undergo a sudden abnormal regression and the duct portion will be greatly stimulated by the excess follicular hormone. This process in the already hyperplastic lactating breast could so easily become a lawless progressive growth of epithelium. The excess formation of the follicular hormone (theelin) would continue for a time until it in turn stimulate the pituitary gland to the production of the anterior pituitary sex hormone, which would slowly balance the follicular hormone by causing normal luteal development, allowing the normal estrus cycle to be restarted. Experimental work investigating this theory further is in progress at the present time and will be reported in other papers in the future.

PLATE VII

Fig. 1 Nipple from rat in estrus. Note desquamation and cornification of duct epithelium.

Fig. 2 Vaginal mucosa of rat in estrus. Note marked cornification with superficial stratum corneum and desquamated cornified epithelium.

Fig. 3 Mammary gland from rat in estrus. Note numerous small ducts with epithelial buds.

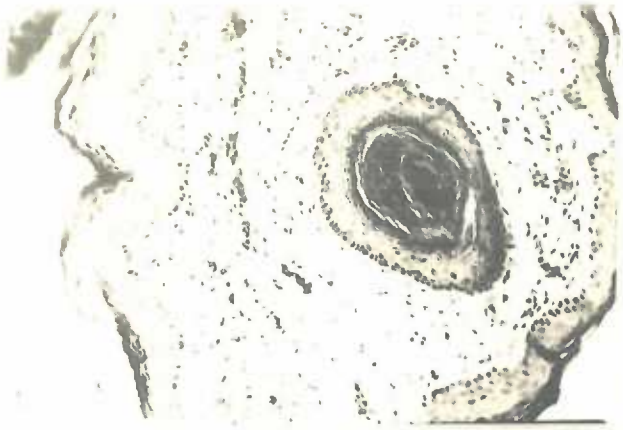
Fig. 4 Vaginal mucosa from animal in metoestrus. Note low epithelium with complete loss of stratum corneum.

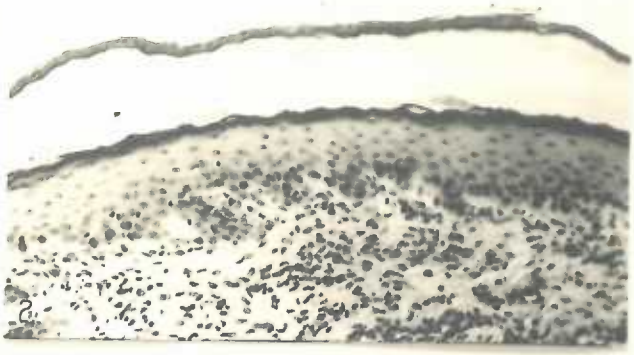
Fig. 5 Mammary gland from rat in metoestrus. Note decrease in number and size of ducts with absence of epithelial buds. Compare with Fig. 3 above.

Fig. 6 Nipple from rat in pro-estrus stage. Note the piling up of epithelium and slight squamous character.

Fig. 7 Vaginal mucosa from rat in pro-estrus. Note high epithelium with beginning cornification of the stratum corneum.

Fig. 8 Mammary gland from rat in pro-estrus. Note increase in branching of ducts and thickness of epithelium. More than in Fig. 5 and less than Fig. 3.

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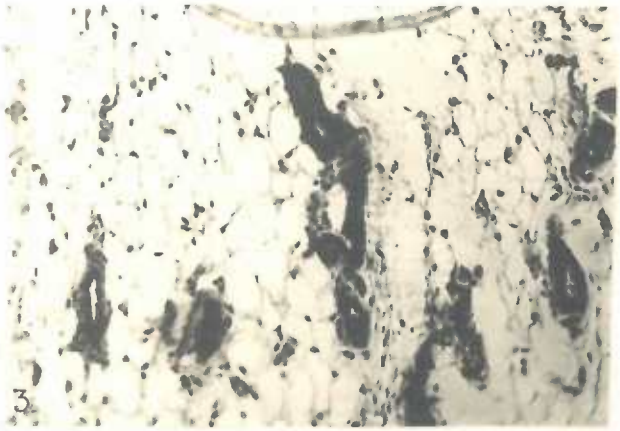
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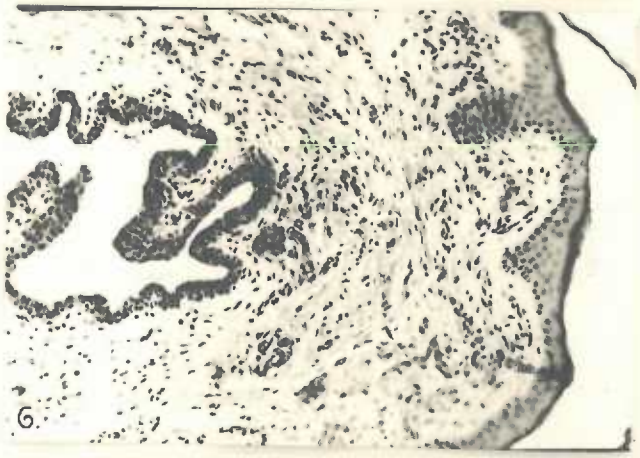
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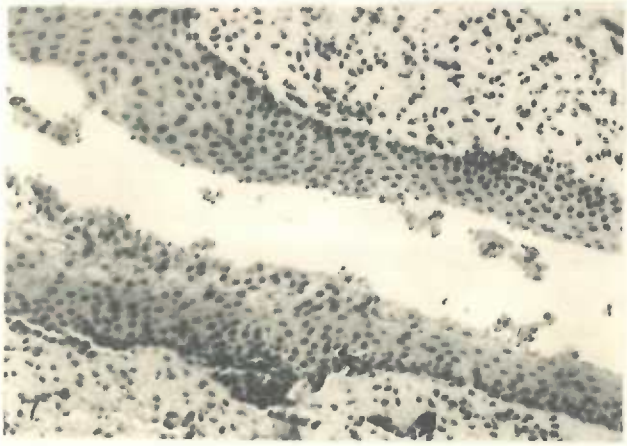


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PLATE VIII

Fig. 1 Nipple from rat in diœstrus. Note thin layer of duct epithelium and numerous leucocytes.

Fig. 2 Vagina from rat in diœstrus. Note thin layer of epithelium with slight squamous transformation.

Fig. 3 Mammary gland from rat in diœstrus. Note decrease in number of ducts and size of epithelium.

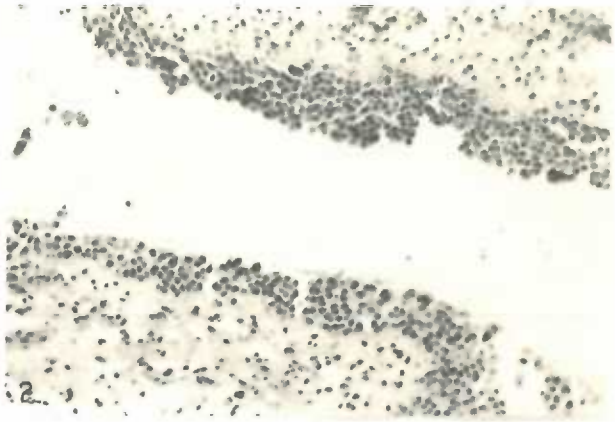
Fig. 4 Mammary gland of virgin female given Theelin. Note prominence of epithelial buds.

Fig. 5 Mammary gland of virgin female given Antuitrin "S". Note duct proliferation and lobule formation with vacuolization.

Fig. 6 Mammary gland of ovariectomized virgin female rat given Theelin and Lipo-Lutin. Note lobule formation with vacuolization.

Fig. 7 Mammary gland of ovariectomized virgin female rat given Lipo-Lutin. Note absence of lobule and alveolar formation. Compare with Fig. 6.

Fig. 8 Mammary gland of hyperthyroid female rat, pseudo-pregnant for 9 days. Note increase in duct branching and budding but no definite alveoli. At a later stage vacuolization was present.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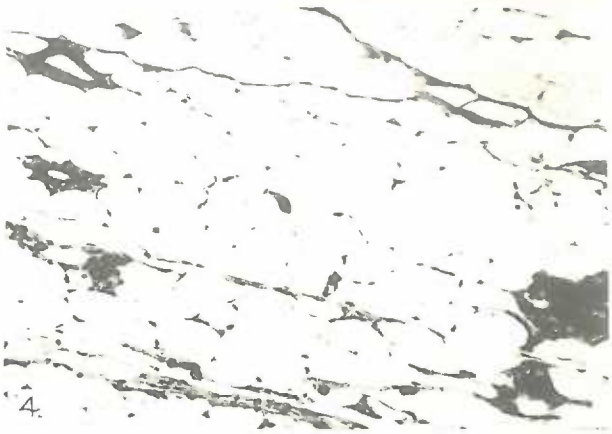
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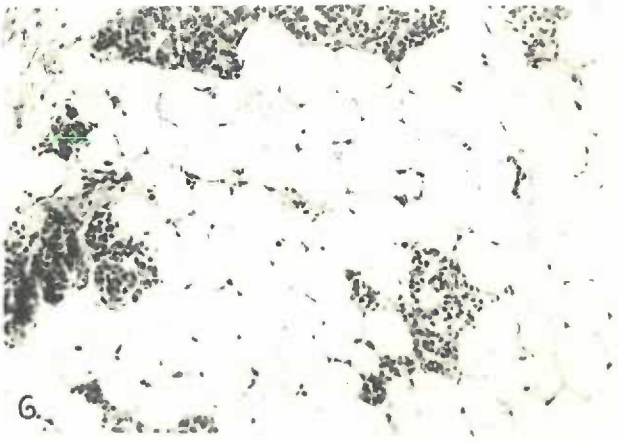
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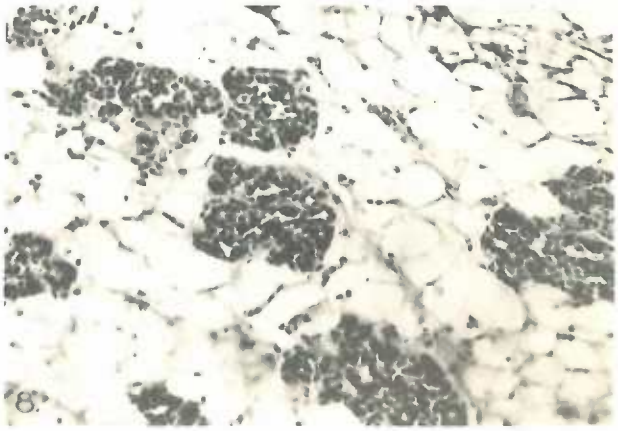
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PLATE IX

Fig. 1 Mammary gland of hyperthyroid female rat, pseudo-pregnant for 7 days and receiving Antuitrin "S". Compare with Plate VIII, Fig. 8 and note similarity.

Fig. 2 Mammary gland from ovariectomized female rat, 28 days after operation. Similar to normal animal in dioestrus.

Fig. 3 Mammary gland from ovariectomized female rat fed thyroid for 21 days. Note diminution in fat. Gland structure similar to normal ovariectomized animal.

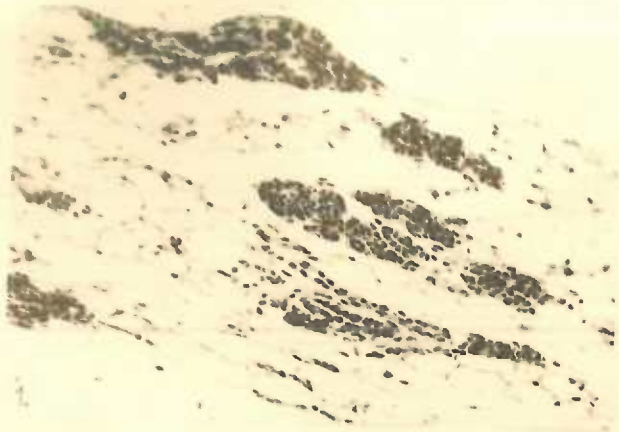
Fig. 4 Mammary gland from ovariectomized female rat given Theelin. Duct system equal to that of normal gland in estrus. See Plate VII.

Fig. 5 Mammary gland from hyperthyroid pseudo-pregnant female rat given Theelin. Note regression and compare with Plate VIII, Fig. 8.

Fig. 6 Mammary gland from ovariectomized virgin female rat given Antuitrin "S". No development beyond normal animal in dioestrus, but they are more prominent than an untreated spayed animal.

Fig. 7 Mammary gland from hyperthyroid pseudo-pregnant female rat given Theelin. Note more marked regression than in Fig. 5.

Fig. 8 Mammary gland of hyperthyroid pseudo-pregnant female rat receiving Antuitrin "S". Note increase in fibrous tissue about duct.



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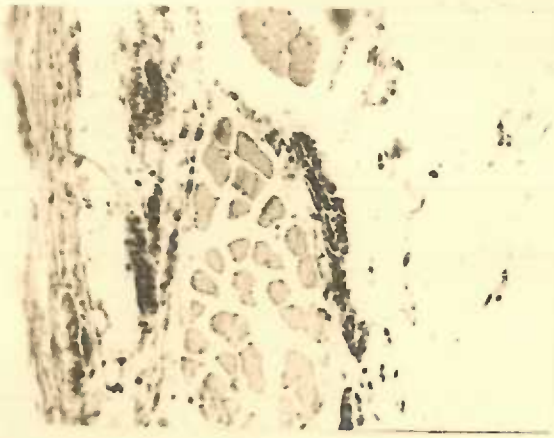

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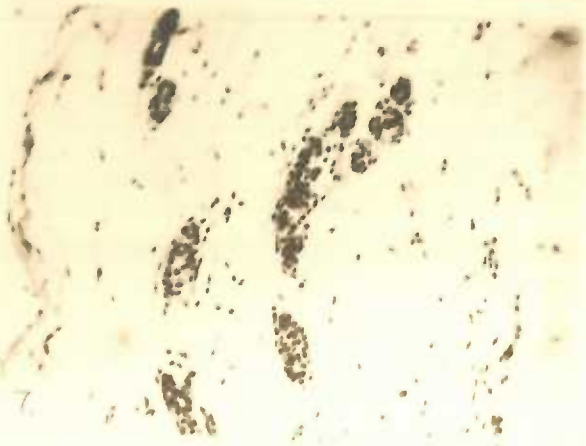

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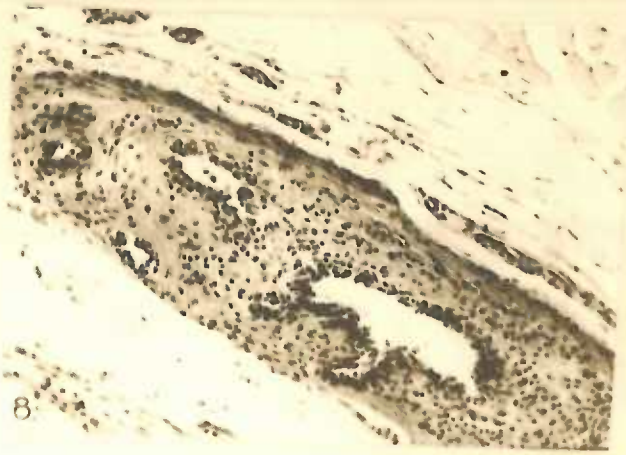

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PLATE X

Fig. 1 Mammary gland from ovariectomized female rat given Theelin and fed thyroid at same time (14 days). Changes similar to spayed animal given theelin alone.

Fig. 2 Same as above only given Theelin and thyroid for 28 days. Note similarity to spayed animal given Theelin alone.

Fig. 3 Mammary gland from normal lactating female rat 24 hours post partum. Note alveoli filled with secretion and single layer of epithelium.

Fig. 4 Mammary gland from normal lactating female rat 2 weeks post partum. Gland still functioning.

Fig. 5 Same as above only 1 week post partum. Note alveoli filled with milk.

Fig. 6 Mammary gland from normal lactating female rat given Theelin. Note epithelial hyperplasia with desquamation of cells. One week post partum.

Fig. 7 Same as above only 2 weeks post partum. Much more regression of mammary function with proliferation of duct epithelium. Theelin for 2 weeks.

Fig. 8 Mammary gland from hyperthyroid, 15-day pseudo-pregnant, female rat given Antuitrin "S" (total of 50 rat units). Note marked alveolar and lobule formation with secretion.


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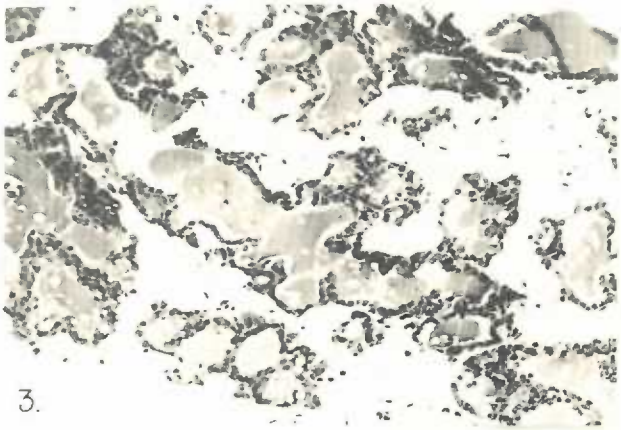
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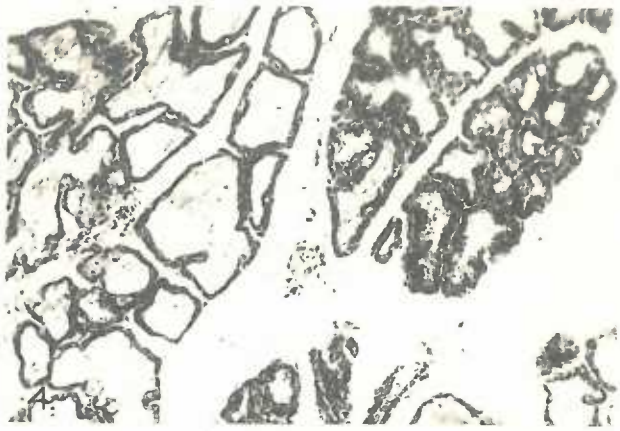
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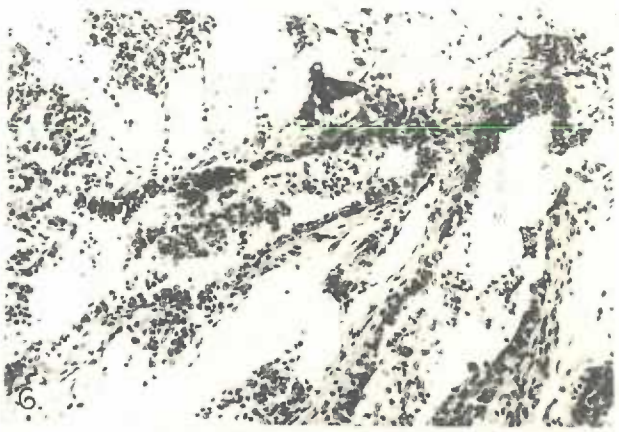
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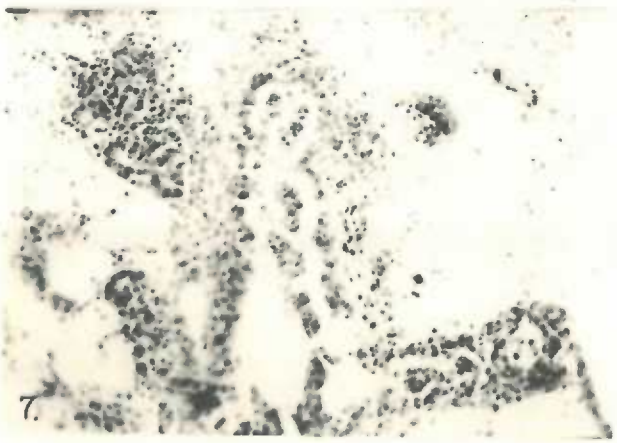
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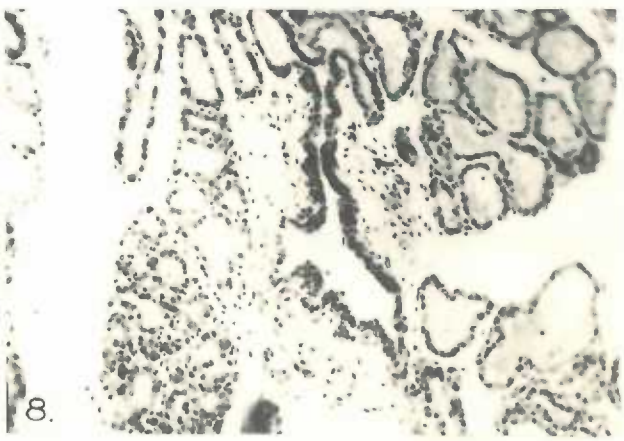
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PLATE XI.

Fig. 1 Mammary gland from normal lactating female rat 48 hours after weaning. Secretion still present in functioning gland. Vacuolization of material in alveoli is marked.

Fig. 2 Mammary gland with cut galactophores from a lactating female rat, 4 weeks post partum. Shrinkage in size of alveoli with atrophy of secretory epithelium.

Fig. 3 Same as above only 5 weeks post partum. Marked regression of glandular tissue with increase in fibrous tissue. Still some dilated ducts.

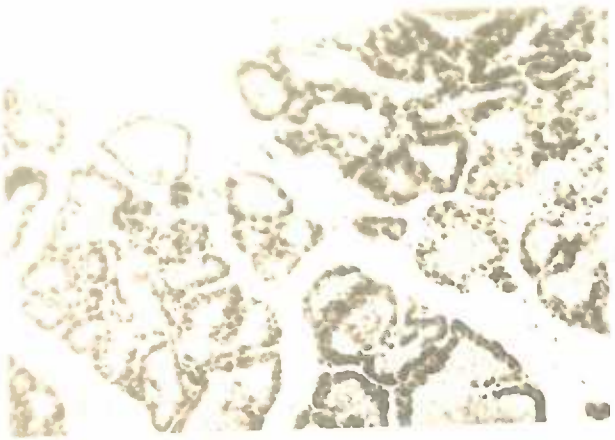
Fig. 4 Mammary gland with cut galactophores from a lactating female rat given Theelin 1 week. Note regression in size of alveoli compared with uninjected animal. Also thickness and hyperplastic character of epithelium.

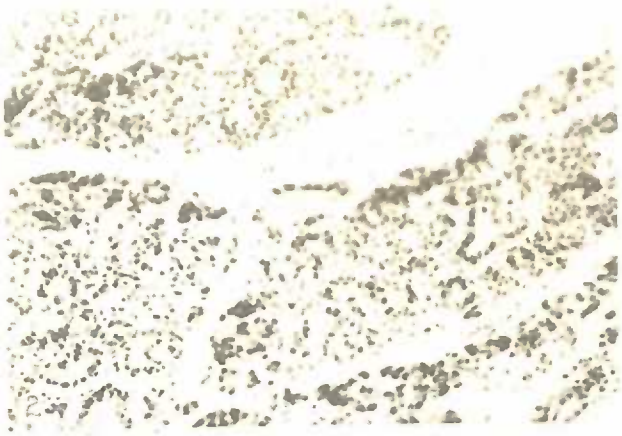
Fig. 5 Mammary gland from lactating female rat given Antuitrin "S". One week post partum. No change from normal lactating animal.

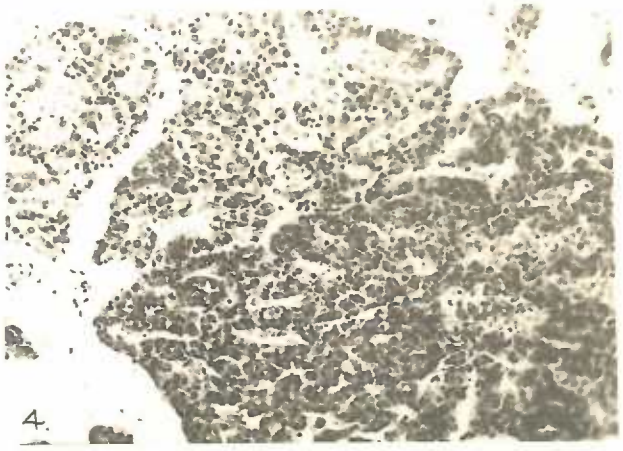
Fig. 6 Mammary gland from lactating female rat given Theelin 1 week. Note decrease in amount of glandular tissue and dense cellular structure.

Fig. 7 Same as above but after 2 weeks of Theelin. Note marked regression. Few ducts still contain secretion.

Fig. 8 Mammary gland of lactating female rat given Antuitrin "S" for 2 weeks. No apparent change differing from an uninjected animal 2 weeks post partum.



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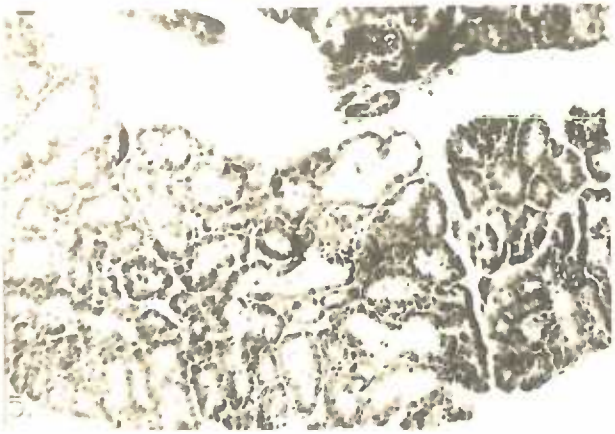

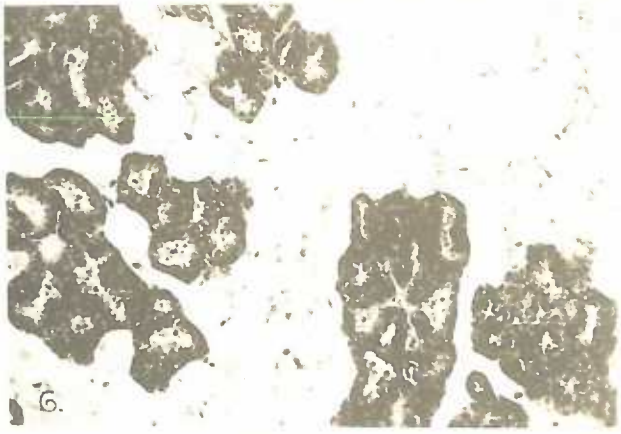
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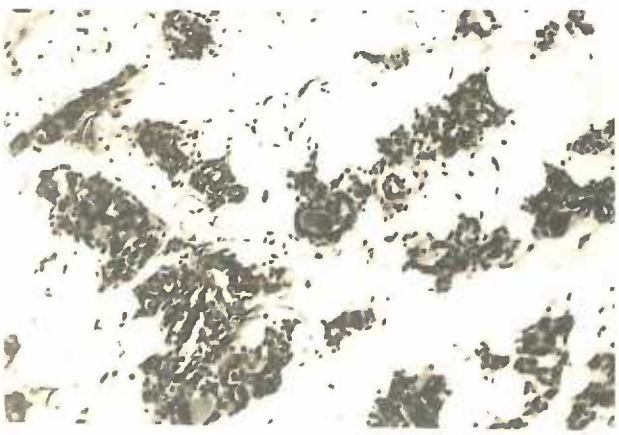
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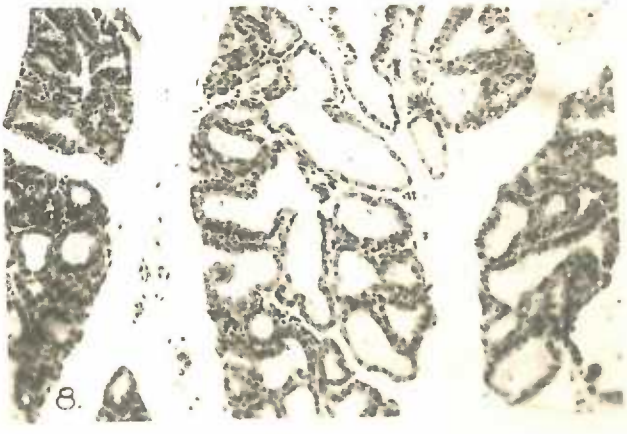
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PLATE XII

Fig. 1 Mammary gland from lactating female rat 1 week post partum. Normal control for Fig. 2.

Fig. 2 Mammary gland with cut galactophores from a lactating female rat 1 week post partum. Note dilatation of alveoli and ducts with atrophic changes in epithelium.

Fig. 3 Mammary gland with cut galactophores from a lactating female rat after 1 week of Theelin and 1 week post partum. Note regression in size of alveoli with increase in number of epithelial cells. Hyperplastic type of growth.

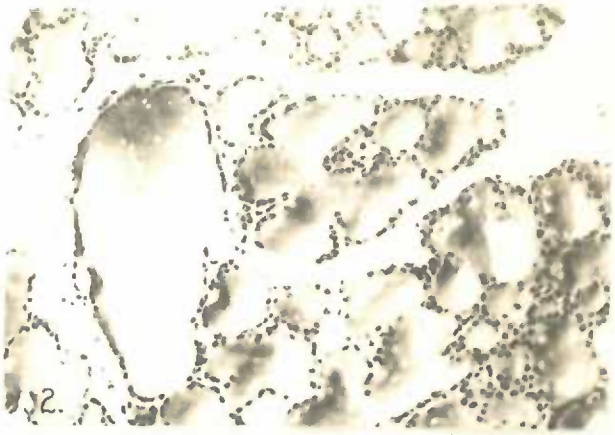
Fig. 4 Same as Fig. 2 only 2 weeks post partum.

Fig. 5 Ligation of the nipple caused obliteration of the ducts with hyperplasia of the epithelium. Lactating rat.

Fig. 6 Mammary gland with cut galactophores from lactating female rat after 3 weeks of Theelin. Note absence of all secretory epitholium with dense cord-like ducts.

Fig. 7 Same as above only after 2 weeks of Theelin. Note absence of alveoli with hyperplastic growth of duct epithelium.

Fig. 8 Mammary gland with cut galactophores from a lactating female rat, three weeks post partum. Most of alveoli are empty with atrophic walls.



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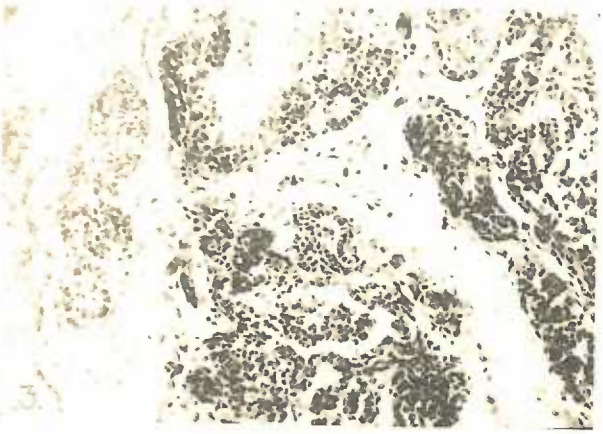

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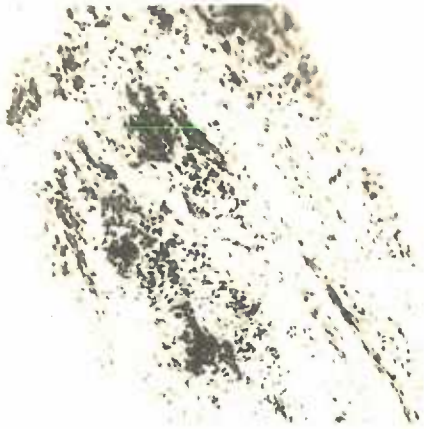

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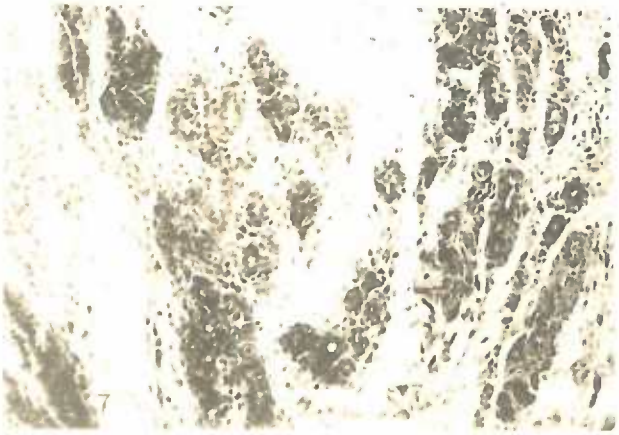

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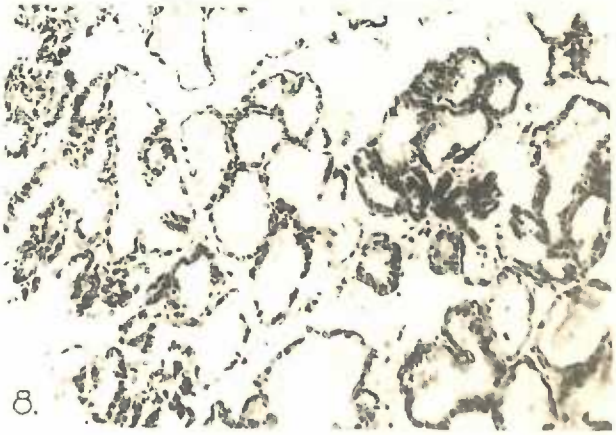

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