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A Study of the Causes of the Normal Development of the Mammary Glands of the Albino Rat

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PREFACE

The growth and development of the udder of heifers pregnant for the first time is a matter of great interest to breeders of dairy cattle. Will the udder be small and ill shaped or large and symmetrically proportioned? Of even greater importance than the external conformation is the extent of gland tissue developing in the udder. The growth of secretory tissue may be limited, resulting in small future milk production; or extensive, resulting in large production.

It is a natural question to ask—what causes the udders of some heifers to remain small and others to grow large? One answer would be the difference in the inheritance received from their sires and dams. While that answer is correct as far as it goes it does not indicate the nature of the factors which cause the variation in the growth of the udder and the secretion of milk.

While much work still remains to be done, encouraging progress has been made during the past few years in obtaining an answer as to the physiological questions involved.

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ABSTRACT

Continuing a study of the hormones concerned in the development of the mammary gland and the initiation of milk secretion, there is reported a detailed anatomical study of the normal development of the gland of the albino rat. This is followed by studies of the influence of the estrus-producing hormone, corpus luteum extracts, and an extract from the anterior pituitary.

It was found that small doses of the estrus-producing hormone produced only duct growth, while larger doses caused some lobule proliferation. Corpus luteum extracts alone caused no changes in the mammary gland of the albino rat. Corpus luteum extracts, when injected with large doses of the estrus-producing hormone (5 r. u. or more) produced lobule proliferation greater than that produced by the estrus-producing hormone alone; but not equal to that characteristic of a gland of a rat in advanced pregnancy.

Thus far the extracts of the anterior pituitary of sheep have been ineffective.

A Study of the Causes of the Normal Development of the Mammary Glands of the Albino Rat

C. W. Turner and A. B. Schultze*

During recent years much study has been directed toward an inquiry into the nature and sources of the stimuli causing the characteristic developmental changes of the mammary glands. The most extensive and definitely controlled work on the effect of hormones on the mammary gland has been carried on with the rabbit as the experimental animal.

Possibly there exist differences in the physiological reaction of various species to certain hormones. That such may be the case is indicated by the work of Allen (1931) in a study of the comparison of endometrial changes in the uterus of the rat with those of the rabbit. He found that the changes characteristic of pregnancy or pseudo-pregnancy in the rabbit were not similar to the endometrial changes in the rat. The possibility that there are differences in the relation between the hormones which stimulate the changes in the mammary glands of the rabbit from those acting on the mammary glands of other animals, makes desirable a comparative study of the mammary gland. Further, a study of variations between different experimental animals may lead to the discovery of fundamental facts which would remain unobserved were only one experimental animal used.

For this reason a study of the hormones which have been shown to be effective in producing changes in the mammary glands of the rabbit was initiated using the albino rat as the experimental animal.

REVIEW OF LITERATURE

Studies on the Causes of Mammary Gland Growth

Before 1900 the causes of mammary gland growth were thought to be due to nervous stimuli. This theory was discarded as a result of experiments carried out by Goltz and Ewald (1896) and others.

After severing the nervous connections to the pelvic organs in a bitch, they found that normal pregnancy development followed by parturition and lactation occurred as in the normal animal. Ribbert (1898) and Pfister (1901) observed that normal

*The data presented in this paper formed part of a thesis presented by A. B. Schultze in partial fulfilment of the requirements for the Degree of Master of Arts in the Graduate School of the University of Missouri, 1931.

growth of the mammary gland occurred when the gland tissue was transplanted into the skin of the ear. The results of these experiments led to the theory that the stimulus causing mammary gland development is in the nature of hormones circulating in the blood stream.

Lane-Claypon and Starling's (1906) experiments with injections of aqueous extracts of placenta, ovaries, and fetuses were the first to attempt to determine the source of the hormones necessary for gland growth. Very slight results were obtained in these early experiments where aqueous extracts were used. In 1912 Iscovesco discovered that more potent extracts of hormones could be obtained by lipoid solvents.

The introduction of the rat unit test for the estrus-producing hormone by Allen and Doisy (1923) made the further study of specific hormones possible. More recently extracts of the corpus luteum have been prepared by Allen (1930) which cause the "progestational proliferation" of the rabbit uterus. Hisaw, Fevold and Meyer (1930) have extracted a water soluble hormone that has similar physiologic properties. These fundamental studies on hormones of the ovary make possible a more accurate determination and relationship of these hormones to the growth of the mammary gland.

EXPERIMENTS WITH THE ALBINO RAT

Effects of Various Extracts.—Experimental studies of the hormones stimulating the growth of the mammary gland in the albino rat are considerably less extensive than of other experimental animals. Results in the rat have been less definite and clear cut than in the rabbit. Loeb (1923) states that other than during pregnancy no noteworthy growth of the mammary gland in the rat has been noted. This, he says, corresponds to the fact that the lutein phase of the sexual cycle is lacking in the rat. The fact that various tissue extracts are so much less efficient in the rat than in the rabbit indicates that there may exist a greater inertia on the part of the mammary tissue in the rat.

Frank and Unger (1911) failed to produce any changes in the mammary gland in white rats by the injections of aqueous extracts of fetuses. However, injections of ovarian and hypophyseal extracts caused considerable increase in gland development. They concluded from these experiments that the ovarian cycle bears a close relation to gland growth and that the persistent corpus luteum of pregnancy is probably an important factor in gland development.

In studies on the effects of placental and corpus luteum extracts on castrated rabbits and rats, Frank and Rosenbloom (1915) found only slight effects on the mammary gland. Extracts of the corpus luteum substance of pregnant animals appeared to be more effective than those of non-pregnant animals.

In work on rats and mice in determining the action of the ovarian follicle hormone (estrus-producing hormone) Allen, Francis, Robertson, Colgate and Johnston (1924) observed that during artificially induced sexual cycles changes occurred in the mammary glands.

Laqueur, DeJongh and Tausk (1927) report an increase in the size of the mammary glands of rats, both males and females, with subcutaneous and intraperitoneal injections of menformon (estrus-producing hormone). Control animals not injected or injected with NaCl solutions showed much less gland growth than the animals injected with menformon. The treated male glands showed thicker ramifications and more numerous small sprouts; but the exact nature of the growth, whether duct or alveolar formation characteristic of pregnancy, is not stated. The animals were not castrated. The injections were continued over a period of three weeks.

Effects of Artificially Induced Pseudo-Pregnancy—Pseudo-pregnancy caused by mechanical stimulation of the cervical canal (Long and Evans 1922) was observed to cause changes in the mammary gland of the albino rat with advance of the pseudo-pregnant condition (Freyer and Evans 1923). The gland was found to resemble the pregnant gland at about the eleventh day of pregnancy. There was a multiplicity of small twig-like buds and the beginning of true alveolar clusters.

Effects of Ovarian Transplants on the Male Gland.—Ovaries grafted in 184 day old male rats resulted in increased glandular development (Engle 1928). By subsequently giving daily transplants of the anterior lobe of the hypophysis ovarian grafts were greatly increased and large follicles and in many cases, lutein tissue, was formed. In controls that received pituitary transplants but no ovarian grafts, the mammary gland showed less extensive growth.

Effect of Anterior Pituitary Hormones.—Evans and Simpson (1929) observed that the mammary glands of the adult virgin rat underwent some hyperplasia after a month of daily treatment of alkaline extracts of the anterior hypophysis. By more chronic treatment with these extracts true milk secretion could be brought about. In 24 day old female rats, two daily implants of anterior hypophysis resulted in increased complexity of the mammary tree when observed on the fourth day. They state that the mammary

gland growth was always more marked in cases in which corpora lutea were coincident with hypertrophied follicular apparatus.

Recent Studies on the Rabbit

Work by Turner and Frank (1930) confirmed by Parkes (1930) shows that with the injection of the estrus-producing hormone alone, the production of growth characteristic of continued estrus is obtained in castrate male and female rabbits. Alveolar growth characteristic of pregnancy could not be induced.

By the simultaneous injection of both the estrus-producing hormone and a corpus luteum extract in castrate male and female rabbits, Turner and Frank (1931) were able to produce the growth of the mammary gland characteristic of pregnancy. The effect of the estrus-producing and corpus luteum hormone was thus shown to bear the same relation to mammary gland growth as the two bear in their action on the uterus to produce "progestational proliferation" in the rabbit. (Hisaw and Leonard 1930). Administration of corpus luteum hormone alone failed to produce any changes in the gland (Corner 1930) Turner and Frank (1931).

Normal Development of the Mammary Gland in the Albino Rat

To critically study the effect of hormones on the development of the mammary gland in experimental animals, it is essential to understand the anatomic changes during normal development. In the animal studied here (the albino rat) the normal development may be divided into the following phases.

1. Embryonic and fetal development, which includes development from the first appearance of the mammary line up to development at birth.

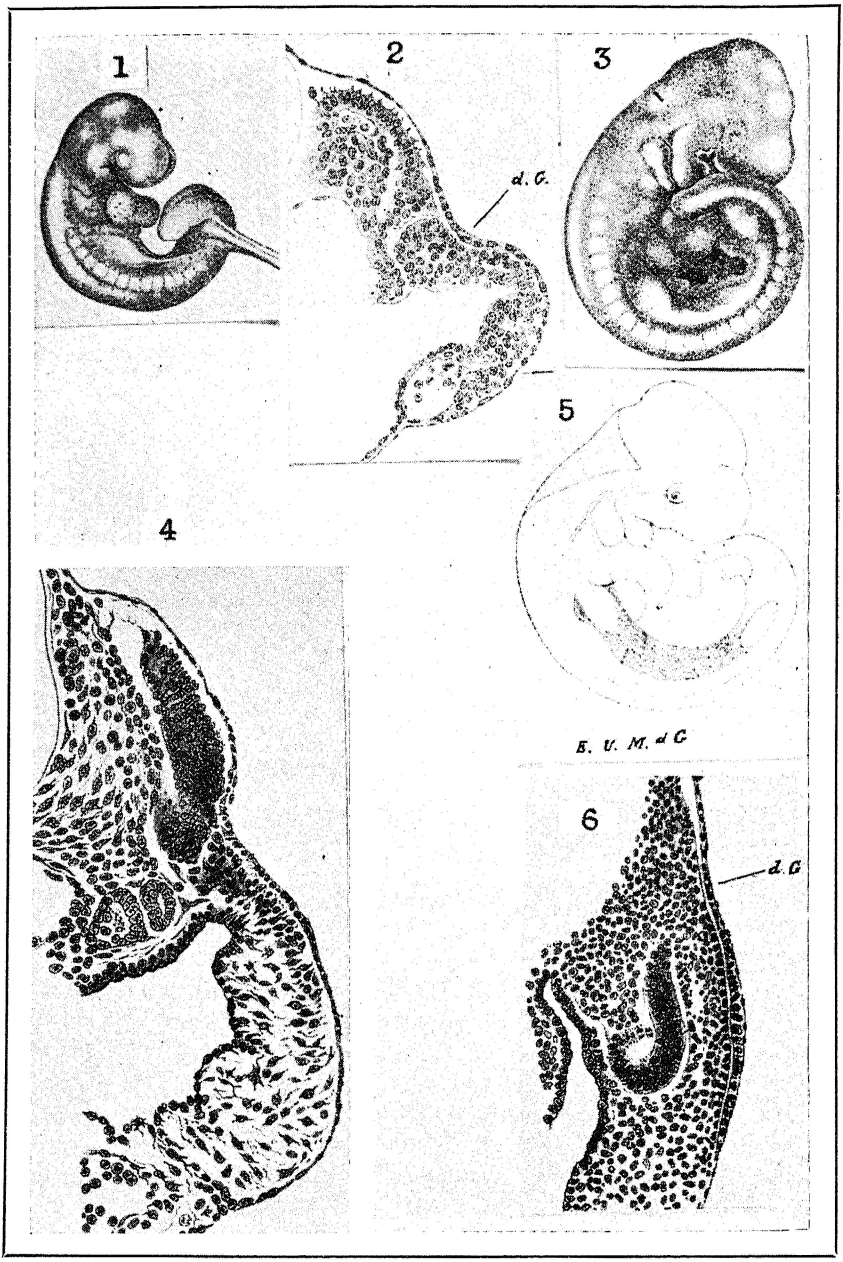
2. Development from birth to puberty. During this period there is an extension and increase in duct growth. No alveoli have appeared up to first ovulation.

3. Development during recurring estrus cycles. This phase is characterized by a cyclic change in the mammary gland following the ovarian cycle.

4. Development during pregnancy. Increase in size and thickness of the gland with lobule formation, followed by milk secretion at parturition, characterizes this phase. Parturition is followed by lactation and later involution.

Embryonic and Fetal Development

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 larger cuboidal cells at the border of the trunk or body zone. The streak or line extends from the shoulder region



Figures 1 to 6 from Henneberg; (1) Lateral aspect of rat embryo 11 days old. (2) Anlage of milk streak in embryo of Fig. 1. (3) Lateral aspect of embryo 12 days old. (4) Cross section through the anlage of the milk streak in 12 day embryo. (5) Lateral aspect of 13 day, 1 hour embryo. The milk streak is indicated through darker shading. (6) Cross section through developed milk streak in 13 day, 1 hour embryo.

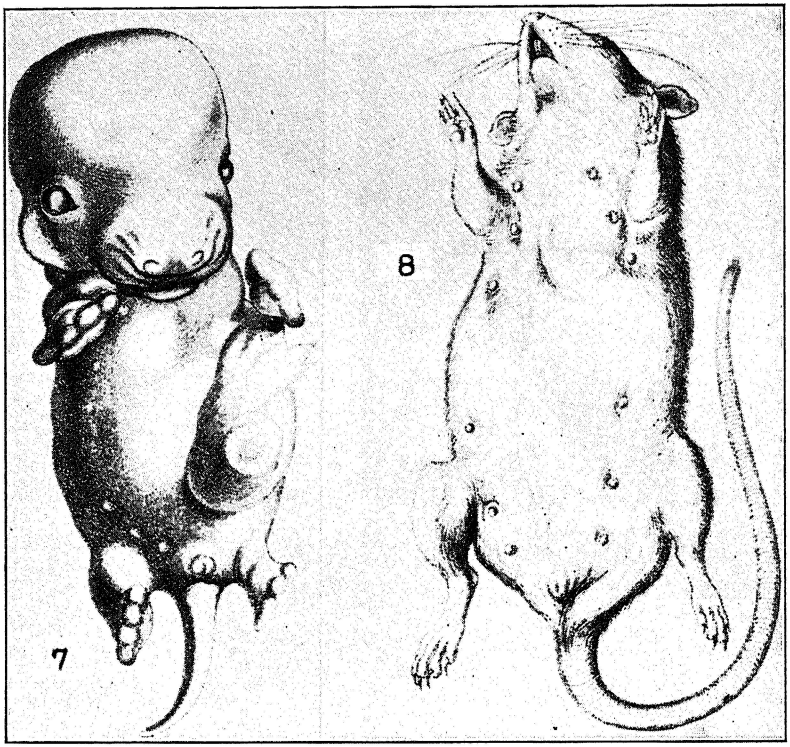
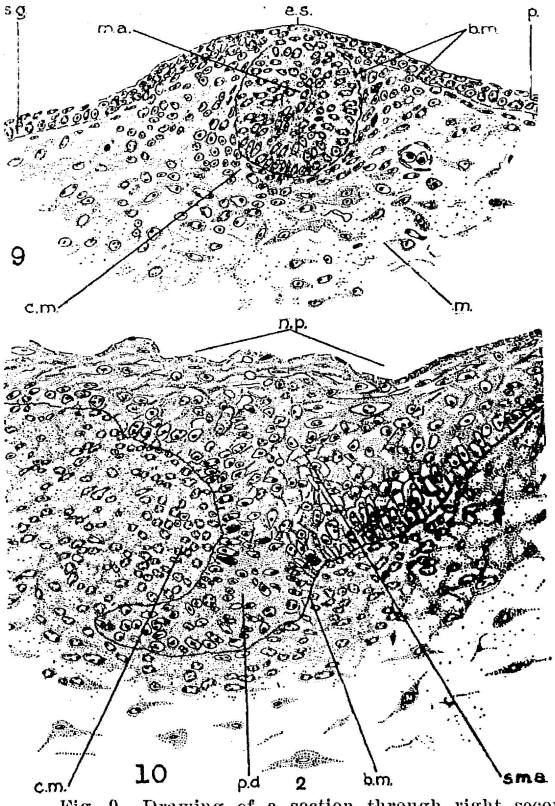


Fig. 7.—Abdominal view of the artificially stretched 15 days. 20 hour embryo with six milk-points on each side. (Henneberg). Fig. 8.—Abdominal view of a suckling rat. (Henneberg).

caudad to the inguinal region. It gradually forms a two cell layered streak in the 13-day-old embryo. The final differentiation of the mammary streak is called the mammary line. In the 14-day-old embryos the inguinal anlagen reach this stage. The gland anlagen from the milk streak in the pectoral region. In 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. The milk streak has disappeared.

Mammary bud stages.—Myers (1917) describes 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 and the Malpighian layer. This mass of cells is attached to the epidermis by a short, constricted neck.



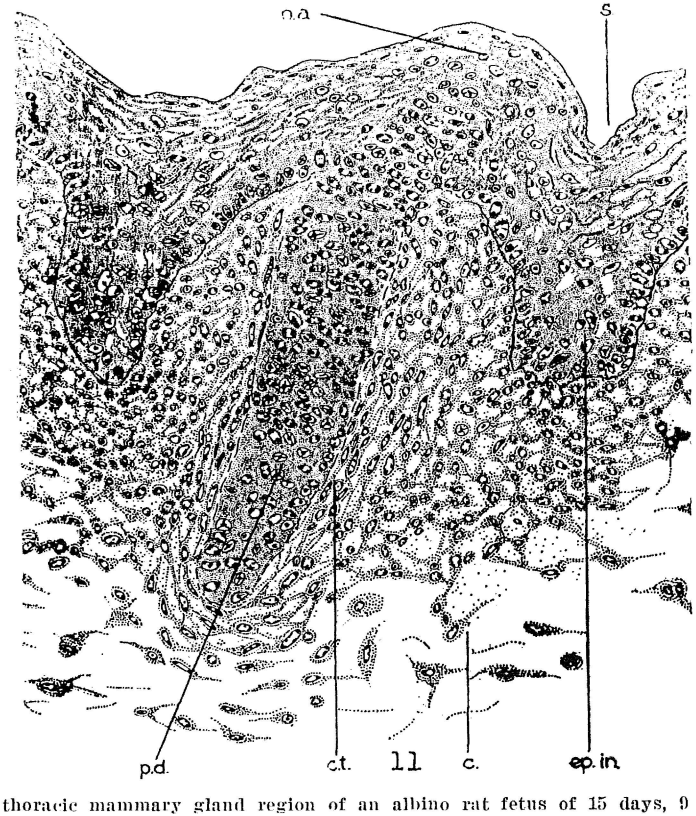
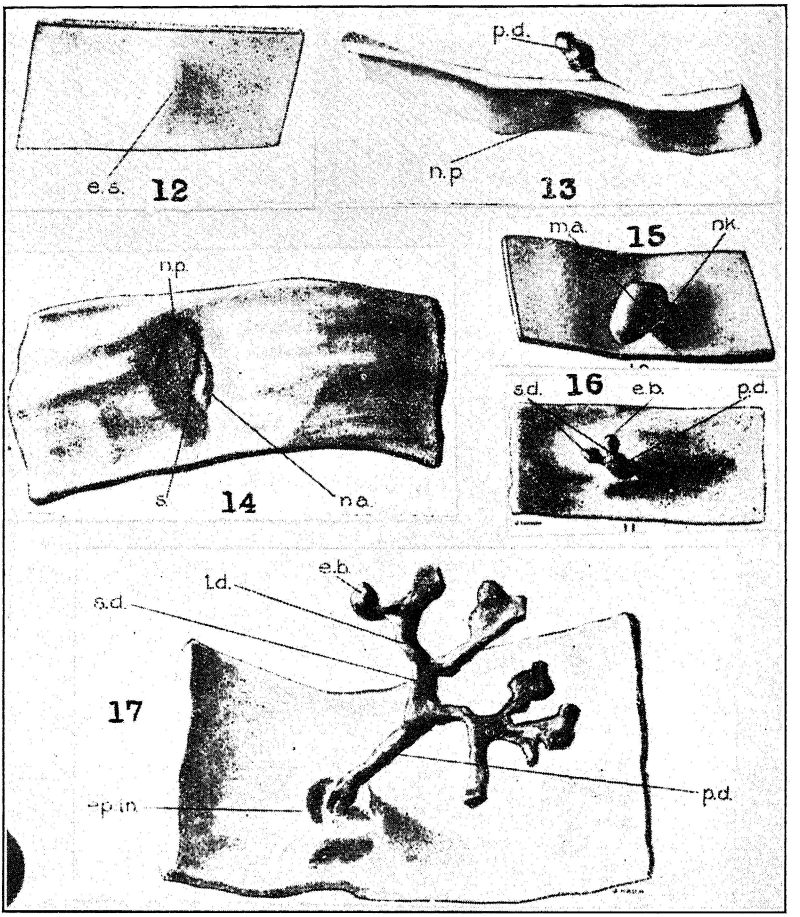


Fig. 9.—Drawing of a section through right second thoracic mammary gland region of an albino rat fetus of 15 days, 9 hours e.s., eminence (mammary hillock); m.a., mammary gland anlage. (Myers). Fig. 10.—Drawing of a section through the left second thoracic developing mammary gland of a female albino rat fetus of 18 days, 9 hours, m.p., early appearance of mammary pit; p.d., primary duct; s.m.a., mammary anlage becoming cornified. (Myers). Fig. 11—Section through the right first thoracic developing mammary gland of a female albino rat fetus of 20 days and 6 hours, c. and c.t., connective tissue cells; ep. in., epithelical hood; n.a., nipple anlage; p.d., primary duct; s., sulcus surrounding nipple anlage. (Myers).



Figures 12 to 17 from Myers: (12) A gland of an albino rat fetus (15 days and 9 hours). e.s., mammary hillock. (13) The first inguinal gland of a female albino rat fetus (18 days and 9 hours). m.p., mammary pit; p.d., primary duct anlage. (14) The left second inguinal gland of a female rat fetus (20 days and 6 hours). n.a., nipple anlage; m.p., mammary pit. (15) The right first thoracic gland of an albino rat fetus (15 days and 9 hours). m.a., mammary gland anlage . (16) The right abdominal gland of a female albino rat fetus. (18 days and 9 hours). e.b., end buds; p.d., primary duct; s.d., secondary ducts. (17) The left first inguinal gland of a female albino rat fetus (20 days and 6 hours). t.d., tertiary duct; ep. in., epithelial hood.

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 toward 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 anlage is directed caudad. The free end of each gland anlage is directed to the area which the future gland will occupy. In the male of 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 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 male fetus there is no indication of an epithelial hood.

Canalization of sprouts.—Myers (1917) noted that in the thoracic and abnominal 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 proceded 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 conspicious in postnatal stages. The cephalic glands are further developed than those more caudally located. The female gland areas are very conspicious 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 ten to fourteen. Myers (1915) observed only one supernumerary gland in an examination of one hundred rats. Schickele (1899) observed eleven teats in 6.66 per cent of the rats observed; twelve teats in 80 per cent of the cases; and thirteen teats in 13.33 per cent of the cases. Donaldson reports that Stotsenburg has noted cases where only six of the twelve 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 absence of the upper pectorals. Of the six pairs of normal glands there are three pairs of thoracic glands, one pair of abdominal glands, and two pairs 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 of the V 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 of the V 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. (Fig. 18)

The ducts of all glands except the last pair of inguinal glands are directed 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 new born the gland area in the female is evidenced by a lighter colored area (Myers 1916). There are no external indications of the mammary gland in the male.

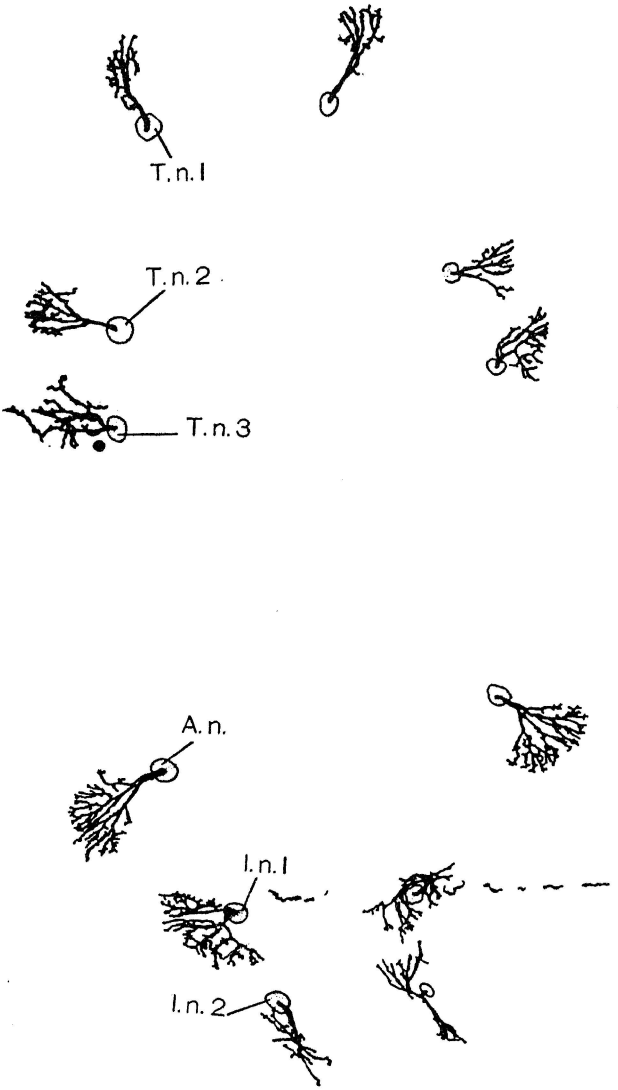


Fig. 18.—Drawn from a cleared preparation of a two weeks old albino rat (internal view) to show the general arrangement of the nipples and the branching of the mammary ducts. T.n.1., T.n.3, first, second, and third thoracic nipples; a.n.1., abdominal nipple; I.n.1., I.n.2, first and second inguinal nipples. (Myers).

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 pair. Also, there is variation between different rats of the same age and weight.

As the age advances to ten weeks there is an increase in 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 week 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 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 mid-line and the last inguinal pair, both cephalad and caudad to the vagina, meet in the mid-line. 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 9 week stage studied there is a slow increase in size of the nipple. Rein (1881) states that in a 3 week 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.

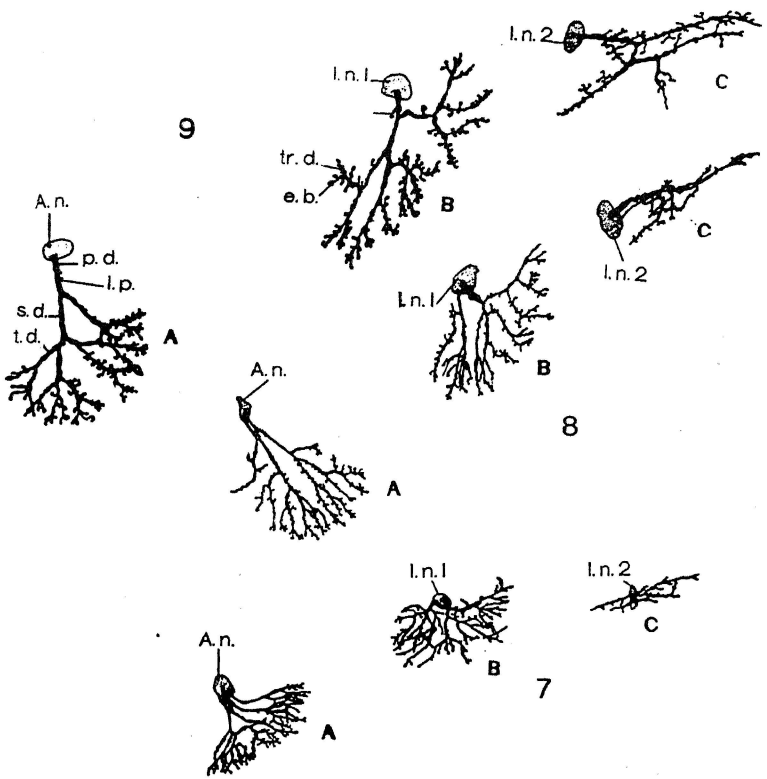


Fig. 19.—(7) Cleared preparation of an albino rat one week old showing distribution and relations of left abdominal gland (A); left first inguinal gland (B); and left second inguinal gland (C); A.n., abdominal nipple; I.n., inguinal nipple; p.d., primary duct; s.d., secondary duct; t.d., tertiary duct; tr.d., terminal duct; e.b., end bud. (8) Same as 7 but from an albino rat two weeks old. (9) Same as 7 but from an albino rat three weeks old. (Myers).

Canalization of the sprouts.—At birth a small irregular slit-like lumen is present in the primary sprout (Myers 1916), which disappears in the intra-dermal 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 Sutter (1921) shows that there is a relation between the appearance of the mammary gland and the stage of estrum. During proestrum the mammary tree exhibits long, slender branches which

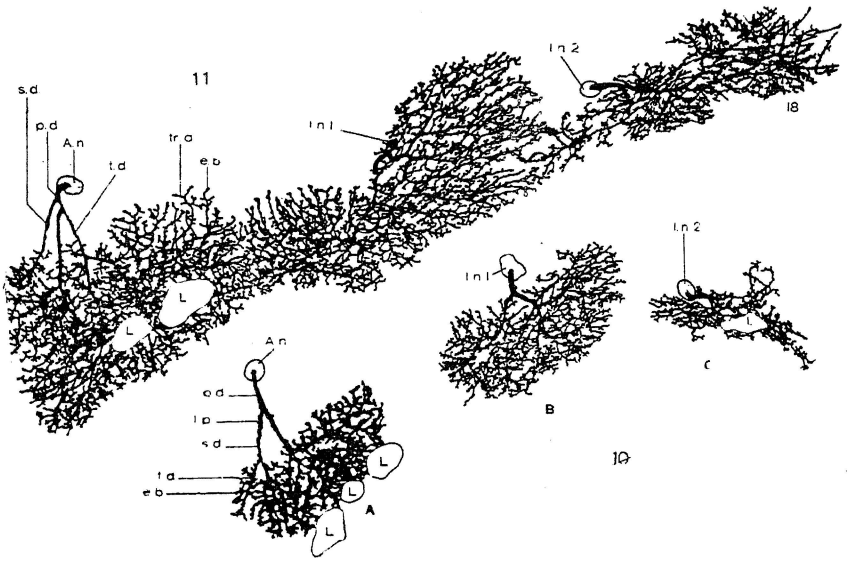


Fig. 20.—(10) Cleared preparation of an albino rat four weeks old showing distribution and relations of ducts of left abdominal gland (A); left first inguinal gland (B); and left second inguinal gland (C); L, lymph-node. (Myers). (11) Same as 10 but from an albino rat five weeks old. (Myers)

have a few almost naked twigs projecting from them. During estrum the small buds, or the 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

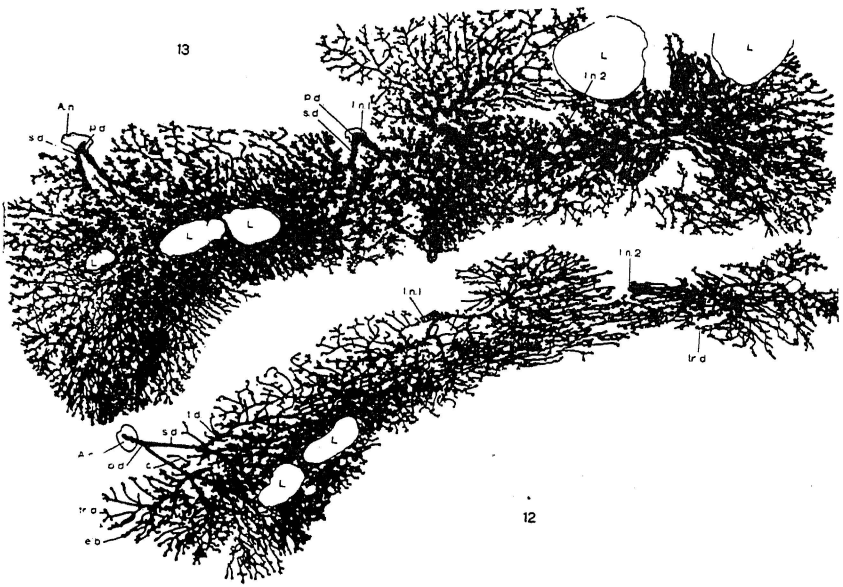


Fig. 21.—(12) Cleared preparation of an albino rat seven weeks old showing distribution and relations of ducts of left abdominal gland, first inguinal and second inguinal gland. (Myers). (13) Same as 12 but from an albino rat nine weeks old. (Myers)

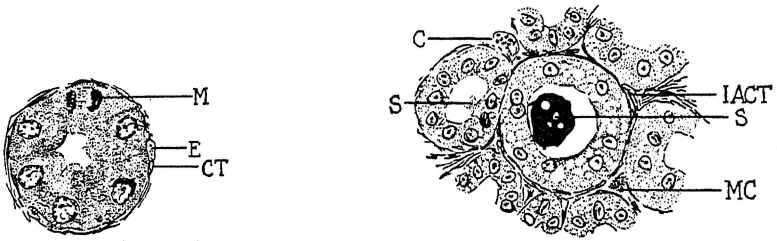


Fig. 22.—(left) Alveolus from a gland of a rat pregnant thirteen days. Connective tissue, C.T., is relatively loose. (Right) Alveoli from gland of a rat eighteen days and twelve hours pregnant. I.A.C.T., interalvolar connective tissue; S., mass of secretion; C., distended capillary. (Roberts).

the thirteenth day of pregnancy. 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 alveoli. In the latter 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.

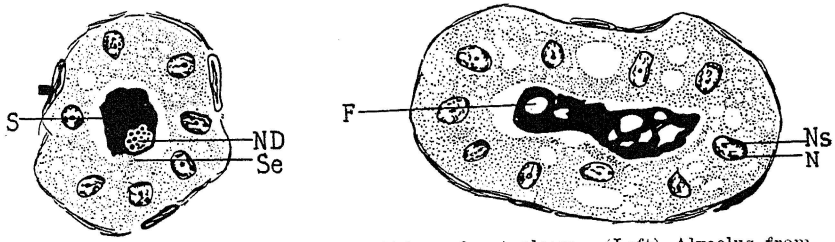


Fig. 23.—Shows vacuolated condition of cytoplasm. (Left) Alveolus from the gland of a rat eighteen days and twelve hours pregnant. S., secretion mass; Se., secretion. (Right) Alveolus from the glnad of a rat nineteen days pregnant. F., fat vacuoles. (Roberts)

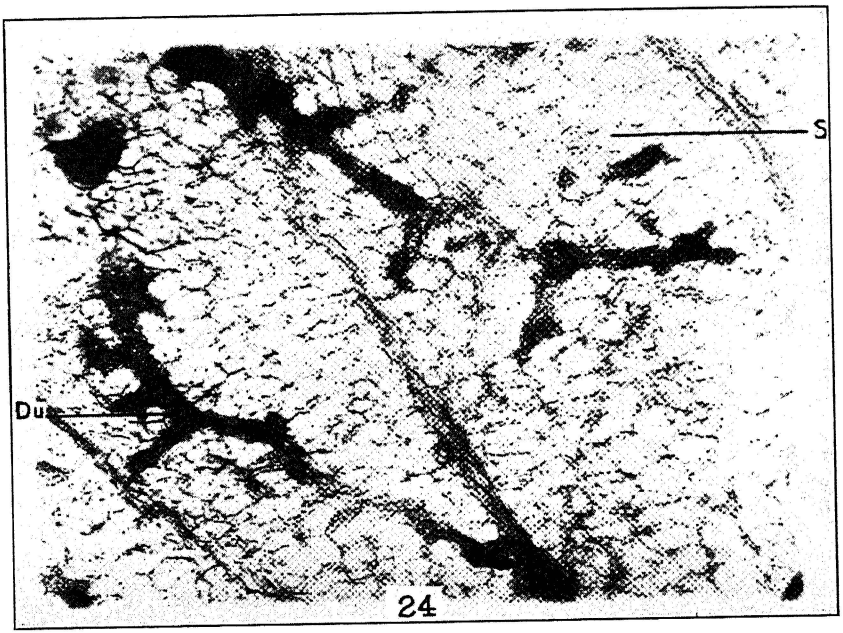


Fig. 24.—Transverse section of the mammary gland of an adult virginal rat. Du., ducts; s., fatty connective tissue stroma. (Maeder)

Vacuolization of the cytoplasm of the alveolar cells, which is indicative of secretory activity, increases from the thirteenth day of pregnancy and is 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.

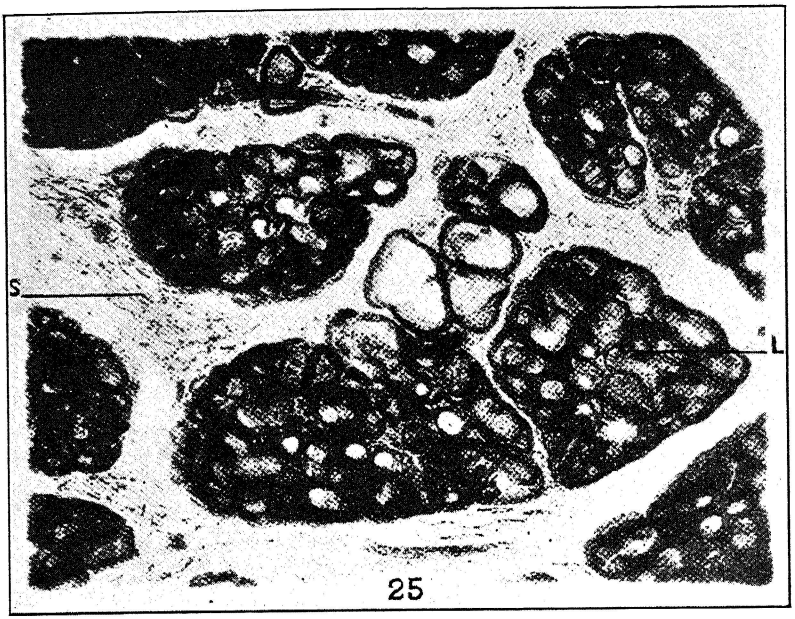


Fig. 25.—Transverse section of the mammary gland of a rat in tenth day of lactation. L., lobules; S., connective tissue septa. (Maeder)

Changes during lactation and involution.—The macroscopic aspect of the mammary gland throughout lactation remains unchanged and is the same as at the end of pregnancy (Maeder 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 twenty-four hours of lactation, but that the young invariably 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 Maeder to occur until the eighth day after weaning, when the stroma once more exceeds the parenchyma in amount. At the ninth day the

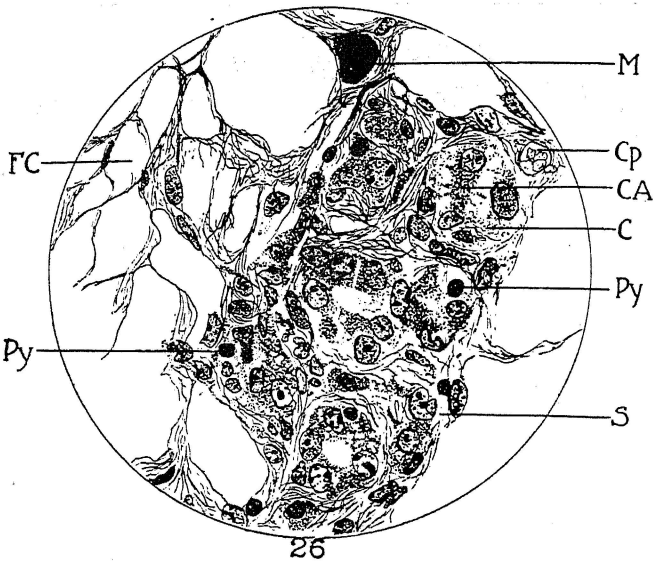


Fig. 26.—Transverse section of the mammary gland of a rat eight days after weaning. Py., pycnotic nuclei. (Maeder).

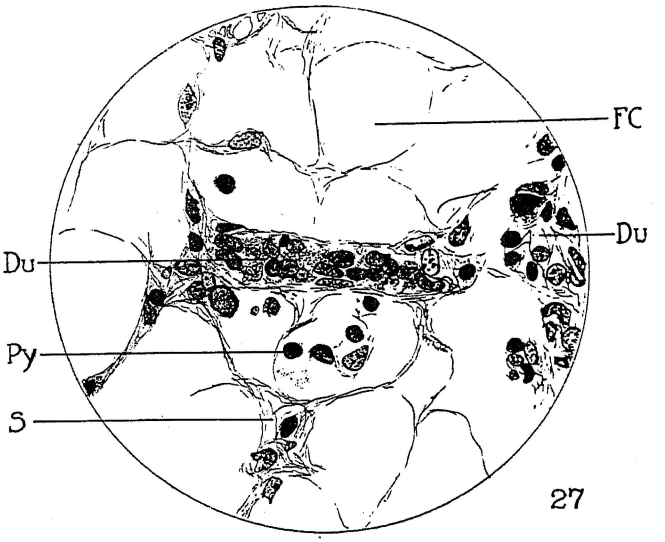


Fig. 27.—Transverse section of the mammary gland of a rat twenty-eight days after weaning. (Maeder)

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 the estrus cycle (corpora lutea) must be kept in mind.

Kuramitsu and Loeb observed the first sign of decided retrogression in the rat gland 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-eighth 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 of their size at the forty-eighth hour stage. At the end of two and three weeks the glands very closely simulate those of the adult virgin animal.

PRESENTATION OF EXPERIMENTAL DATA

Comparison of Developmental Stages in the Rat and Rabbit

In a study of this kind, it is necessary to obtain a complete picture of the normal development at various stages of maturity, not only as controls for the experiment but to determine the stage at which the development present is best suited for certain experiments. As the macroscopic characteristic of total mounts of stages beyond ten weeks of age have not been reported, it was necessary to obtain a series of glands representing the progressive stages beyond that age.

It was found, in agreement with Myers' studies, that the development of the mammary gland in the rat from birth to puberty differs from that observed in the rabbit at this period. During this period in the rat, duct growth predominates. However, there is an indication of sparse lobule growth in the adult virgin female much the same as in the rabbit in continuous estrum.

As is found in the female rabbit, pregnancy in the rat is characterized by marked lobule growth. The greatest formation of lobules apparently occurs between the tenth and fifteenth day of pregnancy.

The gland during lactation is characterized by a preponderance of distended lobule formation and is much thicker than in the resting stage. During involution these lobules take on a feathery appearance probably due to the shrinkage of the ducts and lobules.

Immature male rats, unlike immature male rabbits, undergo considerable development of the gland after birth. In the mature males examined marked lobule proliferation was found. In the limited number of animals examined, this lobule proliferation seems to be absent in males up to 70-80 days of age. In 122-day-old males there is evidence of proliferation. This characteristic is further developed in more mature males, although mature males are found that have no such extensive proliferation. Because of this characteristic of mature males, immature males up to about fifty days of age are most suitable for studies on the growth of the mammary gland.

Variability of the Mammary Gland

In using the glands from normal animals as control glands to compare with glands from experimental animals, some difficulty was encountered because of the variations between animals of the same age, weight, and even between litter mates. In females past the age of puberty some glands were found that had only duct growth, while others had the beginning of lobule formation.

Effect of Castration

Because the males and females used in the experiments were castrated, it was essential to determine the effect of castration on the development of the mammary glands.

Castrated males were compared with non-castrated males of approximately the same age at monthly intervals after the beginning of the experiment. Apparently castration neither inhibits nor hastens the characteristic lobule proliferation. Neither is the continuation of duct growth in the limited number of animals studied retarded.

There is a possibility that duct growth may continue or there may be a different type of growth develop as a result of castration in the female. To investigate this question, spayed females were divided into two groups, one group was allowed to remain untreated; while the second group was used for experiments. The untreated animal was compared to the experimental animal at the end of the experiment. In the animals compared in this way, spaying seems to cause no apparent change in the type of growth during the time the experiments were continued, nor does duct growth continue. It is concluded from these observations that spaying, for short periods of time (20 to 25 days) at least, results in a very slight or no increase in size or character of the mammary gland.

Individual Controls

It was thought that results could be more accurately determined in many experiments by removing a gland before the ex-

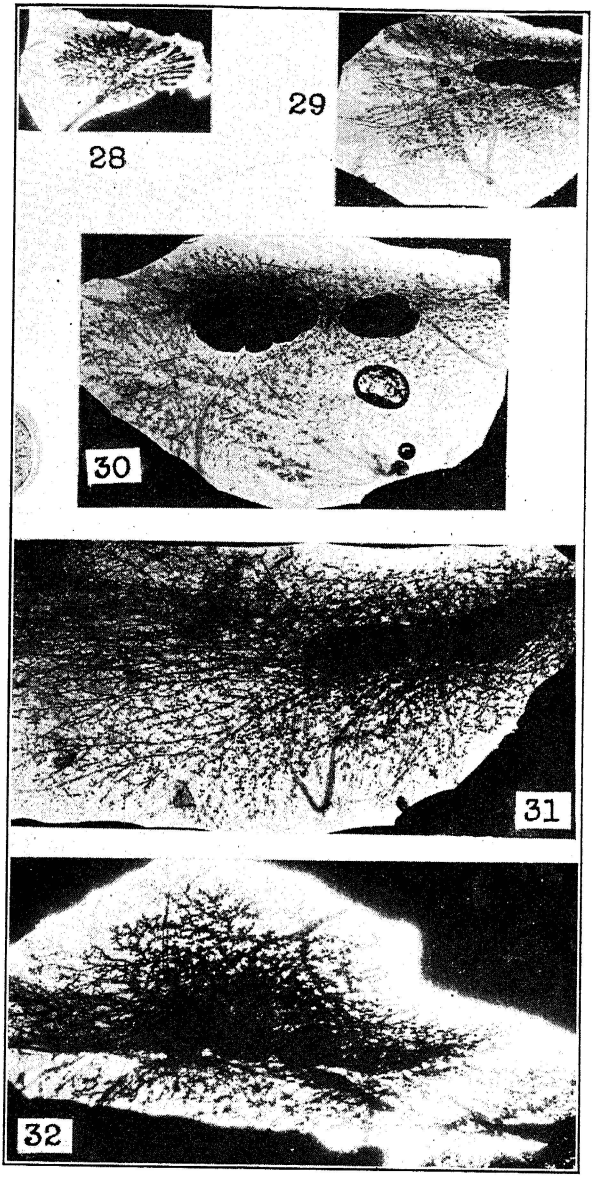


Fig. 28.—Gland from a female rat 33 days of age. X 2. Fig. 29.—Gland from a female rat 65 days of age, but castrated at the age of 40 days of age. X2½. Compare with Fig. 30.—Gland from a female rat seventy days of age. X2½. Fig. 31.—Gland from an adult virgin female rat. X2½. Fig. 32.—Gland from a female rat pregnant for six days. X2½.

periment was begun, and removing the opposite gland after completion of the experiment. This method was used in later experiments. Here again control animals in which no injections were made were run to determine any changes due to the removal of the gonads.

In removing the gland from the live animal, the entire gland was not removed, so that changes in the type of growth rather than the increase in size of the gland were determined.

Extracts Used

1. Estrus-producing hormone. The estrus-producing hormone was obtained from the urine of pregnant cows by a method described by Turner and Frank (1930). This extract was used in previous studies on the growth of the mammary gland in the rabbit. The potency of the hormone is measured in terms of rat units. The rat unit is defined as the minimum amount of the hormone necessary to produce a full change from the negative to the estrual vaginal spread in a castrate rat of approximately one hundred and forty grams (± 20 grams) in weight.

2. Corpus luteum hormones.

(a) A lipoid extract of sow's corpora lutea prepared according to Allen's (1930) method. This extract probably contained some estrus-producing hormone. (One cubic centimeter equals 50 grams of fresh tissue).

(b) A water soluble extract of sow's corpora lutea prepared according to Hisaw, Fevold and Meyer's method. This extract was free of the estrus-producing hormone.

3. An alkaline extract of sheep's pituitary prepared by a method described by Turner and Gardner (1931).

Procedure

The albino rat was used in all the experiments conducted. Injections were made subcutaneously. The animals were killed one day after they had received the last injection. The entire skin was removed, stretched on a cork and fixed in Bouin's fluid. The connective tissue layer containing the gland tissue was removed, stained in Mayer's haematoxylin, dehydrated, and mounted in gum damar. The glands were examined macroscopically and with the dissecting microscope.

The ages of the animals as given in the following reports are the ages at the time the animals were castrated or before injections were begun.

The effect of small and large amounts of the estrus-producing hormone on castrated animals was determined. The effect of the water-soluble and lipoid extract of the corpus luteum of sow's

ovaries was determined when administered simultaneously with varying amounts of the estrus-producing hormone. Also the effect on the mammary gland of the albino rat of an alkaline extract of sheeps' pituitaries and whole pituitaries when implanted subcutaneously was determined.

RESULTS OF THE EXPERIMENTS

The effect of small amounts of the estrus-producing hormone. —To determine the effect of the estrus-producing hormone on immature female rats, five 21-day-old females of the same litter were used. One of these was killed and the gland removed as a control. The other four animals were spayed. One was injected with one rat unit of the estrus-producing hormone daily for five days, one with one rat unit daily for ten days, one with one rat unit daily for fifteen days, and one with one rat unit daily for twenty days.

The control gland showed relatively few branches and little extension of growth and the gland of the rat injected for five days showed a more extensive duct growth than the control. The gland of the animal injected for ten days was not as extensive as the five-day gland, though much more so than the control. The ten-day gland was characterized by numerous club-shaped, deeply staining end buds. The glands of the females treated for fifteen days and twenty days showed much more extensive duct growth than any of the other glands, the gland area being approximately three times that of the control. The end buds of all ducts were characterized by thick, club-shaped growing ends which, in the gland of ten days, gave the whole gland a dense appearance. In the larger glands, the ducts making up the larger part, were relatively fine, giving the area a less dense appearance. All glands were composed entirely of ducts and were free of any alveolar formation.

The effect of one rat unit of estrus-producing hormone on immature males was determined. Five male litter mates 28 days old were used. The glands of one were removed as a control. As in the females, a male for each five-day interval up to twenty days was treated with one rat unit of estrus-producing hormone daily. The animals were all castrated.

The control gland showed a very small extension of growth. The gland of the male treated for five days was approximately twice the diameter of the control gland. Relatively thick, deeply staining, club-shaped end buds were numerous, giving the gland a characteristic, dense appearance. The gland removed from the male treated for ten days showed greater extension than the five day gland. The fifteen and twenty-day glands were most ex-

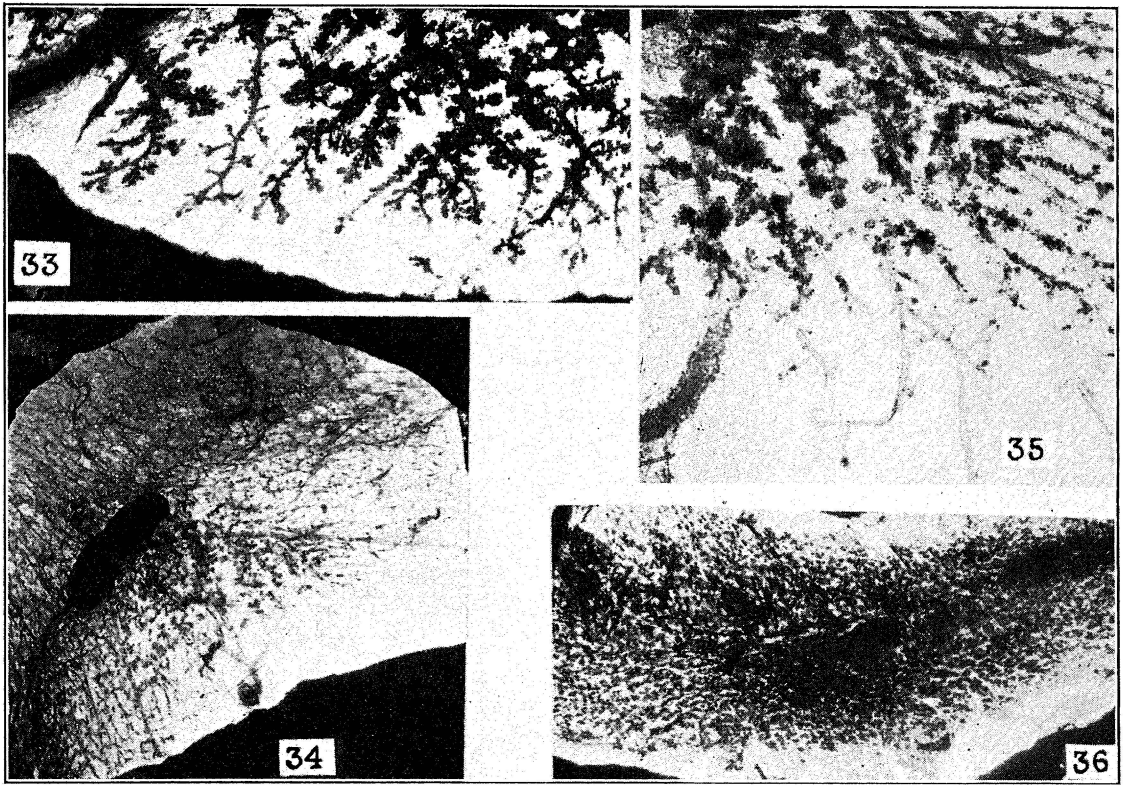


Fig. 33.—Enlarged section of the gland shown in Fig. 32. X10. Fig. 34.—Gland from a female rat pregnant for ten days. X2½. Fig. 35.—Enlarged section of the gland shown in Fig. 34. X10. Fig. 36.—Gland from a female rat pregnant fifteen days. X2½.

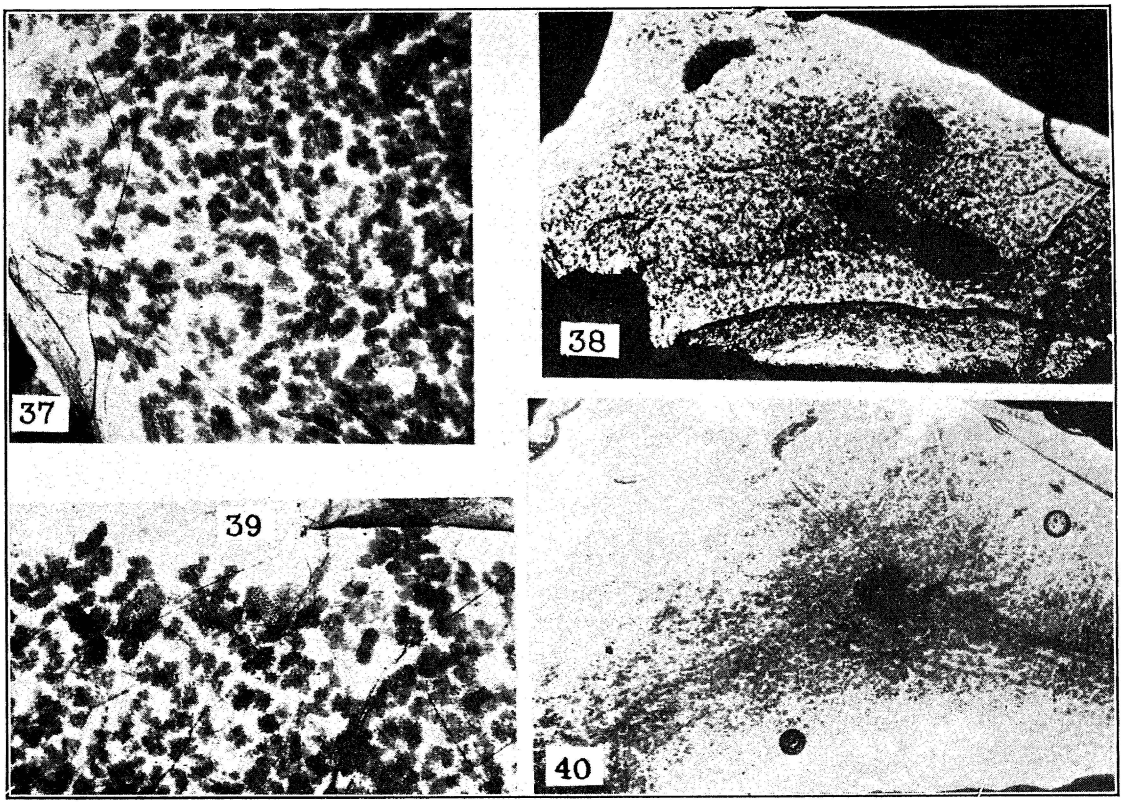


Fig. 37.—Enlarged section of the gland shown in Fig. 36. X10. Fig. 38.—Gland from a female rat pregnant for 20 days. X2½. Fig. 39.—Enlarged section of the gland shown in Fig. 38. X10. Fig. 40.—Gland from a female rat one day following parturition (not as dense as later in lactation). X2½.

tensive in growth. The end buds in all glands were relatively thick as compared to the ducts; thus the larger glands with fewer end buds in relation to the size of the gland area show a less dense structure. In all glands duct growth only occurred.

In view of the fact, as was previously noted, that duct growth apparently continues after castration in the male, much of the growth in the males was due probably to continued growth irrespective of the estrus-producing hormone. However, the gland of the male injected for 20 days shows approximately twice as extensive growth as a gland from a normal male of the same age. In reality the extension of the duct growth in the twenty-day treated experimental gland is greater than that observed in any normal mature male. However, the type of growth is not similar to that of the mature male, consisting entirely of ducts.

The effect of large amounts of estrus-producing hormone.—To determine the effect of larger amounts of the estrus-producing hormone, castrated rats of various ages were treated with increasing amounts of the hormone, as reported in the following protocols.

A 28-day-old castrated female was injected for ten days with five rat units of estrus-producing hormone daily. The gland removed from the rat so treated showed the characteristic thick, club-shaped end buds of a rapidly growing gland. The gland area was considerably more extensive than that of a normal 28-day-old female. No alveoli were present.

Castrated 33-day-old females were injected with ten rat units daily for ten days. The glands removed at the end of the treatment were characterized by the presence of numerous large, club-shaped end buds. These buds were present not only at the extremities of the gland, but also as outgrowths from secondary and tertiary ducts. Some lobules with true aveoli were present. The preponderance of growth consisted of large numerous end buds, and gave the gland its dense appearance. When compared to a gland of a female pregnant for 15 days, the lobules were not nearly as numerous nor were the lobules composed of as many alveoli. The extension of the gland was not any greater than that observed in the gland of a rat receiving only one rat unit of estrus-producing hormone for ten days.

A castrated male in which extended duct growth had been induced by the injection of one rat unit of estrus-producing hormone for ten days, was treated for an additional ten days with the estrus-producing hormone increased to ten rat units daily. The growth resulting was characterized by a much extended gland area, much greater than in any normal mature male. Numerous developing end buds were present throughout the gland area. However, the end buds were not as numerous as in the glands of the females treated with ten rat units of estrus-produciing hormone for ten days.

Castrated females 94 to 97 days of age were treated with ten to fifteen rat units of the estrus-producing hormone for twenty days. Their glands were compared to the control gland removed at the time of castration or before the injections were begun. The extension of growth due to the hormone could not be determined because the entire gland area was not removed in the control. In these glands after treatment lobules with true alveoli were present. The number of lobules relative to the size of the gland was not great. However, they appeared more fully developed and more numerous than was found in any normal nullipara females.

The results of these experiments indicate that large amounts of the estrus-producing hormone, when injected into rats cause extreme end bud proliferation and limited formation of lobules in both immature and mature females. This lobule proliferation is not nearly equal to that present in a gland of a normal female pregnant for fifteen days, but the lobules are more fully developed than in a virgin female. With the larger doses of estrus-producing hormone only extensive end bud proliferation with no alveolar formation was induced in castrate males.

The effects of corpus luteum extracts.—Corpus luteum extracts, both the lipoid and water-soluble extracts, were injected into castrate male and female rats. The glands resulting from the effect of these extracts were compared to normal glands in some cases and to a control gland removed before the treatment in others. The experiments are described in detail below.

Two 33-day-old castrated females were injected for ten days with 0.1 c.c. of a lipoid extract of the corpus luteum. The glands from the treated animals were characterized by duct development. Large club-shaped end buds were numerous throughout the gland area. As stated previously, the estrus-producing hormone was probably present in this extract and may have been the cause of the growth produced. There is reason for believing this because the growth was identical in many respects to that produced by the estrus-producing hormone alone. No lobules were present.

Three 95-day-old virgin female castrates were treated with 0.1 c.c., 0.2 c.c., and 0.3 c.c. respectively, of the water-soluble corpus luteum extract. The injections were continued for a period of twenty days. All glands of the animals thus treated showed no visible changes whatever when compared with a control gland removed before the experiment. The ducts were very fine and no increase in end bud formations or alveoli proliferation occurred.

Two castrated males between the ages of 44 and 50 days were treated with 0.1 c.c. of the lipoid extract of the corpus luteum for a period of ten days. These castrated males had received an injection of one rat unit per day of the estrus-producing hormone and had somewhat extended duct growth. The resulting glands showed no apparent changes except possibly a few more end buds than the normal gland. These end buds were not as numerous as in the glands of the females treated in the same manner as noted previously.

The results of the injection of corpus luteum extracts alone indicate, with one possible exception, that these extracts cause no change in the mammary gland of the albino rat. No changes whatever resulted in the 95-day-old castrated females as a result of these injections. Some control glands removed before the treatments in this group of animals showed more development than they did after the treatment. The proliferation obtained in the two 33-day-old castrated females treated with 0.1 c.c. of the lipoid

extract was due, no doubt, to some hormonal influence because no gland of any normal nullipara female having such proliferation has ever been observed during this study.

The effect of the estrus-producing hormone and extracts of the corpus luteum.—A series of experiments were run wherein male and female rats of various ages were treated with varying proportions of the estrus-producing hormone and the extracts of the corpus luteum. The glands removed from the animals treated were compared with glands of normal animals of the same age, and with glands of animals treated similarly with estrus-producing hormone alone, or the corpus luteum extracts alone. The corpus luteum extracts used here have been shown (Turner and Frank 1931) to be effective in producing changes in the mammary gland of the rabbit when injected simultaneously with the estrus-producing hormone.

Two 30-day-old castrated females were treated for five and ten days respectively with five rat units of the estrus-producing hormone plus 0.1 c.c. of the lipoid extract of the corpus luteum. The glands of the animals thus treated showed some extension of growth and proliferation of numerous end buds. This proliferation in some places was so marked that apparently the beginning of lobule formation was present. The condition, however, was almost identical as far as could be determined, with the glands of the 28-day-old castrated females that were treated with five rat units of estrus alone. The lobule proliferation was not as far advanced nor was the spacing of lobules nearly as close as in the untreated gland of a female pregnant fifteen days. The gland of the rat treated for ten days was somewhat denser than the one treated five days, but the same type of growth was present.

Two 44-day-old castrated males that had received one rat unit daily of the estrus-producing hormone for ten days and therefore had extended duct growth, were treated with five rat units of estrus-producing hormone plus 0.1 c.c. of the lipoid extract of the corpus luteum simultaneously for five and ten days, respectively. The resulting gland growth was very similar to that of the two 28-day-old females injected with five rat units of estrus-producing hormone plus 0.1 c.c. of the lipoid corpus luteum extract. It was somewhat less dense in appearance and the proliferation of end buds and slightly developed lobules, i. e., lobules with only a few alveoli, did not extend to the outer margins of the glands as in the females. When compared to a gland of a female pregnant for fifteen days, the lobules were not as well developed, having fewer alveoli and the number of lobules per unit area was much less. There was no apparent difference between the glands from the animals injected five days or ten days.

Two castrated males 44 days of age were treated for a period of five days, one with one and one-fourth rat units of estrus-producing hormone plus 0.2 c.c. of the lipoid extract of the corpus luteum, the other with one and one-fourth rat units of estrus-producing hormone plus 0.15 c.c. of the same corpus luteum extract. Both of these animals had glands as a result of the treatment that had no lobules, but were characterized by numerous large end buds.

Four castrated males 44 to 50 days old were treated with one and one-fourth rat units of estrus-producing hormone with various amounts of the lipoid extract of the corpus luteum. One received 0.25 c.c., another 0.1 c.c., and another 0.2 c.c. The glands resulting from these injections

were characterized by end buds though not very numerous but extremely large at the extremity of the terminal ducts.

One 40-day-old castrated female was treated with one and one-fourth rat units of the estrus-producing hormone plus 0.3 c.c. of the water-soluble corpus luteum extract. (This extract had been shown to be effective in producing changes in the mammary gland of the rabbit when administered simultaneously with the estrus-producing hormone.) The treatment was continued for 25 days. The gland resulting from this treatment showed considerable extension when compared to the control gland. The numerous end buds were small and very fine as compared to the glands described above. No lobules were apparent.

A group of castrated females between the ages of 90 and 97 days of age were used, injecting the estrus-producing hormone simultaneously with the water-soluble extract. A control gland was removed from each individual before injections. were begun.

One such female was treated with five rat units of estrus-producing hormone plus 0.1 c.c. of the corpus luteum extract daily for a period of ten days. This individual had been treated for a twenty day period previously with 0.1 c.c. of the corpus luteum extract alone. A gland was removed after the twenty day treatment and compared to the gland produced as a result of the following ten day period of treatment where estrus-producing hormone was used with the corpus luteum extract. The gland showed more numerous end buds and the ducts were much thicker than the control. Very few lobules were apparent.

A second female which had been previously treated with 0.2 c.c. of the corpus luteum extract alone for twenty days was treated for a following ten day period with five rat units of estrus-producing hormone plus 0.2 c.c. of the corpus luteum extract. The gland was removed at the end of this ten day period and compared with the one removed after the twenty day treatment of corpus luteum extract alone. This gland, as a result of the ten day treatment, showed considerably more numerous end buds and much wider ducts. Some few lobules were present. In the same animal injections were continued for another ten day period, increasing the estrus-producing hormone to seven and one-half rat units with 0.2 c.c. of the corpus luteum extract. The gland resulting from this last ten day treatment was considerably denser in appearance and numerous lobules with a greater number of alveoli per lobule were present than in any of the glands removed from this animal as a result of the former treatments. This lobule proliferation was not equal in appearance to a gland of a female pregnant for fifteen days. The lobules were not as numerous nor as fully developed.

A third female of this group was treated following castration with two and one-half rat units of estrus-producing hormone plus 0.3 c.c. of the water-soluble corpus luteum extract. This first treatment was continued over a period of twenty days. There were little or no change as a result of this treatment. Numerous end buds were present, but these were present in the control gland removed before the treatment. Following this twenty-day treatment, a treatment in which the esrus-producing hormone was increased to five rat units daily plus the same 0.3 c.c. of corpus luteum extract, was given. The gland removed after this treatment was much denser and lobules were present. (The proliferation was not equal to that of a gland of a female pregnant for fifteen days.) In an additional ten-day period of injection of seven and one-half rat units of estrus-producing hormone plus 0.3 c.c. of the corpus luteum extract no apparent additional change occurred in the gland.

A fourth female was treated following castration with two and one-half rat units of estrus-producing hormone plus 0.1 c.c. of the corpus luteum extract for twenty days. No apparent change was noted that was not present in the control. This twenty-day period of treatment was followed by a twenty-day period of treatment during which the corpus luteum extract was increased to 0.5 c.c. daily, but the estrus-producing hormone continued at two and one-half rat units. Little or no apparent change resulted from the last period of treatment. Apparently the amount of estrus-producing hormone was too small, even with various amounts of the corpus luteum extract, to cause any changes in the mammary gland.

Apparently, the administration of the estrus-producing hormone in sufficient amounts with the two extracts of the corpus luteum is capable of producing in immature and mature castrated males and females, duct growth and lobule formation. This lobule proliferation, even when injections lasted for twenty and thirty days, was not equal to that characteristic of the gland of a female pregnant for fifteen days. The estrus-producing hormone in amounts of two and one-half rat units or less plus the corpus luteum extracts in amounts up to 0.5 c.c. seem incapable of producing any visible change in the mammary gland of the rat. When larger amounts of the estrus-producing hormone replaced the smaller amounts, proliferation of the mammary gland resulted even when the amount of corpus luteum extract remained constant.

The Effect of Pituitary Hormones

Alkaline extract of sheep's pituitary.—The effect of the alkaline extract of sheeps' pituitaries on the mammary gland of the rat was studied using multipara females. Castrate virgin females and castrate and normal mature males were also treated with this extract. The results obtained with these animals were compared to those obtained with rabbits using the same extracts.

Two multipara females were treated with 0.2 c.c. of the alkaline extract for ten days. In one of these females the treatment was begun at the time of weaning of the young and the gland was in full flow of milk. In the other female the gland was partially involuted. A control gland was removed from each individual before injections were begun. The glands removed after treatment were much more involuted than the control glands. They showed no significant difference from glands on control animals that were in approximately the same degree of activity at the beginning of the experiment and had undergone normal involution for ten days.

Two mature males, one castrated, the other not castrated, were treated with 0.2 c.c. of the pituitary extract for ten days. A control gland was removed from each animal before treatment. The glands removed after treatment when compared to the controls showed a slightly more dense appearance because there were a few more lobules. However, both the controls and the glands removed after treatment had lobules present and the differences did not seem to be significant. Certainly the

thick and distended condition of the lobules characteristic of active milk secretion was not present.

Two 95-day-old castrated females were treated with 0.2 c.c. of the alkaline extract of the pituitary daily, one for ten days and the other for twenty days. The rat treated for ten days had previously received a twenty-day treatment of ten rat units of the estrus-producing hormone daily. The gland removed after the ten-day tretament with the pituitary extract showed no significant change. The small change that did occur tended toward an involution of the lobules present before treatment, rather than an increased proliferation or distension. The gland of the female treated for twenty days with the pituitary extract showed no apparent change when compared to the control gland removed before injection.

A castrated male and female both thirty days of age were each treated with 0.2 c.c. of the alkaline extract of the sheep's pituitary for a twenty-day period. The gland of the female after the experiment showed an extension of duct area approximately equal to that of a normal female 50 days old, but no lobules or secretion was evident. The castrated male's gland showed no lobule growth nor was any secretion evident. The gland in the castrate male had continued to develop, and was approximately equal to that of the normal male of the same age.

Apparently the effects of this alkaline extract in the amounts used on the mammary glands in the animals tested were negligible. No changes excepting possibly an extension of duct growth in an immature castrated female resulted. No lobule proliferation was induced nor was milk secretion initiated in the animals studied. These extracts were shown to be effective in initiating milk secretion in the rabbit. (Turner and Gardner 1931).

Implants of the pituitary.—To determine the effect of the whole pituitary implants on the mammary gland, the pituitary from castrated male and female rats was used. The pituitary of castrate animals has been shown to be considerably more potent in stimulating the ovaries of immature animals than those of normal animals (Evans and Simpson 1929).

A pituitary was implanted subcutaneously each day for varying periods of time. The animal on test was killed the second day after the last implant was made, as the effects of the implant were thought to last for at least two days after implanting. The effects of these implants were determined on normal and castrated females.

One 30-day-old normal female received one implant daily for three days and was killed on the fifth day. The gland of the female after treatment was characterized by numerous extremely large club-shaped end buds. No true alveoli were present, however. The extension of the gland area was significantly greater than that of a normal female of the same age. Another normal female 30 days old and a litter mate of the one above received one implant daily for nine days and the gland examined on the eleventh day. The gland resulting from this treatment showed a dense mass of lobules with numerous alveoli. The gland showed as extensive lobule proliferation as that of a normal female pregnant for fifteen days.

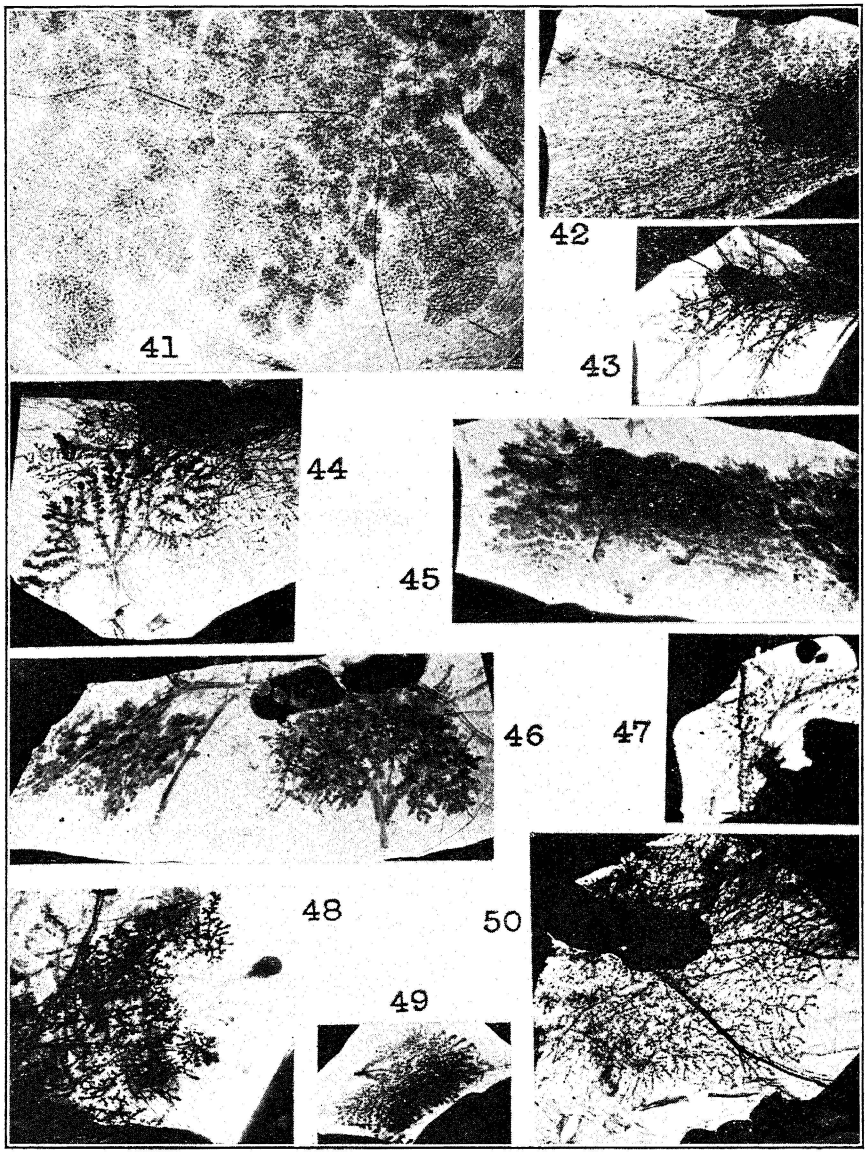


Fig. 41.—Enlarged section of the gland shown in Fig 40. X10. Fig. 42.—Gland from a female rat in process of involution. Removed ten days after weaning young. X2.

Fig. 43.—Gland from a male rat 44 days of age X2. Fig. 44.—Gland from a normal male rat 70 days of age. X2½. The earliest age at which lobule proliferation was observed. Fig. 45.—A normal mature male gland. X2½. Extreme lobule pro-

liferation is present in this individual. Fig. 46.—Gland from a castrated male. X2½. Lobule proliferation is less extreme than in the gland shown in Fig. 45. The proliferation here is more or less characteristic of the mature male's glands.

Fig. 47.—Gland removed from a 21-day-old female as a control on glands of Fig. 48, 49, and 50. X2½. Fig. 48.—Gland from a female castrated at 21 days of age and treated with one rat unit of estrus-producing hormone for five days. X2½. Fig. 49.—Gland from a female castrated at 21 days of age and treated with one rat unit of estrus-producing hormone for ten days. X2½. Extremely thick and numerous end buds are present. No lobules are present. Fig. 50.—Gland from a female castrated at 21 days of age and treatd with one rat unit of estrus-producing hormone for 20 days. X2½. No lobules are present.

A 33-day-old castrated female received a daily implant for six days and was killed on the eighth day. The gland of the female thus treated showed considerable end bud formation and wide ducts. The gland area was not much more extensive than that of a normal female of that age.

Three females castrated when 51 days of age each received an implanted pituitary daily for seven days, nine days, and ten days respectively. The glands of these females thus treated showed an abnormal end bud formation, but no lobule formation.

The whole pituitary, when implanted in normal females, causes marked lobular proliferation of the mammary gland. When these implants were continued daily for nine days, growth equal to that of the gland of a female pregnant for fifteen days was induced. As no marked changes could be induced in the glands of castrated female rats with whole pituitary implants, the results produced in the glands of normal females in all probability were due to hormones secreted by the ovary as a result of the stimulation received from the pituitary implants. The luteinizing effect of the pituitary on the ovary possibly results in the production of a corpus luteum hormone, which together with the estrus-producing hormone, produces lobule proliferation of the mammary gland.

DISCUSSION

Mammary gland development of animals differs from that of most other anatomical structures in that it is delayed until after most other organs have attained considerable development.

In all mammals there is somewhat more or less parallel developmental change of the mammary gland; however, some differences may exist between different domestic and laboratory animals. A study of these differences together with a study of the effects of certain hormones or extracts on the gland development in castrated animals of various species should indicate some of the possible causes of the development of the mammary glands and the characteristic developmental changes.

Limited gland development occurs before birth in the albino rat. This growth, which is much the same as in the rabbit at the

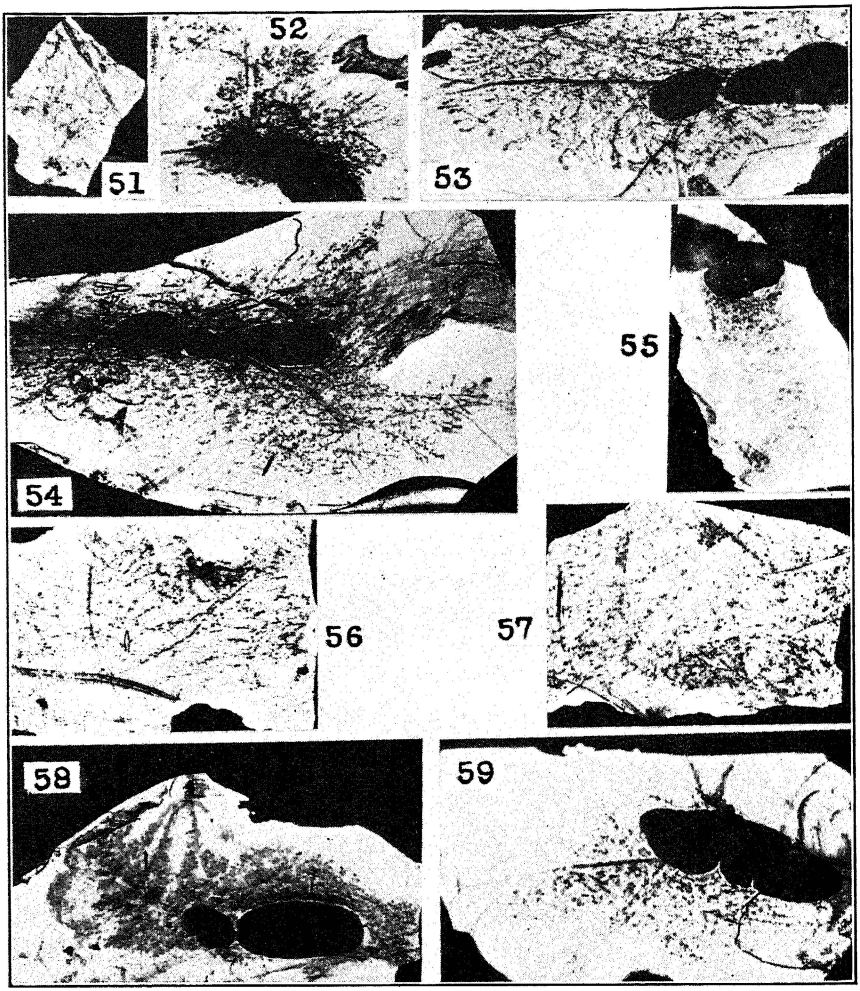


Fig. 51.—Gland from a normal male rat 28 days of age. Used as a control for glands of Fig. 52, 53, and 54. Fig. 52.—Gland from a male castrated at 28 days of age followed by a treatment of one rat unit of estrus-producing hormone for five days. X2½. Large, numerous end buds but no lobules are present. Compare with Fig. 51. Fig. 53.—Gland from a male castrated when 28 days of age followed by a treatment of one rat unit of estrus-producing hormone or ten days. X2½. Compare with Fig. 51. Fig. 54.—Gland from a male castrated when 28 days of age followed by a treatment of one rat unit of estrus-producing hormone for 20 days. X2½. Unusually great extension of ducts is present. Compare with Fig. 39 to compare size of gland area and with control. Fig. 51.

Fig. 55.—Gland from a female castrated when 33 days of age followed by a treatment of one rat unit of estrus-producing hormone for ten days. X2½. Compare lobules are present. Fig. 56.—Gland removed from a 95 day old nullipara female

before treatment as a control on gland shown in Fig. 54. No lobules are present. Fig. 57.—Gland removed after treatment with 10 rat units of estrus-producing hormone for 20 days. X2. Same individual from which control gland shown in Fig. 53 was removed. Limited lobule proliferation is present. Fig. 58.—Gland from a castrated 30 day old female treated with five rat units of estrus-producing hormone plus 0.1 c.c. of the lipoid extract of sow's corpora lutea for 5 days. X2½. Lobules are present. Fig. 59.—Gland from a castrated 44 day old male treated for 5 days with 5 rat units of estrus-producing hormone plus 0.1 c.c. of the lipoid corpus luteum extract. X2½. Duct growth had been induced previously by injecting one rat unit of estrus-producing hormone for ten days. A few lobules are present.

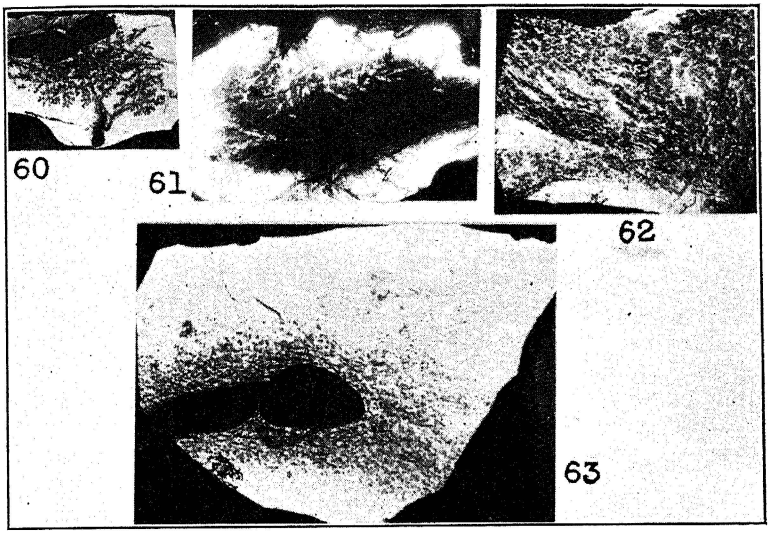


Fig. 60.—Gland from a 33 day old castrated female receiving a rat pituitary implant daily for six days. X2½. Compare with Fig. 28. Fig. 61.—Gland from a thirty day old normal female receiving nine rat pituitary implants. Dense lobule proliferation. Fig. 62.—Gland from a partially involuted female. Control on gland shown in Fig. 63. Fig. 63.—Gland from the same individual whose control gland is shown in Fig. 62 after receiving a ten day treatment of 0.2 c.c. of the alkaline extract of sheep's pituitary. Involution has continued.

time of birth, may possibly be accounted for as due to hormonal stimuli received from the maternal organism. From birth to puberty in the rat there is a continuous increase in duct development. During the fourth and fifth weeks of postnatal life and again during the tenth week the gland undergoes rapid growth. Myers offers an explanation of the cause of the increased growth at ten weeks of age as due to the hormones associated with the attainment of puberty. However, no such explanation can be offered for the continuous duct growth from birth to puberty or the increased duct growth during the fourth and fifth weeks.

Small amounts of the estrus-producing hormone in castrated immature rats produced duct growth in all respects similar to that produced in the normal rat from birth to puberty. If this hormone is the only cause of this characteristic duct development, then the estrus-producing hormone must be present in the immature normal rat in sufficient quantities to produce this duct growth. The fact that this duct growth occurs in immature males indicates that the ovary is not necessary for the elaboration of the hormone necessary for this growth.

The growth characteristic of the gland of the virgin female may be due to hormones secreted by the ovaries during recurring estrus cycles. The estrus-producing hormone and the hormone of the corpus luteum possibly cause the increased duct growth and limited lobule proliferation. However, the effect of the corpus luteum of this period in the rat may be negligible because of the shortness of the estrus period, as is indicated by a lack of inhibitory effect on ovarian function. This growth may be due entirely to the estrus-producing hormone as more marked lobule proliferation than that present in a virgin female has been produced experimentally by the administration of large doses of estrus-producing hormone.

During normal pregnancy the beginning of the proliferation of lobules was observed in the gland of a female pregnant for ten days. By fifteen days marked proliferation is present and is then, as far as can be ascertained macroscopically, approximately as fully developed as the gland of a female at the time of parturition. In the rabbit, this growth characteristic of pregnancy similar to that produced during pregnancy in the rat has been produced (Turner and Frank 1931) experimentally by the administration of the estrus-producing hormone with extracts of sows' corpora lutea. These extracts of sows' corpora lutea administered simultaneously with the estrus-producing hormone in the rat produced lobule proliferation, but not as great as that characteristic of advanced pregnancy. The corpus luteum extracts and the estrus-producing hormone used in these experiments were the same as to source and methods of extraction as those used on the rabbit. However, new extractions of the corpora lutea were made at frequent intervals from a supply of corpora lutea in alcohol; and as some of the experiments with rats were run after the extract had been shown to be effective in producing complete pregnancy development in the rabbit, there is a possibility that the later extractions did not have as great a potency as the first extractions. However, a castrate female treated with a small amount of the estrus-producing hormone (one and one-fourth rat units) plus the first extraction of the corpora lutea which was effective in the rabbit,

produced no lobule proliferation in this individual. This result may have been due to the small amount of the estrus-producing hormone used, because in no experiment was significant lobule proliferation produced when less than two and one-half rat units of the estrus-producing hormone were used in conjunction with corpus luteum extracts.

Apparently, fairly large amounts of the estrus-producing hormone are necessary in conjunction with the corpus luteum extracts to produce marked lobule proliferation in the albino rat gland. That the reverse of this is true is indicated by recent work on the rabbit (unpublished). However, even when large doses of the estrus-producing hormone and the corpus luteum extract were administered, the lobule proliferation produced was not equal to that present in the gland of a rat in advanced pregnancy. The lobule proliferation produced by estrus-producing hormone plus the corpus luteum extract was greater than that produced by the administration of large amounts of the estrus-producing hormone alone.

The administration of the corpus luteum extracts alone produced no significant changes in the mammary glands of the rat.

Evidently the potency of the corpus luteum extracts was insufficient to produce lobule proliferation characteristic of advanced pregnancy, or the amounts of the estrus-producing hormone and the hormones of the corpus luteum extracts were not given in the proper proportions, or the corpus luteum extracts together with the estrus-producing hormone is not all that is required to cause development of the mammary gland equal to that characteristic of advanced pregnancy in the albino rat.

Probably shortly before parturition milk secretion is initiated in the mammary gland of the rat. In the experiments in which the estrus-producing hormone or corpus luteum extracts or a combination of these two, no evidence of milk secretion could be noticed in the glands. Evidently these hormones are not capable of initiating milk secretion. Also the administration of an alkaline extract of sheep's pituitary which has been shown to initiate milk secretion in the rabbit (Turner and Gardner 1931) did not produce this phenomenon when amounts of 0.2 c.c. daily for twenty days were used in the rats treated thus far.

The marked lobule proliferation present in the mammary glands of mature male rats indicates that the hormone or hormones causing this growth may be produced by some organ other than the ovary in the female. The production of these hormones by some other organ in the rat might be an explanation of the lobule proliferation induced in the glands of castrated rats with large doses of the estrus-producing hormone; this is different

from the less proliferation obtained by administration of the estrus-producing hormone in the rabbit.

Pituitaries of castrate rats implanted daily for ten days did not show evidence of being capable of inducing this lobule proliferation in castrated animals. This gland development in the male undoubtedly requires a relatively long period of time as compared to the time required to produce full lobule development in the female during pregnancy. This lobule hormone in the male must, therefore, be slow in its action or liberated in very small amounts; while in the female during pregnancy, this hormone must be present in relatively large amounts or of great potency.

Although the results of this study throw some light on the causes of the changes occurring in the mammary glands of the normal albino rat, many points remain to be investigated and the exact nature and source of the stimuli producing certain changes have yet to be determined.

SUMMARY AND CONCLUSIONS

1. A study and review of the developmental changes in the normal albino rat is presented. Also a study of the effects of hormones and extracts which have been shown by other workers to be effective in producing certain changes in the mammary gland of the rabbit has been made to determine the nature of the hormones causing the growth of the mammary gland in the albino rat.

2. Small amounts of the estrus-producing hormone, when administered to castrate immature male and female rats, results in extension of the duct system similar to the growth occurring during the period from birth to puberty.

3. When relatively large amounts of the estrus-producing hormone were administered, limited lobule proliferation resulted. This proliferation was somewhat greater than that characteristic of the mammary gland of virgin adult female rats, but not nearly as great as that characteristic of the gland in advanced pregnancy.

4. The administration of a lipoid extract or a water-soluble extract of sows' corpora lutea alone did not produce significant changes in the mammary gland of the albino rat.

5. The simultaneous injection of estrus-producing hormone (five rat units or more) and the lipoid or water-soluble extract of sows' corpora lutea produced lobule proliferation greater than that produced by the estrus-producing hormone alone; but in no case was it as great as that present in the gland of a female in advanced pregnancy.

6. The study indicates that proper proportions of the estrus-producing hormone and the corpus luteum extract seem to be necessary for the production of extensive lobule proliferation.. Amounts of the estrus-producing hormone of two and one-half units or less with corpus luteum extracts seem incapable of inducing lobule proliferation even when large amounts of the corpus luteum extract are used.

7. Implants of whole pituitaries of castrate rats for short periods of time at least seem to be incapable of producing significant changes in the mammary gland of castrated female rats.

8. Thus far in the animals studied, the administration of an alkaline extract of sheep's pituitary, which was shown to be effective in initiating milk secretion in the rabbit, has produced no such result in the albino rat.

9. The presence of marked lobule proliferation in the mammary gland of the adult male rat may indicate that some organ other than the ovary is able to elaborate the lobule proliferation hormone.

BIBLIOGRAPHY

Allen, E., and Doisy, E. A., *An ovarian hormone. Preliminary report on its localization, extraction and partial purification, and action in test animals.* Jour. Amer. Med. Assn. Vol. 81 (1923), pp. 819-821.

Allen, E., Francis, B. F., Robertson, L. L., Colgate, C. E., and Johnston, C. G. *The hormone of the ovarian follicle, its localization and action in test animals and additional points bearing upon the internal secretion of the ovary.* Amer. Jour. Anat. Vol. 34 (1924), pp. 133-179.

Allen, W. M., *Physiology of the corpus luteum. V. The preparation and some chemical properties of progestin, a hormone of the corpus luteum which produces progestational proliferation.* Amer. Jour. Physiol. Vol. 92, No. 1 (1930), p. 174.

Allen, W. M., I. *Cyclic alterations of the endometrium of the rat during the normal cycle, pseudo-pregnancy and pregnancy. II. Production of deciduomata during pregnancy.* Anat. Rec. Vol. 48, No. 1 (1931), p. 65.

Corner, G. W., *The hormonal control of lactation. I. Non-effect of the corpus luteum. II. Positive action of extracts of the hypophysis.* Amer. Jour. Physiol. Vol. 95, No. 1 (1930), p. 43.

Donaldson, H. H., 1924. *The Rat: Data and reference tables.* Pub. Wistar Institute.

Engle, E. T., *Hypertrophy of mammary glands in adult male rats with experimental ovario-testes.* Proc. of the Soc. for Exp. Biol. and Med. Vol. XXV, No. 8 (1928), p. 715.

Evans, H. M., and Simpson, M. E., *Hyperplasia of the mammary apparatus in precocious maturity induced by anterior hypophyseal hormone.* Proc. Soc. for Exp. Biol. and Med. Vol. 26, No. 7 (1929), pp. 597-598.

Evans, H. M., and Simpson, M. E., *Hyperplasia of the mammary apparatus of adult virginal females induced by anterior hypophyseal hormones.* Proc. Soc. for Exp. Biol. and Med. Vol. 26, No. 7 (1929), p. 598.

Evans, H. M., and Simpson, M. E., *A comparison of anterior hypophyseal implants from normal and gonadectomized animals with reference to their capactiy to stimulate the immature ovary.* Am. Jour. Physiol. Vol. 89 (1929), p. 375.

Fevold, H. L., Hisaw, F. L., and Meyer, R. K., *Isolation of the relaxative hormone of the corpus luteum.* Proc. Soc. for Exp. Biol. and Med. Vol. 27 (1930), pp. 604-606.

Frank, R. T., and Rosenbloom, J., *Physiologically active substances contained in the placenta and in the corpus luteum.* Surg. Gynec. and Obst. Vol. 21 (1915), p. 646.

Frank, R. T., and Unger, A., *An experimental study of the causes which produce the growth of the mammary gland.* Archives of Internal Medicine Vol. 7 (1911), pp. 812-839.

Freyer, M. E., and Evans, H. M., *Participation of the mammary gland in the changes of pseudo-pregnancy in the rat.* Proc. Am. Assn. of Anatomists in Anat. Record Vol. 25 (1923), p. 108.

Goltz, F., and Ewald, J. R., *Der Hund mit verkurztem Ruckenmark.* Pfluger's Arch. Vol. 68 (1896), pp. 362-400.

Hisaw, F. L., Fevold, H. L,. and Meyer, R. K., *The corpus luteum hormone. II Methods of extraction.* Physiol. Zoology Vol. III, No. 1 (1930), p. 135.

Hisaw, F. L., and Leonard, S. L., *Relation of the follicular and corpus luteum hormones in the production of progestational proliferation of the rabbit uterus.* Amer. Jour. Physiol. Vol. 92, No. 3 (1930), p. 574.

Iscovesco, H., *Morcellement des differents lipoides constitutifs des organes. Technique general.* Compt. Rend. des Seances de la Soc. de Biol. Vol. 73 (1912), 858-860.

Kuramitsu, C., and Loeb, Leo, *The effect of suckling and castration on the lactating mammary gland in rat and guinea pig.* Amer. Jour. of Physiol. Vol. 56, No. 1 (1921), p. 40.

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

Laquer, E., De Jongh, S. E., and Tausk, M., *Ueber weibliches sexual hormon, menformon V. Ueber den feminisierenden einfluss des menformons auf die unentwickelte Brustdruse. (On the female sexual hormone, Menformon. V. The effeminative influence of menformon on the undeveloped mammary gland).* Deutsche Med. Wochenschrift Vol. 53 (1927), p. 867.

Loeb, L., *The mechanism of the sexual cycle with special reference to the corpus luteum.* Amer. Jour. Anat. Vol. 32 (1923), p. 305.

Long, J. A., and Evans, H. C., *The oestrus cycle in the rat and its associated phenomena.* Memoirs of the University of Calif. Vol. 6 (1922).

Maeder, L. M., *A change in the mammary gland of the albino rat (mus norvegicus albinus) during lactation and involution.* Amer. Jour. Anat. Vol. 31, No. 1, 1922, pp. 1-26.

Myers, J. A., *Studies on the mammary gland. III. A comparison of the develop- the milk ducts and the development of the nipple in the albino rat from birth to ten weeks of age.* Amer. Jour. Anat. Vol 19 (1916), pp. 353-377.

Myers, J. A., *Studies on the mammary gland. II. The fetal development of the mammary gland in the female albino rat.* Amer. Jour. Anat. Vol. 22, No. 2 (1917), p. 195.

Myers, J. A., *Studies on the mammary gland. III. A comparison of the developing mammary glands in male and female albino rats from the late fetal stages to ten weeks of age.* Anat. Rec. Vol. 13, No. 4 (1917), p. 205.

Myers, J. A., *Studies on the mammary gland. IV. The histology of the mammary gland in male and female albino rats from birth to ten weeks of age.* Amer. Jour. of Anat. Vol. 25, No. 4 (1919), p. 395.

Myers, J. A., *Studies on the mammary gland. V. The effects of inanition on the developing mammary glands in male and female albino rats from birth to ten weeks of age.* Amer. Jour. of Diseases of Children. Vol. XVII (1919), pp. 311-328.

Myers, J. A., *Studies on the mammary gland. VI. The development of the mammary gland from its earliest appearance until the period of pregnancy.* Amer. Jour. of Diseases of Children, Vol XVIII (1919), pp. 4-13.

Myers, F. J., and Myers, J. A., *Studies on the mammary gland. VIII. Gross changes in the mammary gland in the female albino rat during the period of involution.* Proc. Amer. Assn. Anat. Rec. Vol. 21, No. I (1921), p. 74.

Parkes, A. S., *The function of the corpus luteum. IV. The relation of oestrin to the luteal phase of the oestrus cycle.* Proc. Roy. Soc. B. Vol. 107 (1930), p. 188.

Pfister, M., *Ueber die reflektorischen beziehungen zwischen mammae und genitalia muliebria.* Beitrage zur Geb. und Gynak. Vol. 5 (1901), pp.421-445.

Rein, G., *Untersuchungen über die embryonale Entwicklungeschichte der Milchdrüse. (Investigation concerning the embryonic development of the mammary gland).* Archiv, fur Mikr. Anatomie Vol. 20 (1881), p. 431.

Ribbert, H., *Ueber transplantation von ovarium, Hoden und mamma.* Arch. f. Entwicklungsmechn. Vol. 7 (1898), pp. 688-708.

Roberts, F. L., *Changes in the mammary gland of the albino rat during the second half of pregnancy.* The Graduate School of the University of Minn., Papers from the Mayo Foundation for Medical Education and Research and the Medical School, Vol. 1, W. B. Saunders Co., Philadelphia and London (1921).

Schickele, G., *Beiträg zur morphologie und entwicklung der normalen und uberzahligen milchdrüsen. (Contributions to the morphology and development of the normal and supernumerary mammary glands).* Zeitschrift f. Morphologie u. Anthropologie Vol. 1 (1899), p. 507.

Sure, B., *Dietary requirements for fertility and lactation. I. The role of fat soluble vitamines in fertility and lactation.* University of Arkansas, College of Agriculture, Bulletin 250 (1930).

Sutter, Monroe, *Cyclic changes in the mammary gland of the rat associated with the estrus cycle.* Proc. Amer. Assn. of Anatomists, Anat. Rec. Vol. 21 (1921), p. 59.

Turner, C. W., and Frank, A. H., *The effect of the estrus-producing hormone on the growth of the mammary gland.* Mo. Agri. Exper. Sta. Res. Bul. 145 (1930).

Turner, C. W., and Frank, A. H., *The relation between the estrus-producing hormone and a corpus luteum extract on the growth of the mammary gland.* Science Vol. 73, No. 1889 (1931), p. 295.

Turner, C. W., and Gardner, W. U., *The relation of the anterior pituitary hormone to the development and secretion of the mammary gland.* Mo. Agr. Exp. Sta. Res. Bul. 156, 1931.

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