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# The Normal Development of the Mammary Gland of the Male and Female Albino Mouse

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# The Normal Development of the Mammary Gland of the Male and Female Albino Mouse

## I. INTRAUTERINE

C. W. TURNER AND E. T. GOMEZ

ABSTRACT.—This paper describes the normal development of the mammary gland of the male and female albino mouse from its earliest appearance as a mammary streak in 10-day-old embryos up to the time of birth. Following the appearance of the mammary streak, the mammary line develops in 12-day-old embryos. At intervals along the lines the further proliferation of the stratum germinativum results in the formation of the mammary hillocks which gradually round off to become mammary buds by the 14th day. In females, the primary sprout begins to grow from the distal end of the mammary bud, beginning the 18th or 19th day. By the 20th day a few secondary sprouts may begin to form. The primary sprout develops slightly earlier in the male than in the female. The nipple, which is present only in the female, is formed by an epithelial ingrowth surrounding the mammary bud and primary sprout.

The study of the humeral mechanism responsible for the coordination and synchronization of the various parts of the female reproductive system has been greatly stimulated during the past few years following the discovery of the estrogenic hormone of the ovary and the establishment of a rapid biologic assay. Other hormones of the ovary present in the corpus luteum have since been discovered. Further, the discovery of the relation between the ovary and the pituitary has done much to advance knowledge in this field.

Since the pioneer work of Lane-Clayton and Starling (1906) there has been periodic attempts to determine the hormones responsible for the growth and lactation of the mammary gland without marked success, until the last few years. As the mammary gland is an integral part of the reproductive system, it is only natural that the studies of the ovarian and pituitary hormones should stimulate renewed interest in this problem.

As an introduction to the study of the experimental development of the mammary gland, it is of the utmost importance to have available clear anatomic pictures of the normal changes occurring in the gland during successive sexual epochs.

The excellent description of the normal development of the mammary gland of the rabbit by Ancel and Bouin (1909-12), Schil (1912), Hammond and Marshall (1914) and others, undoubtedly accounts for the use of the rabbit in much of the early experimental work. The observations on the normal development of the rabbit have been reviewed and extended recently by Turner and Frank (1930). In addition, ob-

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servations on the influence of the estrogenic hormone on the growth of the gland have been presented.

The careful studies of Myers (1916-17) on the early development of the mammary gland of the albino rat, together with the observations of Roberts (1921), Maeder (1922), and, more recently, Weatherford (1929) and Turner and Schultze (1931) presents a comprehensive picture of the developing gland of this common laboratory mammal.

Considering the numerous studies of the rabbit and rat, it is surprising to find an almost complete lack of study concerning either the normal or experimental development of the mammary gland of the common laboratory mammal, the albino mouse. This is especially true considering the advantages of a small animal in experimental work with expensive hormones.

In addition to the advantage of size in the case of the mouse, there are other considerations which indicate the desirability of extending the studies of the normal and experimental growth of the mammary gland to as many mammals as possible. In the first place, there are certain species differences in the normal sexual cycle which may be taken advantage of in the study of the problem. The variation in the estrus cycle may be cited as an example. The rabbit ovulates only upon coitus, whereas the rat, guinea-pig, and dog have cycles varying greatly in the duration of the luteal phase.

In the second place, the experimental evidence already accumulating is tending to show important species differences in the response of the hormones in different mammals. As a consequence, it is impossible to generalize after observing the effect of the hormone in one or two species. While this limits the application of the observations, the finding of species differences will undoubtedly make possible a more complete analysis of the interrelationship of the various hormones.

For these reasons an examination of the normal development of the mammary apparatus of the albino mouse was initiated as a part of a larger study of the comparative anatomy and experimental development of the mammary apparatus. It is the object of the present paper to present descriptions of the successive changes in the formation of the glands during embryonic and fetal life. While in a second paper the changes from birth to puberty, during the recurrence of estrum, pregnancy, lactation, and involution will be traced.

### MATERIALS AND TECHNIQUE

Embryos and fetuses of known age after conception were obtained by taking vaginal smears daily of a group of adult nulliparous female mice (usually early in the morning) and at the sign of estrum placing the animals in cages permanently occupied by the males (Danforth,

1916). Six hours later, the vagina of each animal was examined for the presence of a definite gelatinous plug, indicating copulation. As soon as a vaginal plug was observed, the female was returned to an individual cage.

Contrary to the report of Sobotta (1895), a definite gelatinous plug was almost without exception observed closing the vaginal orifice shortly after copulation. However, the plug was found to persist after coitus for a rather short period, varying from four to six hours.

In all cases of pregnancy, conception was considered as dating from the time of discovery of the vaginal plug. The possibility of error in the age of the embryo is obvious, but our observations indicate that it is but a matter of a few hours.

In collecting the embryos, the primiparous animals were sacrificed at various stages of gestation. The embryos were removed from the uteri and fixed immediately in Bouin's fluid or a solution composed of 10 parts of a saturated aqueous solution of  $\text{HgCl}_2$  and one part glacial acetic acid. The specimens were embedded in toto and the sections cut from 6 to 10 microns thick. The sectioned material was stained in Delafield's hematoxylin and eosin, differentiated in acid alcohol, dehydrated, and mounted in balsam.

In the early extra-uterine development (birth to seven days of age) with the exception of the litter collected at birth, the young were no longer sectioned in toto, but rather the skin containing the mammary glands was sectioned. The development of the mammary gland from eight days of age, through pregnancy, lactation, and involution was studied by means of whole mounts of the gland. The glands of the series of animals from which the embryos were obtained were used for the advancing stages of pregnancy as well.

The following method was employed in preparing the whole mounts of the glands. The animals were sacrificed at the desired age or stage of development and skinned. The skin was spread out and tacked upon pieces of flat corks. They were then fixed in Bouin's fluid for 24 hours. The glands were then dissected from the skin, washed in water until the yellow color was largely removed, stained in Mayer's hemalum, washed with 1 per cent potassium alum, followed by further washing in water. The glands were then differentiated in 70 per cent acid alcohol (2 per cent  $\text{HCl}$ ) until the color in the connective tissue had been removed, showing the glands in sharp contrast to the light background, dehydrated in a series of alcohols, and mounted in balsam on slides.

The sex of the fetuses was determined by studying the developing reproductive organs. In the newborn and in later stages after birth, the sexes were determined by the relative anogenital distance described by Jackson (1912) and further corroborated by dissection of the reproductive organs after the skin containing the glands was removed.

Since the pubertal age varies widely with the individuals (ranging from 50 to 70 days), the opening of the vagina was used as a criterion for determining the onset of puberty. The animals were smeared immediately after the opening of the vagina and at estrum (indicated by positive vaginal smear) a gland was removed and daily thereafter throughout the entire length of the estrus cycle. In securing the gland, the animals are placed under ether anaesthesia.

### OBSERVATIONS

The growth and development of the mammary gland has been divided into the following stages:

I. Embryonic and fetal development. This stage includes all phases of development from the earliest appearance of the anlage of the mammary streak to the formation and canalization of the primary mammary sprout (duct).

II. Prepubertal. During this stage the canalization of the primary sprout (duct) is completed, followed by extensive growth and progressive canalization of the secondary, tertiary, and quaternary sprouts in the females. In the male, the primary sprout (duct) remains rudimentary throughout normal life.

III. During the recurring estrus cycles. This stage is characterized by cyclic changes in the mammary-duct system following the ovarian cycle. No alveoli were formed either at the first or succeeding cycles.

IV. Pseudopregnancy. Following a sterile coitus the luteal phase of the estrus cycle is lengthened to about 10 days. When this occurs the hyperplasia of the ducts and lobules is observed comparable to that observed during the first half of pregnancy.

V. Pregnancy. The first half of the gestation period (10 days) is characterized by the hyperplasia of the ducts and lobules constituting the mammary gland. During the second half of pregnancy the growth phase is succeeded by the gradual initiation of secretion causing first the enlargement of the epithelial cells of the alveoli, then the lumen with the discharged secretion. At parturition the gland has become greatly hypertrophied due to the presence of milk.

VI. Lactation and involution. The mammary glands remain essentially the same anatomically, except for the periodic secretion and discharge of the formed milk during lactation. Following weaning, the presence of the formed milk causes a rapid decline of milk secretion, followed shortly by resorption of the milk and eventually involution of the gland.

The present paper of the series will deal with the embryonic and fetal development of the mammary gland, while a second paper will describe the extra-uterine growth and development.

## EMBRYONIC AND FETAL DEVELOPMENT

**Mammary Streak.**—The development of the mammary gland begins quite early in embryonic life. Kallius (1897), Strahl (1898), and Hirschland (1899) noted the enlargement of the cells of the *stratum germinativum* in human embryos extending from the anterior-limb buds to the posterior-limb buds. In cross section these single-layered cells are larger and somewhat more elongated than those of the adjacent epidermis. This, the earliest stage in the development of the mammary apparatus, has been called the mammary band or streak. In the albino rat, Henneberg (1900) observed the early appearance of the mammary streak in embryos of eleven days' gestation.

In the albino mouse, the earliest indication of the initiation of the development of the mammary apparatus appeared in 10-day-old embryos. In a cleared preparation of the whole embryo there was observed on one side in the region of the dorsal limiting furrow a narrow light streak extending from the anterior-limb bud to the anlage of the posterior appendage (Fig. 1). Histologic examination of sections through this streak revealed that it was composed of a single layer of irregularly arranged cuboidal cells. In all probability, these cells compose the mammary streak (Figs. 3 and 4).

In 11-day embryos, the streaks, now observable on both sides, are more conspicuous (Fig. 2). However, sections through these structures indicate that the embryonic ectoderm or Malpighian layer is still composed of a single layer of regularly arranged cuboidal cells which are distinctly larger and somewhat more elongated than those of the adjacent epidermis (Fig. 5).

**Mammary Line.**—The mammary streak is a very transient stage in the development of the mammary glands. Soon the cells begin to proliferate. This process continues until several layers are present. This stage of development was first discovered by Schultze (1892) in the 1.5 cm. stage of swine embryos. Similar structures have since been observed in the early development of many mammals.

In the 12-day embryos, the original faint streak has been converted into a very definite mammary line, which is slightly elevated above the surface of the epidermis. In cross section, the mammary lines show definite signs of growth. In place of the two-layered epidermis composed of the periderm and the *stratum germinativum*, there has now formed by the proliferation of the latter layer, a number of intermediate cells which has caused the Malpighian layer to press downward slightly into the mesenchyme, causing a slight condensation and deeper staining appearance of the latter. Farther away from the line, the cells thin out very gradually, producing a lighter staining tissue. There is a distinct

basement membrane separating the mammary line from the underlying mesenchyme (fig. 6).

The mammary lines are generalized structures which mark quite distinctly the location of the future teats and glands in so far that the teats are always found in orderly linear arrangements corresponding to the position of the lines.

**Mammary Bud.**—With the completion of the proliferation of the mammary lines, the generalized nature of the mammary apparatus development is completed. The new departure in the type of development is first noted by the unequal proliferation of the cells of the stratum germinativum at intervals along the lines. This growth results in the appearance of slight elevations which have been designated as "*mammary hillocks*." It is the first of a series of stages in the development of the mammary bud.

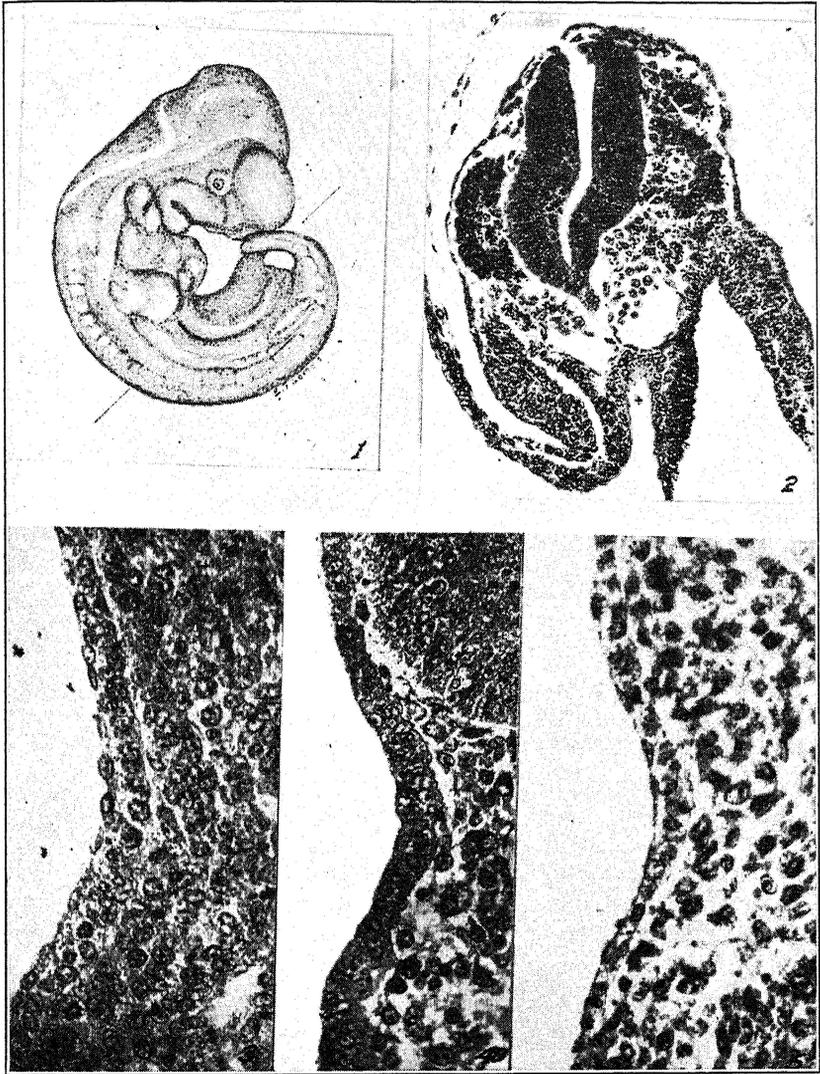
While Langer (1851), Kölliker (1859), Huss (1873), and Gegenbaur (1876) observed stages in the formation of the structure now called the mammary bud, the observations of Rein (1881) on the various stages of bud formation in the rabbit were the first to show that these are simply stages of development of the "Hügelförmige Anlage". The term mammary hillock (Milchhügel) was first used by Bonnet (1892), while Schultze (1892) used the distinctly misleading term "primitive teats". Kallius (1897), a little later, described a slightly earlier stage as the "mammary crest."

In the albino rat, Henneberg (1900) observed the formation of a lens-shaped structure in the cephalic end of the mammary line in a 14-day-old embryo. In other embryos of the same age the second and third pectoral hillocks were beginning to appear. In a 15-day, 9-hour rat embryo, Myers (1917) observed the formation of the complete bud.

In the albino mouse, the hillock stage was already well established in the thoracic region of a 13-day embryo. Histologically, this structure appeared in cross section as a half-moon-shaped mass of cells which project only slightly above the surrounding epidermis, whereas it describes a half circle in contact with the mesenchyme. (Fig. 7). With the proliferation of cells to form an intermediate stratum, the basement membrane had been forced deeply into the mesenchyme tissue.

The number of mammary hillocks corresponds with the number of normal and supernumerary teats or glands in a given species. In the albino mouse there are normally five mammary hillocks in each line, three in the region of the thorax and one each in the abdominal and inguinal regions.

As the mammary hillocks develop, the parts of the mammary lines intervening disappear gradually, leaving the isolated hillocks to represent the anlage of the mammary glands. These structures continue



## Plate I.—Explanation of Figures

Fig. 1. Side view of a 10-day-old albino mouse embryo, showing the mammary streak. In a cleared preparation the mammary streak appeared as a narrow streak of thickened epithelium in the region of the dorsal limiting furrow extending from the axilla to the anlage of the posterior appendage on only one side. X 8 $\frac{1}{2}$ .

Fig. 2. Microphotograph of a section through the mammary streak in the region anterior to the anlage of the posterior appendage in an 11-day-old embryo. The mammary streak which is present on only one side in a 10-day-old embryo now appears on either side. X 42.

Fig. 3. A section through the mammary streak of a 10-day-old embryo at the level indicated in figure 1. X 107.

Fig. 4. A section of the mammary streak of a 10-day-old embryo in the region of the anlage of the posterior appendage. X 107.

Fig. 5. A section of the mammary streak of an 11-day-old embryo at approximately the level indicated in figure 1. X 107.

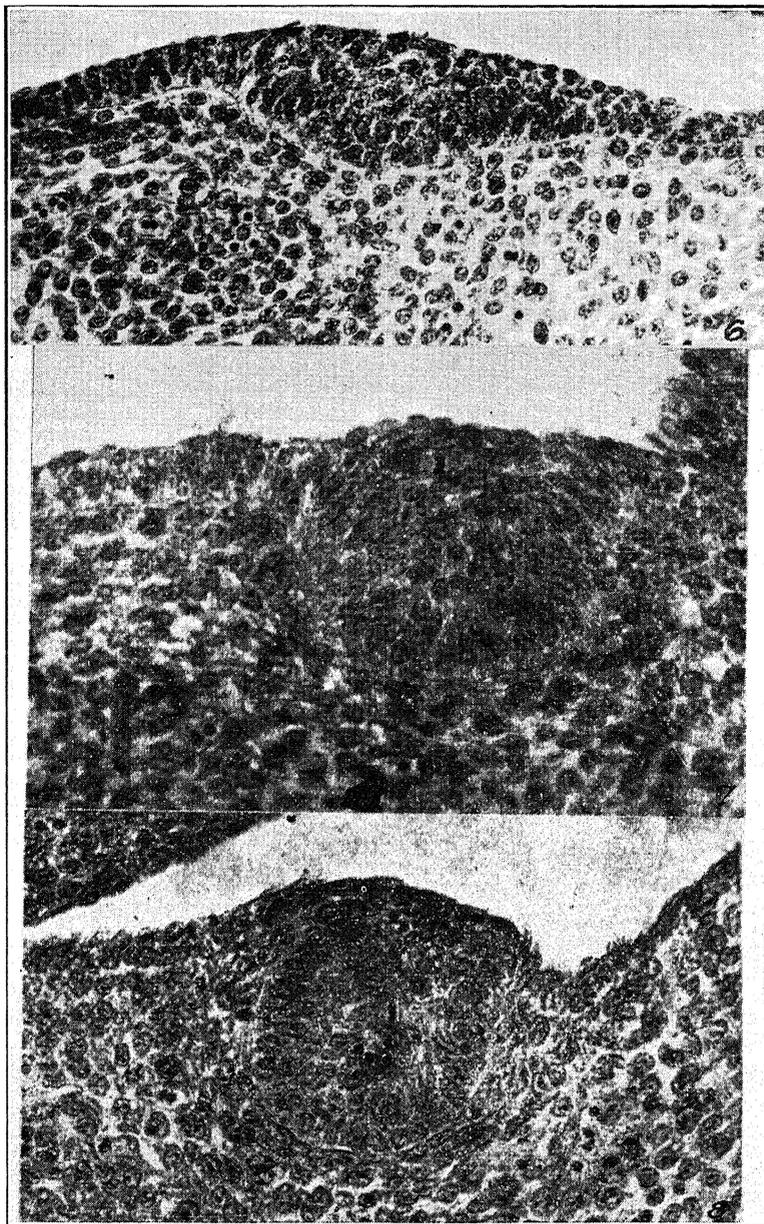
to proliferate and become rounded off and at the same time sink farther into the underlying mesenchyme. Thus in 14-day-old fetuses the mammary hillocks have become transformed into circularly formed mammary buds, although they have not become completely invaginated (Fig. 8). The basement membrane enclosing the bud is quite distinct. The mesenchyme cells below are closely packed together, probably due to the pressure from the invaginating bud. These cells are circularly disposed with their long axes parallel to the basement membrane.

The buds undergo progressive development by sinking deeper into the mesenchyme in 15-day fetuses. At this time the superficial surface of the buds are on a level with the surrounding epidermis. The buds, now completely embedded in the mesenchyme, are either ovoid or spherical in shape and are in contact with the epidermis only at the outer poles. This stage marks the completion of the development of the mammary bud. From the 15th day until the 17th day, the buds continue to sink into the mesenchyme, but maintain contact with the surrounding epidermis by a rather constricted neck filled with cornified cells (Fig. 10). The slight depression of the epidermis over the bud has been called the mammary pit. In the epidermis adjacent to the mammary buds, hair buds are beginning to form.

Histologically, the mammary bud is composed of the same cellular elements as the mammary hillock, namely, the Malpighian layer resting on a distinct basement membrane and with the central cell (intermediate cell layer) arranged in rows with the long axes of the cells pointing toward the center of the bud already showing the first signs of cornification.

The mesenchyme cells beneath the basement membrane are densely packed together, forming definite layers. Farther away, the cells are less numerous and irregularly arranged. Several small blood vessels are seen coursing toward the mammary-gland area.

**The Primary Sprout in the Female.**—Until the 16th day of intra-uterine life the sex cannot be distinguished. Therefore, the description of the anlage of the mammary apparatus up to this time is applicable to either sex. At this time the sex can be differentiated, although with some difficulty. In the 17-day female fetus, the mammary bud has increased in length slightly (Fig. 12). Its deep part becomes the anlage of the primary sprout, while its superficial portion gradually undergoes vacuolization, cornification, and desquamation. In the 18-day stages observed, the primary sprout had not yet definitely appeared in the inguinal buds, but in some of the pectoral buds early stages of this new development were observed. From the distal end of the bud, which has been changed gradually into a funnel-shaped structure, a primary sprout was observed (Fig. 14). At 19 days, the sprout showed considerable growth in the nature of a solid bud formation directed downward. The free end



## Plate II.—Explanation of Figures

Fig. 6. The mammary line. Photomicrograph of a section through the mammary line in the pectoral region of a 12-day-old embryo. X 130.

Fig. 7. The mammary hillock. Section through the inguinal region of a 13-day-old fetus. X 130.

Fig. 8. The mammary bud. Section through the pectoral region of a 14-day-old fetus. X 130.

of the primary sprout presented a marked bulbous enlargement in comparison with the proximal end.

Histologically, the primary sprout at this stage possesses a basal layer of cuboidal cells with deeply staining ovoid nuclei. The basal ends of the cells rest on a somewhat indistinct basement membrane (Figs. 15 and 16). The inner portion of the sprout is filled with irregularly shaped cells. As the sprout lengthens and widens out there appears to be a tendency for the central cells to separate, forming a small cavity or lumen. The canalization of the primary sprout to form the primary milk duct proceeds from the distal to the proximal end. Thus the sprout adjoining the rudiment of the mammary bud is last to canalize (Fig. 18).

The upper layers of the epidermis surrounding the residue of the mammary bud which was called the mammary pit in earlier stages is composed of cornified epithelial cells. These cells are gradually cast off by a process of desquamation.

The formation of secondary sprouts was first observed in 20-day fetuses. They appear as solid spherical buds at the terminal (free) end of the primary sprout. No further change in the sprouts up to the time of birth occurs.

**The Primary Sprout in the Male.**—The initial development of the primary sprout in the male may be observed somewhat earlier than in the female fetus. In fresh and cleared preparations of 16-day fetuses, the depression of the epidermis over the mammary bud corresponding to the mammary pit is quite distinct. In cross section there is no indication of the development of the primary sprout; however, the mammary bud has continued to sink deeper into the mesenchyme tissue similar to that observed in the females of the 17-day stage (Fig. 11).

In the 17-day fetuses, the depression above the area of the developing gland is replaced by a slight eminence of cornified cells causing the epidermis at that point to be somewhat lighter than the adjacent epidermis. In sections of the anlage of the gland at this stage is seen an oblong mass of epithelial cells entirely surrounded by a distinct basement membrane located at the distal end of the mammary bud (Fig. 13). This is believed to be the primary sprout which has become detached from the residue of the mammary bud. The intermediate cell layers are loosely packed and are of irregular shape, showing a tendency to separate from each other, thus indicating early stages of canalization. In general, the formation of the lumen in the primary sprout is essentially the same as in the female.

The mammary bud is rapidly undergoing cornification and desquamation so that in 18- and 19-day fetuses indications of the rudiment of the bud are seldom observed. Instead, the epidermis above the

detached primary duct becomes continuous and slightly elevated in comparison with the adjoining epidermis. At the same time connective tissue is beginning to form from the mesenchyme tissue between the upper end of the primary sprout (duct) and the epidermis. The sprout appears as an isolated structure embedded in the corium (Fig. 17). Hair anlage are forming in the epidermis in the immediate neighborhood above the sprout. It may now be more properly called a duct, as a definite lumen is forming. In 20-day fetuses small solid secondary sprouts have developed along the primary duct which has markedly increased in length, being many times greater than its breadth.

**Individual Variation in Gland Development.**—Variations in the rate of development of the mammary gland are of two sorts. There may be variations occurring among individuals in a given litter as well as among the glands of an individual. In the present study observations were made of the mammary glands of all individuals in each litter collected as well as comparing the development of the anterior and posterior glands.

The extent of variation in gland development in the albino rat has been reported by Myers (1917). Similar variation has been noted in the present study. Thus of the six mouse embryos of 10 days gestation collected in a litter, four showed rudimentary mammary streaks, while in the other two they were still absent. Of the seven embryos of the 11-day stage, two showed a well-developed mammary streak on both sides, while in three the mammary streak was present on one side only. In one embryo of this litter the mammary streak had undergone proliferation, forming a definite mammary line.

In embryos of the 12-day stage, well-developed mammary lines were observed in four individuals, while in one the development was somewhat delayed. Of the 7 fetuses collected in the 13-day stage, the glands of four had reached the mammary-hillock stage, one still showed only the mammary line, while one was in the early bud stage.

No significant differences other than the size of the buds were observed in fetuses of 14, 15, and 16 days. At the latter age, however, the sex could be determined and some sex differences were noted.

In most of the fetuses 19 days old, the primary sprout was present. In one fetus of this age a solid outgrowth was observed at the terminal end of the sprout, indicating the early development of the secondary sprout. In most of the 20-day fetuses the anlage of the mammary glands were composed of single unbranched primary sprouts, while in a few cases secondary and tertiary sprouts were beginning to appear. At this stage, the lumen of the ducts begin to form in some fetuses, while in others no trace of the lumen was observed. The lumen usually continues

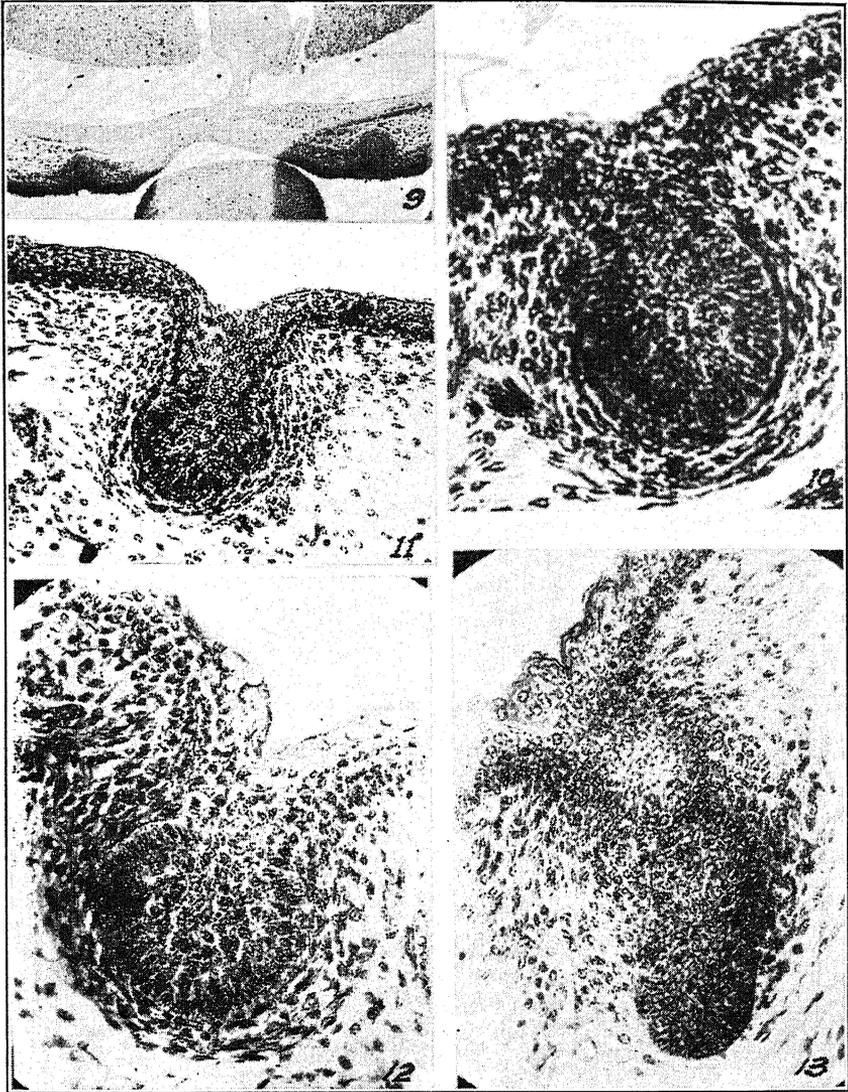


Plate III.—Explanation of Figures

Fig. 9. Section through the inguinal region of a 15-day fetus, showing the position of the pair of mammary buds. X 10.

Fig. 10. Photomicrograph of left mammary bud shown in figure 9. X 130.

Fig. 11. A section through the third pectoral bud of a male fetus 16 days old. X 130.

Fig. 12. A section through the third pectoral bud of a 17-day-old female fetus. X 65.

Fig. 13. A section through the third pectoral mammary bud of a male fetus 17 days old. X 65.

to develop so that at parturition a considerable cavity in some parts of the primary duct may be observed.

Variation in the development of the individual gland anlage was observed by Schultze (1893) in early cat and fox embryos. The early growth and development of the anlage of the mammary apparatus appears to be initiated at the cephalic end and progresses toward the caudal end of the embryo. In the rat, Henneberg (1900) and Myers (1917) observed a similar condition. In the mouse, the most striking difference in development was observed in embryos of the 12- and 13-day stages. At the 12-day stage, the cephalic pairs of mammary anlagen were found to be in the hillock stage, while those of the abdominal and inguinal pairs were still in the undifferentiated or mammary-line stage.

In the 13-day embryos, the cephalic pairs of anlagen are found in a well-defined mammary bud stage, while those of the inguinal pairs are still in the hillock stage. In the 14-day stage, however, there are no significant differences in development between cephalic and inguinal pairs of the mammary buds. Similarly, the first development of the primary sprout appeared in the mammary buds located in the pectoral region. In general, the development of the mammary glands during each succeeding stage of growth and differentiation is observed first in the cephalic end and then proceeds toward the caudal end of the mammary line. Following birth, however, these differences, if present, cannot longer be observed.

**Nipple Development.**—The mode of teat or nipple development has received a great deal of attention in the past, because of the theory advanced by Gegenbaur (1876) that the cow's teat differed from that of the human in that the entire excretory duct of the cow's teat, including the cistern of the gland was formed from the mammary bud, while in the human gland, the floor of the bud rose and formed the upper surface of the nipple. It was later shown that the supposed differences in the mode of nipple formation were due to inaccurate observations on the formation of the primary sprouts. Careful observations of the mammary-gland formation in a large series of animals have indicated that the single primary sprout in ruminants forming a single duct in the teat and the multiple sprouts in certain other animals forming multiple excretory ducts are completely homologous structures.

It seems evident from the studies of a number of species of animals that the part played by the mammary bud and the primary sprout or sprouts in the development of the nipple are quite similar. As a consequence, there is no real basis for a classification of teat development dependent upon the mode of primary sprout formation as postulated by Gegenbaur and others.

Comparative studies of nipple development are believed by the writers to reveal three distinct types which have not been previously classified. While a more detailed presentation of the classification of nipples or teats upon their mode of formation is contemplated, a brief statement is made here because it will aid in explaining the position of the mouse in the classification.

(a) *Eversion teat.* In certain marsupials there has been described a condition in which the teat anlage is formed at the base of a pocket which becomes everted to form the definite teat (O'Donoghue, 1911).

(b) *Proliferation teat.* The proliferation teat is formed early in fetal life as a result of the proliferation of the mesenchyme cells surrounding the mammary bud, thus raising the bud and forming a distinct teat or nipple. This type of teat formation has been observed in most Monodelphia, with the exceptions noted below.

(c) *Epithelial ingrowth teat.* In this case the teats or nipples are formed by epithelial ingrowths surrounding the bud and primary sprout which sink for a considerable distance into the mesenchyma forming a teat deeply embedded rather than raised above the surface. This type of teat development occurs in the rat and mouse. In the albino rat, Myers (1917-19) observed the epithelial ingrowths which he described as an epithelial hood. Apparently, however, the relation of these ingrowths to the formation of the teat were not understood, since he states, "As to the significance of the epithelial hood in the albino rat I have as yet reached no definite conclusion." As will be shown in the following description of the nipple development of the albino mouse, the epithelial ingrowths play an important role in the nipple formation.

**Nipple Development in the Mouse.**—In the albino mouse the earliest indication of the nipple formation becomes evident in fetuses of 18 days. This is indicated in sections by the development of epithelial ingrowths a short distance from the mammary bud forming a shallow furrow or sulcus around the anlage of the gland (Fig. 14). In the 19-day stage the invagination of the malpighian layer has grown considerably, now appearing in sections as a pair of short solid epithelial thickenings on either side of the primary sprout (Fig. 15). This ingrowth is continuous around the mammary bud and primary sprout. Because the entire structure is slightly sunken, the cornified cells accumulate over the nipple anlage as well as at the sides in the invagination, thus giving the structure the appearance of a cap or hood around the developing gland. This structure, called by Myers the epithelial hood, was observed earlier by Gegenbaur (1876), Rein (1882), and Klaatsch (1884). The writers believe that the term epithelial hood is not well chosen. Rather the

emphasis should be applied to the epithelial ingrowth which forms the nipple at the expense of the underlying mesenchyme tissue.

The inner and outer surface of the ingrowth is lined with a smooth-layered stratum germinativum which becomes continuous with the surrounding integument. The epidermis over the nipple area is nearly twice as thick as the adjoining epidermis, due to the cornified layers. This renders the skin over the developing nipple opaque and causes the nipple area to appear much lighter than the surrounding skin upon gross inspection.

From the 20-day stage to the end of the first week of postnatal life, the epithelial ingrowth continues to sink into the corium as well as to increase in thickness (Fig. 18). Hair follicles are very numerous in the skin around the epithelial ingrowth, but in no case was hair observed inside this area. By the end of this period the stratum germinativum of the inner wall of the ingrowth outlines quite definitely the future nipple the apex of which is becoming slightly elevated above the surface of the surrounding skin.

At the beginning of the second week, the cornified cells of the central portion of the epithelial ingrowth begin to degenerate. This, coupled with the growth of the hairs lateral to the nipple area, renders the nipples conspicuous to the naked eye. However, the nipple soon becomes completely masked by the profuse growth of hairs.

The process of desquamation of the cornified cells of the ingrowth continues so that about the middle of the fourth week the sides of the ingrowth are gradually separated, the outer border becoming part of a mammary sulcus surrounding the base of the teat and the inner border becoming the wall of the nipple. The apex of the nipple is slightly elevated above the surrounding skin.

The nipples appear to change little for a time until the sixth week when the nipples begin to protrude from the skin in a conspicuous manner. This change is probably due in part to a growth of elements of the nipple as well as an elevation of the base. These changes coinciding with the approach of puberty and the first estrus cycles are probably due to the estrogenic hormone, for there is a rapid growth of other elements of the gland as well.

Nipples are usually present in most species in the male as well as the female. In the rat and mouse, Schickele (1899) noted the absence of nipples in the male. Because of this absence the development of the mammary apparatus in the male during the period when the nipple is forming in the female is of great interest.

In male fetuses of 17 days the surface of the skin over the developing gland is somewhat lighter than the surrounding epidermis. In some

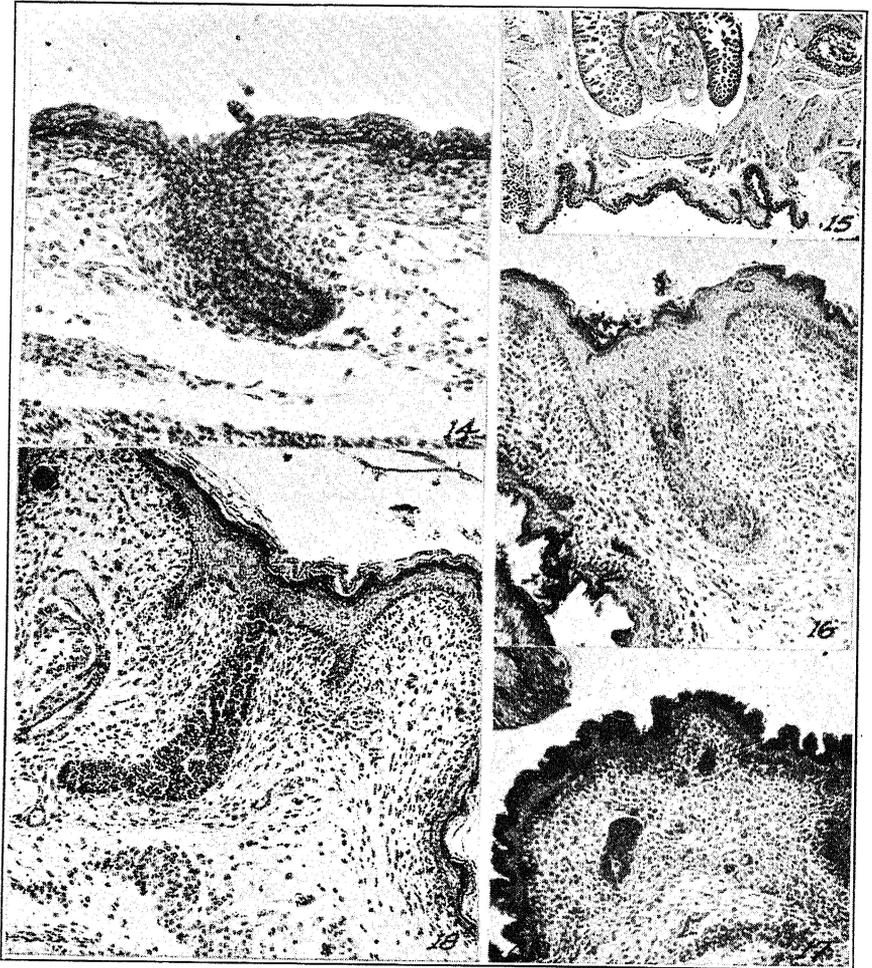


Plate IV.—Explanation of Figures

Fig. 14. Section through the pectoral mammary-gland anlage of an 18-day-old fetus. The primary sprout may be seen developing from the distal end of the mammary bud which is gradually becoming changed into a funnel-shaped structure. It is also at this stage that the anlage of the epithelial ingrowth, a thickening of the ectoderm immediately surrounding the proximal end of the bud becomes evident. X 81.

Fig. 15. Section through the inguinal glands of a 19-day-old fetus. A secondary sprout has already formed on the distal end of the primary sprout of the left gland. The one to the right still remains as a club-shaped structure. The epithelial ingrowth has sunk slightly deeper into the mesenchyme. X 40.

Fig. 16. Photomicrograph of the right gland shown in figure 15. The rudiments of the mammary bud are still present. The epithelial ingrowths appear as a pair of short solid epithelial thickenings. The depression above the developing gland is quite prominent at this stage. X 105.

Fig. 17. A section through the mammary gland of a 19-day male fetus. The primary sprout is detached from the skin, appearing as an isolated structure embedded in the mesenchyme. A lumen has already formed in the distal portion of the duct. X 105.

Fig. 18. Section through the inguinal gland of a 20-day-old female albino mouse. The epithelial growth is shorter and thicker than in the previous stage. A secondary sprout composed of a solid mass of epithelial cells is developing from the distal end of the primary sprout. Small cavities are being formed in the middle of the primary sprout as a result of the rearrangement of the cells in the formation of a lumen. X 105.

instances the area above the developing mammary gland can be recognized. From the 19-day stage throughout postnatal life there is no external indication of the mammary glands.

As already observed, in the male mouse the primary sprout becomes detached from the mammary bud in the 17-day stage and appears as an isolated structure in the corium. The sprout begins to canalize at the 19-day stage and is practically complete at the time of birth. The residue of the mammary bud soon loses its identity and the epidermis over the primary sprout soon becomes continuous with the adjacent skin. The epithelial ingrowth which takes part in nipple development in the female does not form.

### SUMMARY AND CONCLUSIONS

1. The earliest indication of the development of the mammary apparatus of the albino mouse appeared in 10-day-old embryos. At this time a narrow light streak was observed on one side composed of a single layer of cuboidal cells, which extends from the anterior-limb bud to the anlage of the posterior appendage, which is believed to be the mammary streak.

2. In 12-day embryos, the original faint streaks have been converted into very definite mammary lines composed not only of the periderm and stratum germinativum, but also of a number of intermediate cells.

3. At intervals along the lines, further proliferation of the stratum germinativum results in the appearance of slight elevations which have been called mammary hillocks. These half-moon-shaped masses of cells were observed in the thoracic region of a 13-day embryo. The mammary hillock continues to proliferate and become rounded off to form mammary buds by the 14th day. From the 15th day until the 17th day the buds become embedded in the underlying mesenchyme tissue.

4. From the distal end of the mammary bud, which has been changed gradually into a funnel-shaped structure, a primary sprout begins to form during the 18th and 19th days in female fetuses. As the sprout lengthens and widens out there appears to be a tendency for the central cells to separate, forming a cavity or lumen which becomes the primary milk duct. Secondary sprouts were observed at 20 days.

5. In the male, the primary sprout was observed in 17-day-old fetuses. In this case the sprouts become detached from the mammary buds which rapidly undergo cornification and desquamation. The epidermis above the detached sprout become continuous, leaving no trace of the bud, while the sprout appears as an isolated structure embedded

in the corium. Thus there is formed an anlage of a duct system without a nipple.

6. Two types of variation in the rate of development of the mammary gland were noted. Considerable variation was found to occur among the individuals in a litter in the extent of development attained. Considering the development of the gland anlagen in given embryos, it was found that each succeeding stage of growth and differentiation was observed first in the cephalic end and then proceeds toward the caudal end of the mammary line.

7. The mouse is similar to the rat in the peculiar type of nipple development. Instead of a proliferation nipple, the nipple is formed by epithelial ingrowths surrounding the bud and primary sprout which grow for a considerable distance into the mesenchyme tissue, forming a nipple deeply embedded rather than raised above the surface. Only at puberty and during pregnancy do the teats extend above the surface.

## II. EXTRAUTERINE

C. W. TURNER AND E. T. GOMEZ

**ABSTRACT.**—Continuing the study of the normal development of the mammary glands of the male and female albino mouse, there is described the extrauterine changes associated with puberty, pseudo-pregnancy, pregnancy, lactation, and involution.

At the approach of puberty, the mammary ducts begin to grow rapidly in the female, whereas in the male the gland anlage remains rudimentary throughout normal life. During the recurring estrus cycles slight periodic growth takes place.

During the first half of pregnancy or pseudopregnancy, the hyperplasia of the gland parenchyma occurs, whereas after the middle of pregnancy the gland epithelium gradually starts to secrete causing an expansion and unfolding of the alveoli due to the accumulation of the products of secretion.

The removal of the products of secretion causes intense lactation, whereas following weaning, the accumulation of milk inhibits further secretory activity. There follows a gradual resorption of the milk in the lumen of the alveoli and ducts and a consequent progressive collapse of these structures so that on the tenth day the glands appear similar to those at the end of pseudopregnancy.

In the preceding section of this bulletin, the embryonic and fetal growth and development of the mammary gland of the albino mouse were described. The earliest evidence of the anlage of the gland—the mammary streak—was seen in the 10- and 11-day embryos. Soon the streaks were converted into paired mammary lines as a result of the proliferation of the Malpighian layer of the streaks. At intervals along the lines of 13-day embryos, the hillock stage of mammary bud development was observed. The mammary buds were rounded off by the 15th day but continued to invaginate slightly during the two days following. About the 18th or 19th day, the primary sprout begins to grow from the distal end of the bud, while secondary sprouts were starting to form by the time of birth. The epithelial ingrowths which form the teat appear on the 18th day and continue to sink into the mesenchyme for a period after birth.

**Prepubertal Development**—From the time of birth up to the end of the first week of extrauterine life the secondary sprouts of the female continue to grow and canalize to form ducts. Tertiary and quaternary sprouts are also seen. In section, the ducts are observed spreading out in a single plane (Figs. 1-2, 3). Immediately surrounding the ducts are a few concentric layers of connective tissue. The irregularly arranged connective tissue fibers between adjacent ducts may be regarded as representing a part of the gland stroma found in later stages (Fig. 2a).

In the males, the end of the primary sprout adjacent to the epidermis is still uncanalized. The other end of the sprout and the rudimentary secondary sprouts possess lumina slightly more advanced in development than in females of corresponding age. At this stage the mammary

duct ceases to grow and remains rudimentary throughout normal life (Fig. 33-34).

From the beginning of the second week until the approach of puberty the mammary glands of the female continue very slow growth indicated by an increase in length and number of ducts. Examinations of gross mounts of the mammary glands at various stages show one principal (primary) duct coming from the nipple which branches to form a pair of secondary ducts. In the early stages of growth, the ducts undergo a regular dichotomous type of branching. As growth progresses, new lateral branches sprout more or less irregularly from the chief ducts. In some cases secondary branches develop between the nipple and the bifurcation of the chief ducts (Figs. 8-12). At the distal end of each duct or sprout are saccular-like structures composed of a solid mass of epithelial cells. These structures, which are the points of active growth of the duct system, are called "end-buds". During the early stages of development, the end-buds are not conspicuous; however, with advancing age they become more and more prominent due to the deeply staining characteristic in Mayer's hemalum.

The formation of the lumen of the ducts in the female makes its appearance during the first 24 hours after birth. In sections of the ducts of the newborn, the intermediate cell layers of the primary sprout are loosely packed irregularly shaped cells which show a tendency to separate from each other.

Sections of the nipple and ducts taken the second day show a very slight depression at the apex of the developing nipple (Fig. 1). This pit, which represents the future opening to the nipple (the streak canal) deepens with age and becomes continuous with the lumen of the primary duct. During the 2-day stage, however, the upper end of the primary sprout is still composed of a solid group of cells. As the sprout is traced deeper, an occasional lacuna representing the first trace of lumen formation, is encountered. In some glands several lacunae have become joined, forming an irregular outline (Fig. 2). The cells lining the walls of the developing ducts which are three to four cells thick do not have a definite arrangement in layers. The secondary ducts possess more regular lumina with an indication that the cells are becoming arranged into layers. From the secondary ducts up to the terminal ends of the sub-branches, there is in most cases a fairly definite layer of cuboidal cells surrounding the lumen. The free ends of the ducts, called end buds, are composed of solid masses of epithelial cells.

The unique development of the nipple of the mouse has been described in the preceding paper. While considerable development occurs during the period from birth to puberty, it seemed better to treat the entire development at that point.

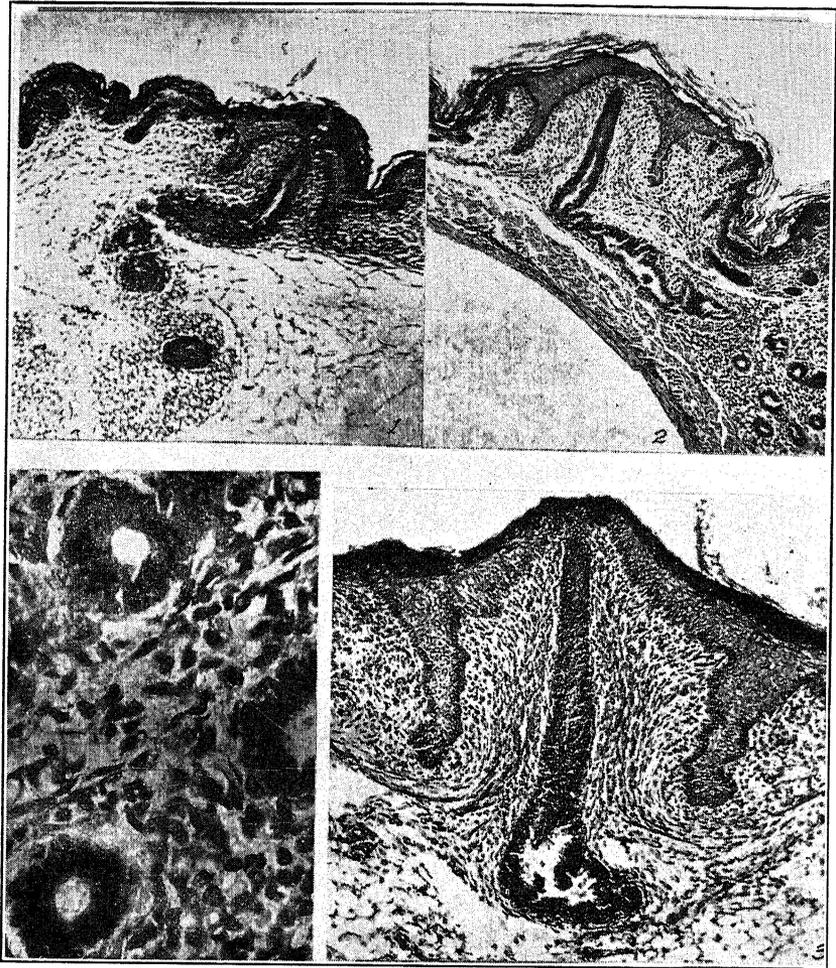


Plate I.—Explanation of Figures

Fig. 1. Section through the inguinal gland of a female mouse on the second day of postnatal life. The epithelial ingrowth had sunk deeper into the mesenchyme. A definite lumen has already formed in the primary sprout. Secondary and tertiary ducts are developing. X 40.

Fig. 2. Section through the inguinal gland of a female albino mouse on the fourth day of postnatal life. The epithelial ingrowth has sunk further into the mesenchyme. Secondary, tertiary, and quaternary ducts have formed. Definite lumina have formed and in some places have flowed together, forming a large irregular cavity. X 40.

Fig. 2a. Microphotograph of a section through the secondary ducts. Each duct is surrounded by a sheath of connective tissue, the future mantle layer. Between the ducts the dense connective tissue, the true gland stroma, may be seen. X103.

Fig. 3. A section through the nipple of an 8-day-old female albino mouse. X 40.

**During the Recurring Estrus Cycles.**—The growth of the duct system during the prepubertal period is only sufficient to keep pace with general body growth. At the approach of puberty, however, the mammary ducts begin to grow rapidly, resulting in a dense arborization. Sutter (1921) found in the rat a relation between the appearance of mammary glands and the stage of estrum. In the albino mouse puberty was usually reached during the eighth or ninth week.

Macroscopic examination of the mammary glands in various stages of the estrus cycle also showed distinct changes. During proestrus the ducts of the gland exhibit long slender branches which have a few light staining end buds along their course. At estrum the small end buds show evidence of growth and new end buds appear. The end buds at this time stain deeply and the ducts show marked distention with fluid (Fig. 13). If pregnancy or pseudo-pregnancy does not occur following ovulation, the glands undergo slight retrogressive changes; however, as the next estrum approaches the glands again show evidence of proliferation (Fig. 15). With each succeeding estrus cycle the growth of the ducts is slightly extended. However, these proliferative changes are more obvious during the first few cycles than later when the growth of the duct system is more extended.

A typical picture of the histology of the mammary gland during puberty was seen in a 61-day old female. The glandular parenchyma at this time consisted of ducts and end buds which were scattered throughout the stroma of fatty connective tissue. The end buds borne at the terminal ends of the ducts and branches consist of solid masses of epithelial cells in the form of a capsule. The lumen of the duct adjacent to the end bud is wider than at other parts of the duct, except in places where branching occurs. The walls of the ducts are composed of irregular layers of cuboidal cells two or three cells thick (Fig. 16).

**Pseudo-pregnancy.**—In animals which ovulate only after coitus as well as in certain animals which have regular estrus cycles, a sterile coitus causes either ovulation and the formation of corpora lutea, or the lengthening of the luteal phases of the estrus cycle. This condition, first described by Ancel and Bouin (1911) in the rabbit, has been called pseudo-pregnancy. The condition has since been observed and the development of the mammary gland described in a number of mammals. Considering the development of the mammary gland, there are two types of pseudo-pregnancy, complete and incomplete. In the former type illustrated by the ferret (Hammond and Marshall, 1930) pseudo-pregnancy continues for the same time interval as normal pregnancy with full growth and the initiation of lactation of the mammary gland corresponding to the condition at parturition. In incomplete pseudo-pregnancy illustrated

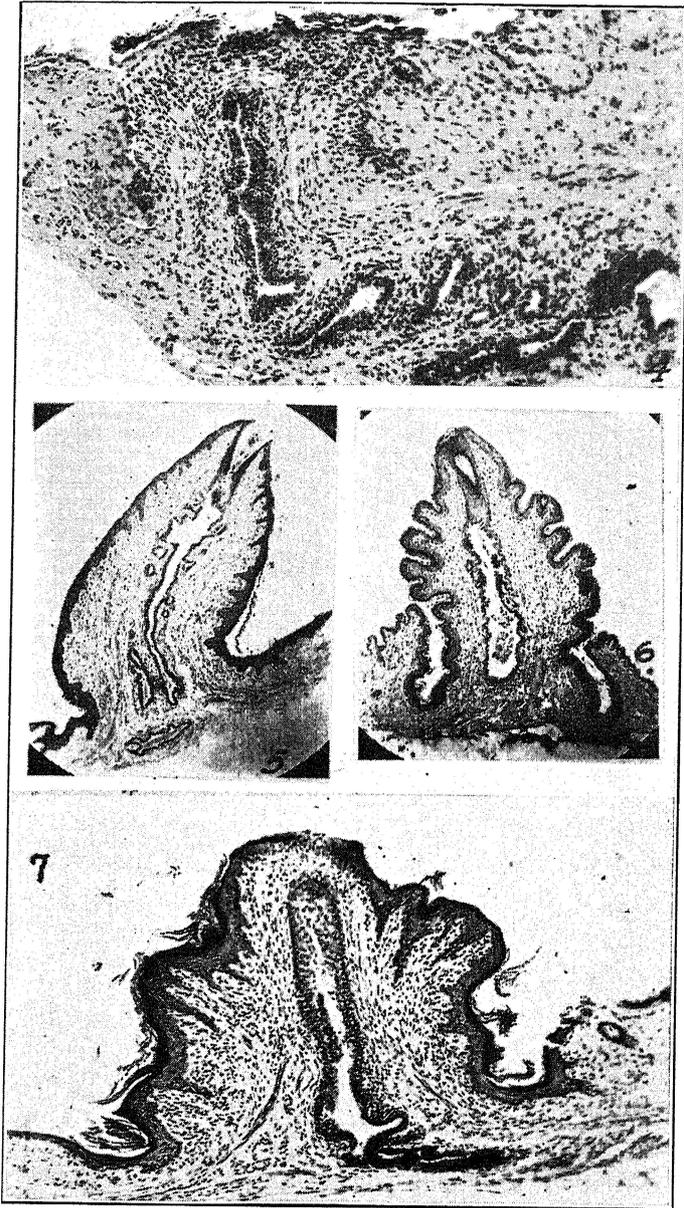


Plate II.—Explanation of Figures

Fig. 4. Microphotograph of a section through the inguinal gland of a 26-day-old female. The cells of the epithelial ingrowth are in the process of degeneration and desquamation. X 130.

Fig. 5. Section through the inguinal nipple during the period of first estrus (61 day old female). At this stage the nipple tissue is quite turgid and the epithelial ingrowth cells are completely cast off so that the base of the nipple is raised somewhat above the surface of the skin. X 40.

Fig. 6. Section of the nipple of a female albino mouse during the period of lactation. The base of the nipple when at rest is sunk below the surface of the skin and thereby forms a deep sulcus around the nipple. The relaxed tissue caused various invagination of the outer wall of the nipple. X 40.

Fig. 7. Section through an inguinal nipple during involution. Retrogressive metamorphosis had taken place so that at this stage the nipple appeared somewhat tortuous and shrunken. Cornified cells beginning to accumulate in the shallow sulcus around the base of the nipple. As in virgin females, these cornified cells are cast off in the ensuing estrus cycle and in pregnancy. X 130.

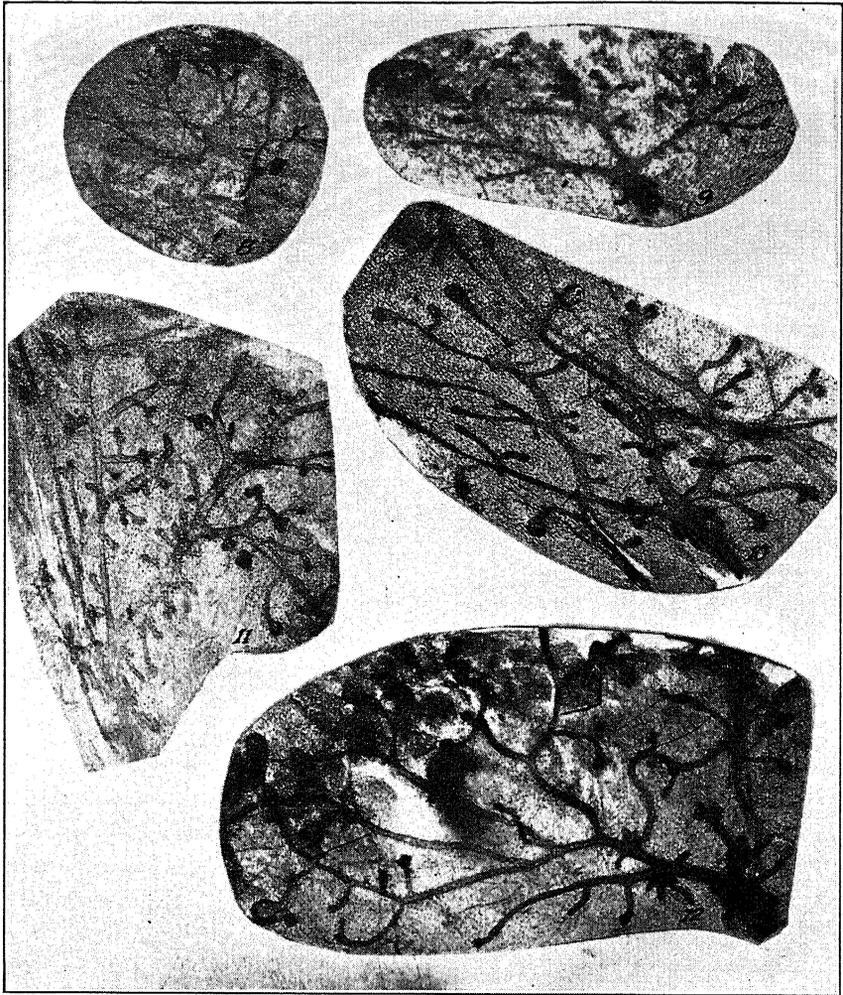


Plate III.—Explanation of Figures

Fig. 8. Mammary gland of an 8-day-old female. Secondary branches arise from the primary duct and from which arises numerous sub-branches in a dichotomous manner. X  $2\frac{1}{2}$ .

Fig. 9. Mammary gland of a 15-day-old female. The ducts are much broader, longer, and with marked deeply staining end-buds. X  $2\frac{1}{2}$ .

Fig. 10. Mammary gland of an 18-day-old female. The ducts have further increased in length and number. The end-buds are more prominent than in the preceding stages. X  $2\frac{1}{2}$ .

Fig. 11. A 36-day-old female gland. X  $2\frac{1}{2}$ .

Fig. 12. A 48-day-old female gland. X  $2\frac{1}{2}$ .

by the rabbit and rat, the condition continues for about half the normal period of pregnancy and only the growth phase of mammary gland development occurs.

A condition of incomplete pseudo-pregnancy occurs in the albino mouse. Following a sterile coitus during estrum, the corpora lutea persist for a period of approximately 10 days instead of 3 or 4 days during a normal estrus cycle. During this period there is initiated a rapid hyperplasia not only of the duct system but of the gland lobules as well. The extent and character of the growth observed is entirely comparable to that observed during an equal interval during pregnancy (Fig. 31). However, the lactation phase initiated during the second half of pregnancy is not produced.

**Pregnancy.**—The mammary gland duct system of the albino mouse becomes slightly extended during each estrus cycle after reaching puberty. After conception, however, instead of the slight regressive changes which normally take place when pregnancy does not occur, the mammary glands enter upon a new phase of growth.

The histological changes in the mammary gland of the rat during pregnancy have been described by Roberts (1921), the cytological changes by Weatherford (1929), and the changes observed in gross mounts of the entire gland by Turner and Schultze (1931). The present study has been confined to the observations of gross mounts of the entire glands and certain histological changes associated with the formation of the gland lobules and alveoli. In general the development of the mouse gland during pregnancy is quite similar to that described in the rat.

In whole mount preparations of the gland of a mouse pregnant 7 days (Fig. 17) a new type of outgrowth or budding from the terminal ends and lateral walls of the mammary ducts and sub-branches may be observed. These latter buds appear quite different from the buds which sprout new branches of the duct system in virgin glands. They differ both in position and morphology. In the case of the budding of branch ducts, the point of bifurcation is almost as wide as the diameter of the bud, consequently they appear as spherical structures. These branches develop most frequently at the ends rather than along the sides of the ducts. In the case of the anlage of the lobes, the buds are somewhat elongated with a constricted neck forming the attachment to the wall of the duct. These buds develop in great numbers along the wall of the ducts as well as at the terminal ends.

The buds representing the anlage of the lobes of the gland increase considerably in length to form the intralobar duct of the future lobe. At the terminal end and lateral walls of this duct appear secondary bud

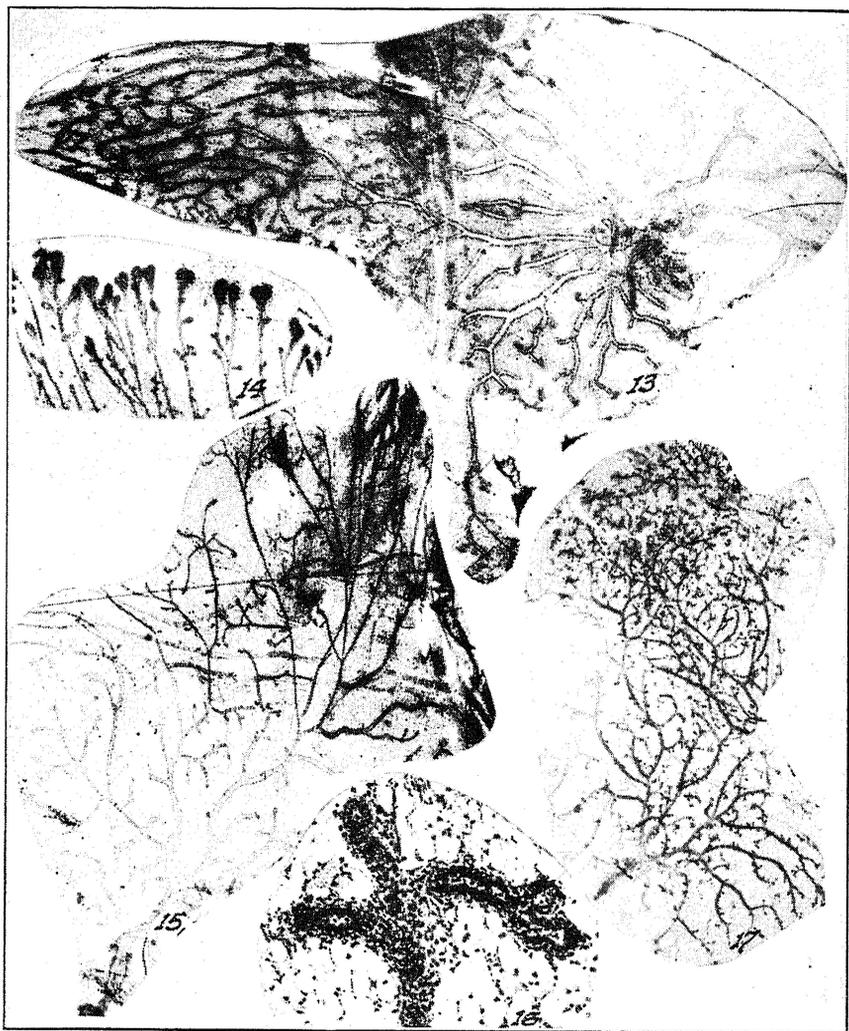


Plate IV.—Explanation of Figures

Fig. 13. Mammary gland of a female albino mouse during the period of first estrus (61 days old). The ducts are well distended with secretion. X  $2\frac{1}{2}$ .

Fig. 14. Microphotograph of the end-buds found in the gland during the period of estrus. X 10.

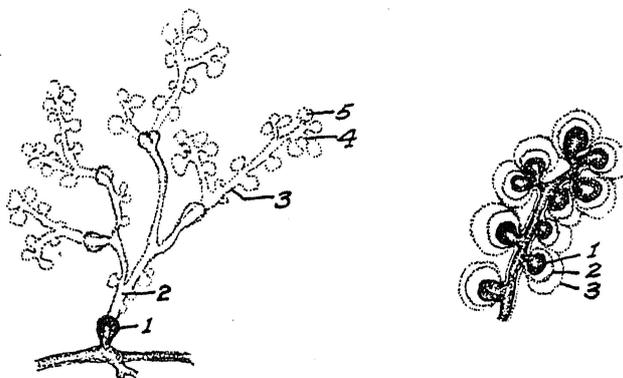
Fig. 15. Mammary gland during anestrus. The gland parenchyma is composed of long slender ducts with deeply staining end-buds. X  $2\frac{1}{2}$ .

Fig. 16. Section through the duct and end-buds. The end-buds are composed of solid masses of epithelial cells forming capsules at the ends of the ducts. The lumen of the buds are wider than those of the ducts. X 113.

Fig. 17. Microphotograph of a 7-day pregnant gland. The ducts are much longer and slender however, with numerous lateral bud outgrowth along the course of the ducts and its sub-branches. X  $2\frac{1}{2}$ .

outgrowths in the gland of an animal pregnant 10 days, from three to five of these buds may be observed at the end of such an intralobar duct. These buds again develop into short interlobular ducts along and at the end of which more buds appear. These latter buds become the alveoli. Thus at the end of 12 days of pregnancy a large number of alveoli buds are present on each of the intralobular ducts. At this time the hyperplasia of the gland parenchyma is complete.

While there are considerable variations in the course of development the typical lobe-bud develops from the wall of the duct, forming an intralobar duct. Usually three (interlobular) ducts branch to form lobules. Each of these ducts (the intralobular) further branch dichotomously and each sub-branch bears from four to nine solid alveoli-buds. The entire development is called a lobe composed of several lobules and they in turn are composed of a number of alveoli and the connecting intercalary ducts.



SCHEMATIC DRAWING SHOWING THE FORMATION OF A LOBE

Fig. A. (1) The anlage of the lobe appears as a bud-like outgrowth on the lateral wall of the duct. The bud is composed of a mass of epithelial cells. A lumen is formed coincident to the growth in length of the bud resulting in the formation of an intralobar duct (2) Interlobular ducts derived from the lateral bud outgrowth, showing secondary buds at the lateral walls and terminal ends of the ducts. (3) Intralobular ducts derived from the lateral and terminal buds of the interlobular ducts. Note the multiplicity of buds representing the true alveoli. (4) Intercalary duct, short duct establishing communication of the lumina of the alveoli to the intra- and interlobular ducts. (5) Alveoli.

Fig. B. Schematic drawings showing the direction of growth of the alveoli. (1) Alveoli seen in section on the 12th day of pregnancy. (2) Boundary of the alveolar wall at the 15th day of pregnancy when the lumen begins to be filled with secretion and (3) further distention of the alveoli and duct at parturition.

In histologic sections, the bud-like outgrowths present in the 8 to 10 day pregnant stage (Figs. 18-21) are composed of solid masses of epithelial cells. With the further advance of pregnancy these duct-buds increase in length with the formation of lumina along their course and the formation of new outgrowths along their lateral walls and sub-branches.

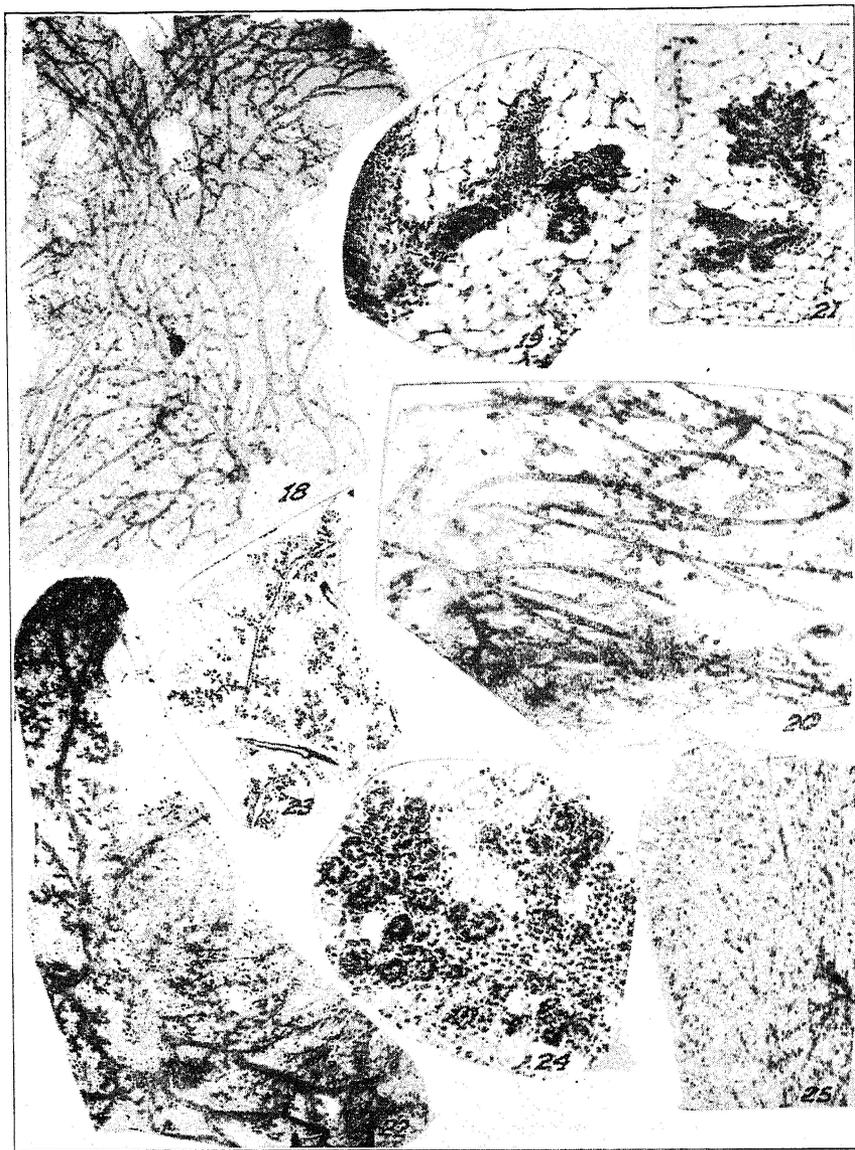


Plate V.—Explanation of Figures

Fig. 18. The gland from a mouse pregnant 8 days. The ducts have grown further in length. The lateral outgrowth seen in 7-day stage has considerably increased in length with lateral and terminal outgrowth developing from them. X 2½.

Fig. 19. Microphotograph of a longitudinal section of the anlage of a lobe. The primitive lateral bud outgrowth increases in length and new outgrowths form both laterally and terminally. X 113.

Fig. 20. Portion of a gland from a mouse pregnant 10 days. There is a distinct arborization of the lateral and terminal outgrowths thus producing a complex glandular structure. X 2½.

Fig. 21. Microphotograph of a section of the developing lobules. A cluster of 3 to 9 buds form at the terminal ends of the sub-branches of the lobular duct. At this stage definite lumina are enclosed by a single layer of cuboidal cells. X 113.

Fig. 22. Portion of the mammary gland of a mouse pregnant 12 days. At this stage the hyperplasia of the gland parenchyma is complete. X 2½.

Fig. 23. Portion of the gland shown in Fig. 22, sectioned through the longitudinal course of the ducts. X 2½.

Fig. 24. Microphotograph of a portion of the gland. Numerous clusters of alveoli are formed from the lateral walls of the intralobular duct. The alveoli are almost spherical with lumina enclosed by a single cell layer. X 113.

Fig. 25. Portion of a gland from a mouse pregnant 14 days. The lobules are much larger due to gradual filling of the lumina of each alveoli with secretion. X 2½.

The alveolar buds abundant in the glands of 12 days of pregnancy, are at first composed of solid masses of epithelial cells. Without further growth, lumen are formed in each of the alveoli, thus in section the lumina of the alveoli are lined by a single layer of cuboidal cells (Figs. 22-24).

The above description of the development of the mammary gland during pregnancy is exactly the same as during pseudo-pregnancy.

A distinct change may be noted in the whole mounts and sections of the glands on the 14th day (Fig. 25). Previously the alveoli were more or less compact deeply staining structures of slightly irregular form, but in general more or less spherical or cuboidal. At this time the alveoli have increased in size, become more irregular in form, and stain less deeply. With the enlargement of the alveoli, the gland stroma becomes less conspicuous.

Each succeeding day, the alveoli show progressive enlargement (Fig. 26-28). By the 19th day the lumen of the individual alveoli begin to show along the margin of the gland where the gland parenchyma is not too dense. These alveoli are spherical in shape with a dark border and a lighter center. The 21st day, after parturition, the gland has become very thick, being filled with milk. It is only along the border of the gland that the structure of the lobules and alveoli may be seen (Fig. 29-30). At this time the alveoli have expanded with milk to a large size. The enlargement of the lumen causes the cuboidal epithelial cells to assume a flattened position, thus increasing greatly the circumference of the alveoli and at the same time stretching the connective tissue sheath surrounding the alveoli and ducts.

The series of whole mounts and sections of the glands during pregnancy show distinctly two phases of development. During the first half of pregnancy the pubertal gland which consists of a duct system, rapidly sprouts a lobule system. After the middle of pregnancy, the process of cellular secretion is initiated, which causes an expansion and unfolding of the alveoli until, at the approach of parturition, the secretory process has reached a very high level with the entire gland engorged with secretion.

During pregnancy the only gross change that can be observed externally in the mammary apparatus of the living animal is the increase in the size of the nipple. At the beginning of pregnancy there is not a remnant of the epithelial ingrowth left except some necrotic debris in the groove which has taken its place. The nipple, which has been more or less embedded, may now be drawn out to nearly double its apparent length. In fact, it is drawn out during the act of nursing, and the purpose of the degeneration of the epithelial ingrowth is doubtless to allow for

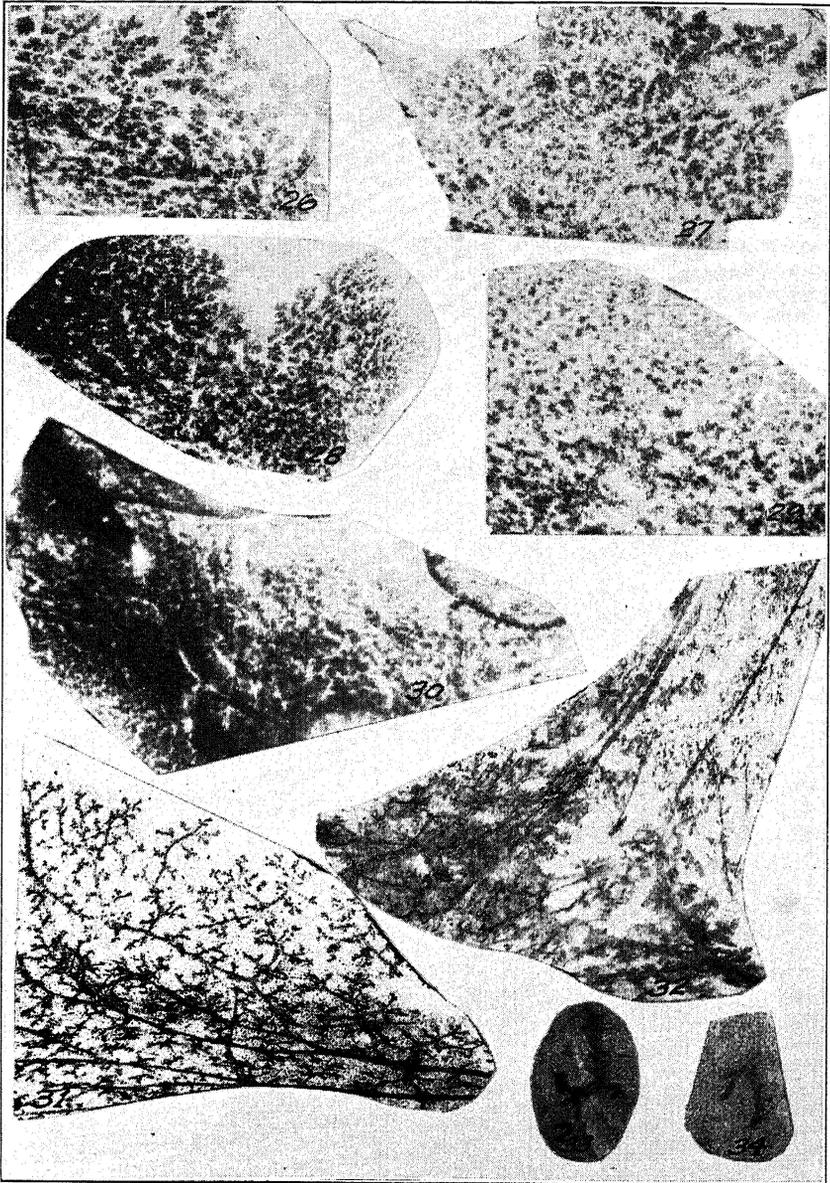


Plate VI.—Explanation of Figures

- Fig. 26. Portion of a gland from a mouse pregnant 15 days. The size of the alveoli increase because of hypertrophy and filling of the lumina with secretion. X 3.
- Fig. 27. Portion of a gland from a mouse pregnant 16 days. Further increase in secretion. X3.
- Fig. 28. Portion of a gland from a mouse pregnant 17 days. X 3.
- Fig. 29. Portion of a gland from a mouse pregnant 19 days. X 3.
- Fig. 30. Portion of a gland at full term (21 days) or immediately after parturition. In fresh preparations the glands appear milky and are considerably thickened due to hypertrophy and distention of the lumina of the alveoli with secretion. X 3.
- Fig. 31. Portion of a pseudo-pregnant gland. The numerous lateral bud outgrowths (lobule anlagen) characteristic of the development during normal pregnancy is observed at the end of pseudo-pregnancy which is about the 11th or 12th day after sterile coitus. Compare this figure with figure 17 and 19. X 3.
- Fig. 32. Portion of a gland involuted 10 days. The lobules and alveoli had undergone retrogressive metamorphosis, so that at the 10th day after weaning the lobules appeared tortuous and shrunken. X 3.
- Figs. 33 & 34. Mammary glands of a mature male albino mouse. The glands of the male remain rudimentary during normal adult life. X 7.

this increase in the length of the nipple to accommodate the young. (Fig. 4-5).

**Lactation.**—Following parturition the new born animals begin to remove the products of the mammary gland. Consequently, the secretory activity of the epithelial cells of the alveoli is stimulated to replace the milk removed (Fig. 35). In comparing mouse glands at the time of parturition with those of animals three days later it was observed that the average size of the alveolus during the interval had increased considerably. This would indicate that the development of secretory activity increases for a period after parturition. Ten days after parturition the sectioned gland showed alveoli and ducts still greatly distended with milk. During the entire period of lactation, the glandular parenchyma predominates with the stroma appearing very slight in amount.

In order to determine the amount of milk removed from the gland at a single nursing, a mouse was separated from her 3 day old young for five hours. A check gland was removed in toto to prevent the loss of milk from the gland. Then the young were allowed to nurse for 30 minutes and a second gland removed for examination. A similar experiment was performed on a mouse with a litter 10 days old.

The glands with the accumulation of milk for five hours were greatly distended, possibly slightly more the 10th day than the third. Upon nursing for 30 minutes, the size of the alveoli had decreased considerably, yet there was still much milk in the gland (Figs. 37-40).

During lactation, the nipples at rest are embedded half way below the surface of the skin of the immediate surrounding gland area, resulting in the formation of a deep sulcus around the nipples (Fig. 6). The sulcus is free of accumulating debris due to the fact that during nursing the nipples are drawn out to their greatest length.

**Involution.**—Following weaning there is an accumulation of milk in the gland for 24 hours or more, then the presence of the milk inhibits further secretory activity. By the fifth day the gland has lost much of the milk from the alveoli and ducts and consequently appears much thinner than during lactation (Fig. 36). The process of involution continues rapidly so that on the 10th day mounts of the entire gland resemble those of 10 to 12 days of pregnancy or at the end of pseudo-pregnancy (Fig. 32). The final fate of the alveolar cells after long periods of involution has not been investigated.

During involution when the position of the nipples is not disturbed, cornified or pycnotic cells accumulate in the sulcus so that in advanced stages only the outer pole of the nipple can be seen with the naked eye (Fig. 7). As in virgin nipples the cells filling the sulcus are cast off during the ensuing pregnancy.

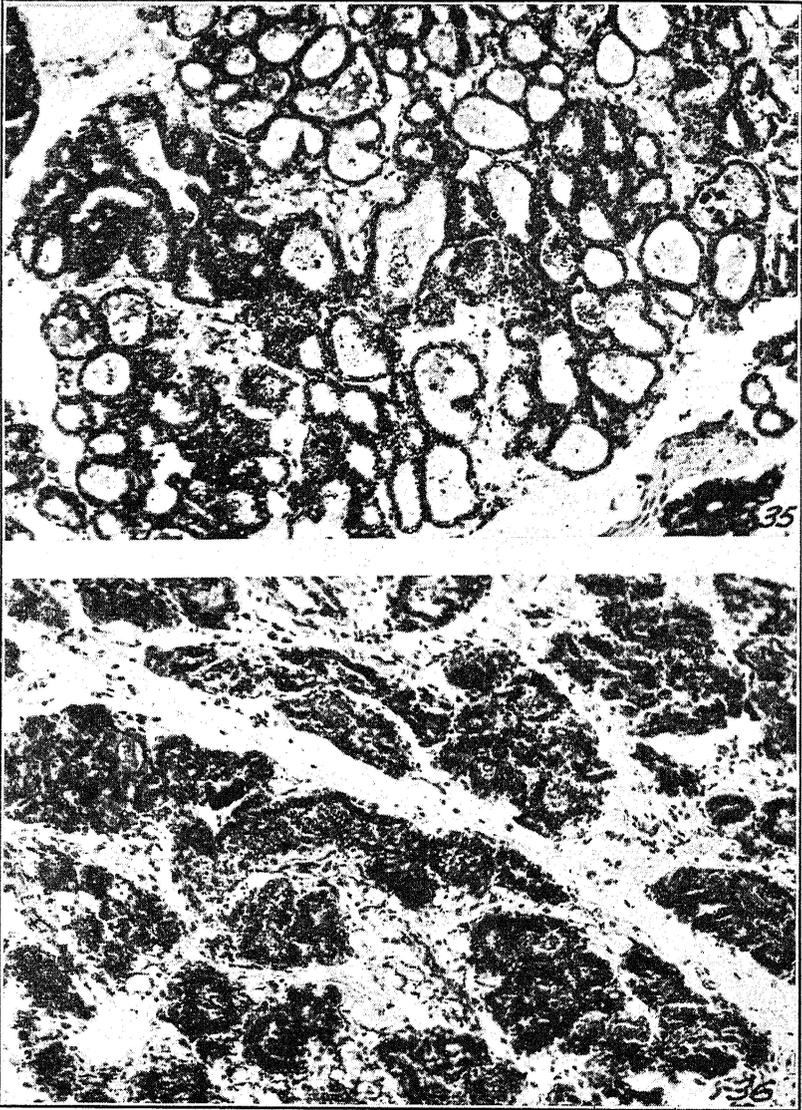


Plate VII.—Explanation of Figures

Fig. 35. Microphotograph of a section of the mammary gland immediately after parturition. The alveoli are greatly distended and the lumina are in most part filled with milk. The gland stroma between the lobules is well stretched and therefore appears somewhat inconspicuous. X 130.

Fig. 36. Microphotograph of a section of a mammary gland involuted for five days. The milk in the lumina of the alveoli has been resorbed with a collapse of the wall. The connective tissue sheaths surrounding the alveoli and lobules are quite distinct and the gland stroma appears more conspicuous between the lobules. X 130.

**The Development of the Gland Stroma.**—The mammary gland may be divided into two parts, the parenchyma or true gland or secreting tissue and the stroma, the supporting connective tissue. The development of the gland stroma in the albino mouse may be traced from the earliest appearance of the mammary anlage at the 13 day stage. At this time the mesenchymal cells lying below the stratum germinativum of the mammary bud are condensed. The cells nearest the bud are somewhat elongated and are arranged in from two to four distinct layers. Following the development of the primary sprout, it becomes surrounded with developing connective tissue representing the mantle layer of stroma.

At the time of birth there are as many as four cell layers and their fibers surrounding each duct, while farther away from the ducts the connective tissue cells and fibers are arranged parallel to the surface of the skin. By the second day, the ducts are covered with a sheath of fibrous tissue. Surrounding the sheath are layers of condensed connective tissue representing the true stroma of the gland. It may be noted that during fetal stages the mantle layer only is evident since at this period the mammary gland consists of a single unbranched club-shaped mass of epithelial cells—the primary sprout. On the second day of extra-uterine life, the stroma is divisible into the mantle layer closely surrounding the ducts and the true stroma between the ducts. Beginning at this time there is a marked infiltration of fat which begins to appear in the region that will later be occupied by the mammary ducts. The fat is deposited in pads, one of which surrounds the ducts of the two inguinal glands on either side. The thoracic fat pads receive the mammary ducts of the right and left second and third thoracic glands, while the ducts of the first thoracic glands ramify the cervical pads of fat.

The pads of fat at first lie lateral and dorsal to all parts of the glands except the terminal ducts. The ducts grow laterally and the pads of fat while increasing in size extend medially until the ducts of the mammary glands are completely embedded in fat.

As the animals advance in age, the mantle layer decreases somewhat in thickness as it passes from the primary to the terminal ducts. Elastic fibers are quite numerous in later stages extending far toward the free end of the system of ducts. The subcutaneous blood supply is quite rich in the region of the stroma.

The gland stroma in lactating animals appears in situ as a fatty connective tissue pad on either side of the body wall and extending upward to within a short distance from the mid-dorsal line (Fig. 42-43). Corresponding to the arrangement of the nipples, the gland stroma may be divided into the inguinal and thoracic groups with a considerable

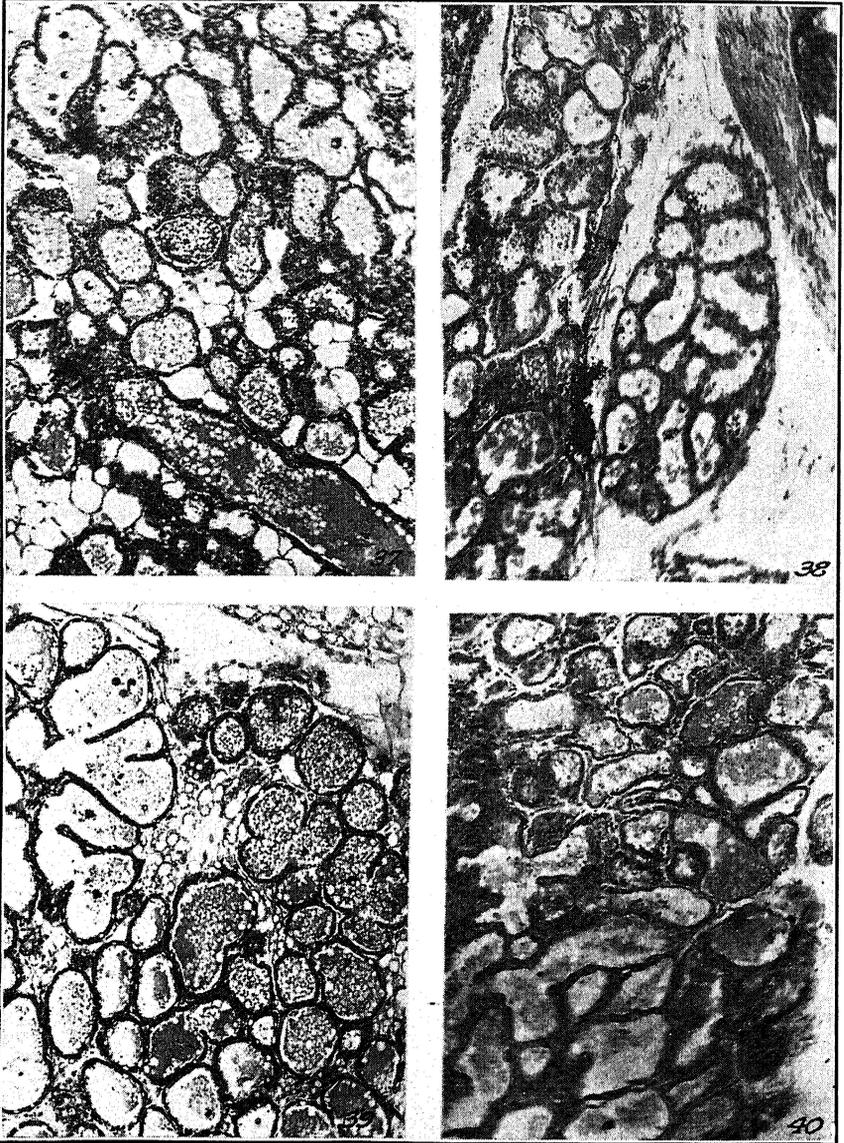


Plate VIII.—Explanation of Figures

Fig. 37. Microphotograph of a section of the mammary gland on the third day of lactation. The young were removed five hours before this gland was removed in toto to prevent the loss of milk from cut surfaces. X 100.

Fig. 38. Microphotograph of a section of the mammary gland on the third day of lactation removed and fixed immediately after the young had nursed it for 30 minutes. While the size of the alveoli has been decreased as a result of the removal of milk, it is not as great as might be expected. X 100.

Fig. 39. Microphotograph of a section of the mammary gland on the 10th day of lactation. The young were removed five hours before this gland was removed in toto to prevent the loss of milk from cut surfaces. X 100.

Fig. 40. Gland on the 10th day of lactation removed and fixed immediately after the young had nursed it for 30 minutes. Alveoli appear somewhat shrunken as a consequence of the removal of milk but not as great a collapse occurred as might be expected. X 100.

space separating the two. Ventrally, the inguinal and pectoral stromal pads never cross the mid-line.

In the male, the gland stroma, like the gland parenchyma, remains rudimentary throughout normal life.

**The Vascular System of the Mammary Glands.**—The ontogeny of the vascular system which furnishes the blood to the mammary glands of the albino mouse was not investigated. However, the origin and course of the blood vessels supplying the blood to the mammary glands was traced and the changes occurring during pregnancy, lactation, and involution observed.

The mammary glands of the albino mouse may be classified into two groups according to their position, namely the thoracic and inguinal glands. They are separated by a considerable space on the abdominal wall. Both sets of glands receive their blood supply from the superficial blood vessels of the thoraco-abdominal wall.

The thoracic glands are supplied with superficial blood vessels from the main arteries on either side, the thoraco-epigastric (external mammary) arteries arising from the subclavians and by internal mammaries arising from the stumps of the external mammary arteries. The internal mammary arteries furnish several small branches which pass between the ribs to the skin, and a large epigastric branch.

The inguinal glands are supplied by vessels from the superficial epigastric arteries arising from the femorals in the region of the inguinal canals. Small branches of the hypogastric arteries pass through the subcutaneous muscles into the inguinal glands. The intercostal arteries furnish very little of the blood supply. Anastomoses between branches of the two systems occurs most frequently among the terminal branches ramifying the gland free space on the abdominal wall.

The larger veins as a rule parallel the arteries. The smaller veins and arteries are less apt than the main trunks to parallel each other. The terminal branches interdigitate in such a way as to insure an even distribution of the capillaries. The main branches lie in the sub-cutis superficial to the cutaneous muscles. From the vessels rami pass toward the skin and over the surface of the musculature. From both sets of branches as well as from the main vessels, rami pass into the mammary glands (Fig. 42).

In early pregnancy the capillary plexuses about the mammary ducts become much more richly developed than in virgin animals. At the same time there is initiated also an active development of arterioles and venules. The subcutaneous vessels in the region of the gland and all the vessels supplying the gland increase rapidly in size. The vessels not only increase in diameter but also increase in length with the growth of the mammary ducts and send rami to the branches which arise from the

ducts. The new ducts are supplied in part by branches derived from the vessels of the parent duct and in part by new vessels which develop in the capillary plexuses of the surrounding stroma.

During the latter part of pregnancy, the chief interest lies in the development of the vascular supply of the gland lobules. At first the anlagen of the lobules are surrounded by networks of capillaries which are continuous with the capillaries of the ducts. As these vessels become highly developed, one or more arterioles and venules develop between them and the arteries and veins of the ducts. As the alveolar ducts develop at the periphery of the lobular ducts the same process is repeated. Frequently, however, the arterioles and less frequently the venules of the capillary plexus join vessels which approach the lobule from the side opposite the duct. Both the veins and the arteries may extend into a lobule from the extremity opposite the duct. The venules and arterioles which supply the alveoli almost invariably extend from the neck of the alveolus outward into the capillary plexus surrounding it.

During lactation the vascular system of the gland is greatly elaborated and enriched, however, the essential features are as described above. As the glands undergo the process of involution after weaning, the glands gradually thin out due to the gradual disappearance of milk from the lobules and ducts. The lobules and alveoli contract and the gland stroma again appears in larger amount in comparison to the gland parenchyma. With these changes the capillaries of the lobules gradually disappear causing the relatively thick-walled venules and arterioles to appear unduly large. The capillaries about the ducts also disappear in part. With the requirement for blood greatly reduced even the larger veins and arteries have become shrunken so that they eventually appear more like the vessels of the gland before pregnancy. However, the involution of the vascular system is not complete for the vessels are more numerous than in the virgin.

**Number and Arrangement of the Nipples.**—The mammary apparatus of the albino mouse normally consists of five pairs of glands. Three of these pairs are thoracic or pectoral glands, while the other two are inguinal glands. An abdominal pair of glands present in the rat is not present in the mouse. On account of this arrangement there is a considerable space on the abdominal wall free of gland tissue.

The teats are located so that two V-shaped areas are formed. The three pairs of thoracic teats form a V with the point of the V directed cephalad. The two inguinal pairs also form a V with the point of the V directed caudad. (Fig. 41).

The variation in the nipple number in the rat and mouse has been reported by Schickele (1899). Of the rats 80 per cent possessed 12

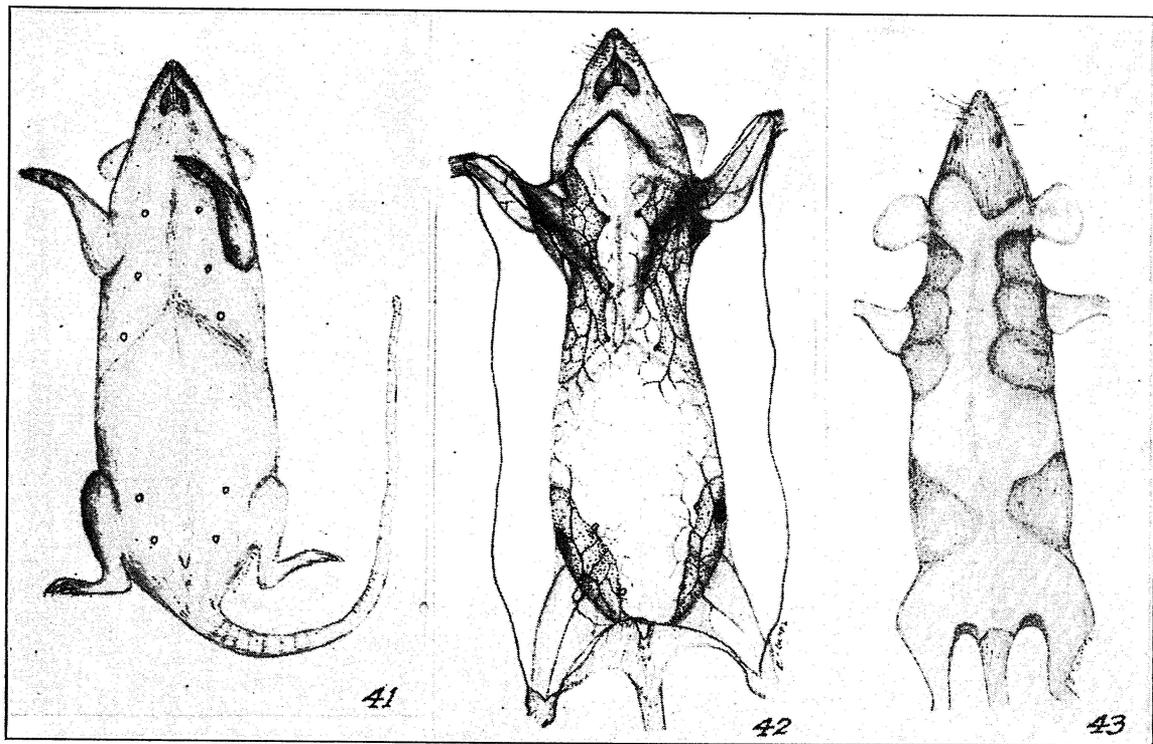


Plate IX.—Explanation of Figures

Fig. 41. Ventral view of the mouse, showing the normal position and arrangements of the nipples. The nipples occurred in pairs, three in the pectoral and two in the inguinal region. Variations also occur in the number and position of the nipples. X  $\frac{3}{8}$ .

Fig. 42. Ventral view of the female mouse, the skin carefully dissected off to show the vascular system and the position of the gland stroma. The more prominent lymph glands are shown. X  $\frac{3}{8}$ .

Fig. 43. Dorsal view of the same animal. (Fig. 42) showing the extent of the gland stroma. X  $\frac{3}{8}$ .

nipples, while 13.33 per cent had 13 and 6.66 per cent had only 11. In the albino mouse 86.96 per cent examined possessed 10 nipples, 7.24 per cent had 9 and 1.45 per cent had 12, 11, 8, and 7 nipples each.

In the present study the nipple number of 156 female albino mice was tabulated. Of these 152 or 97.44 per cent possessed 10 nipples, 3 or 1.9 per cent had 11, and 1 or .65 per cent had 12 nipples. In two of the three animals observed with 11 nipples the supernumerary nipple was in front of the left inguinal pair corresponding to the position of the abdominal nipple of the rat. In the third case the supernumerary nipple was behind the third thoracic nipple. The space between the last normal nipple and the supernumerary was approximately the same as that which exists between the normal nipples. The supernumerary nipples in the animal with 12 nipples were paired structures on the abdomen in front of the inguinal glands.

The nipples are canalized by a single duct. The constricted entrance to the duct is called a streak canal followed by an ampullary dilation corresponding to the cistern of the teat in larger mammals. At the base of the teat the single excretory milk duct turns laterally. It then usually divides into from two to three secondary branches.

### SUMMARY AND CONCLUSIONS

1. From birth until the approach of puberty, the mammary gland anlagen of the female continue to grow very slowly whereas in the male the mammary ducts cease to grow and remain rudimentary throughout normal life.

2. At the approach of puberty, however, the mammary ducts begin to grow rapidly. Changes may be noted in the gland during the recurring estrus cycles. Thus during proestrus the ducts of the gland exhibited long slender branches which have a few light staining end buds along their course. At estrus the small end buds show evidence of growth and new end buds appear. The end buds at this time stain deeply and the ducts show marked distention with fluid. Slight retrogressive changes then occur during metestrus.

3. During pseudo-pregnancy which follows a sterile coitus, there is initiated a rapid hyperplasia not only of the duct system but of the gland lobules as well. The extent and character of the growth observed is entirely comparable to that observed during the first half of pregnancy. However, the lactation phase initiated during the second half of pregnancy is not induced.

4. Two distinct phases of development are observed in the mammary glands during pregnancy. During the first half of pregnancy the hyperplasia of the gland parenchyma occurs, whereas after the middle

of pregnancy the gland epithelium gradually starts to secrete causing an expansion and unfolding of the alveoli due to the accumulation of the products of secretion.

5. The removal of the products of the glands by the newborn animals stimulates the secretory activity of the epithelial cells of the alveoli which continues as long as the milk is removed frequently.

6. Following weaning, the accumulation of milk inhibits further secretory activity. There follows a gradual resorption of the milk in the lumen of the alveoli and ducts and a consequent collapse of these structures so that on the 10th day the glands appear similar to those at the end of pseudo-pregnancy.

7. The gland stroma, the supporting connective tissue, gradually become infiltrated with fat following birth in the females. There are formed two inguinal fatty pads, and two thoraco-cervical fatty pads into which the glands grow. In the male, the gland stroma, like the gland parenchyma, remains rudimentary throughout normal life.

8. While the ontogeny of the vascular system associated with the mammary glands was not investigated, the origin and course of the blood vessels was traced and the changes occurring during pregnancy, lactation, and involution observed.

9. The number and arrangement of the nipples and glands of the mouse was described. Over 97 per cent of the animals examined had 10 nipples, consisting of three pairs of thoracic or pectoral nipples, and two pairs of inguinal nipples and glands.

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