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The Normal Development of the Mammary Gland of the Male and Female Guinea Pig

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ABSTRACT.—The developmental anatomy of the mammary gland of the guinea pig here described, is a part of a study to determine the hormones responsible for the growth and secretory activity of the gland of the common laboratory and domestic animals. The embryonic and fetal development of the mammary apparatus is essentially the same as that previously described in the rabbit. The mammary lines in the 20-day-old embryos quickly develop into mammary buds by the 25th day, followed by the development of a single primary sprout about 5 days later.

The development of the nipples, caused by the growth of the mesenchyme tissue, results in the elevation of the mammary buds beginning at about the time the primary sprout begins to invaginate. Thus the growth of the duct system and the nipple go on simultaneously until birth. During this period the development is remarkably similar in the male and female.

From birth to the approach of puberty only slight growth of the duct system is observed, but with the recurrence of estrum increased sprouting of the ducts occurs. However, the extensive growth of the duct system followed by the development of the lobule-alveolar structures is greatly stimulated by pregnancy. This growth phase is completed largely during the first half of this period. Following the gradual cessation of growth, the second half of pregnancy is characterized by the slow initiation of secretory activity by the gland epithelium resulting in the enlargement of the lumina of the alveoli with secretion.

The stimulus to intense secretory activity begins at about parturition and lactation continues as long as milk is removed frequently. When the young are removed, the gland begins to undergo a steady decline in size with the resorption of milk completed within 10 days or two weeks.

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The Normal Development of the Mammary Gland of the Male and Female Guinea Pig

C. W. Turner and E. T. Gomez

As an integral part of the study of the hormones responsible for the normal growth and function of the mammary gland which has been in progress in this laboratory for a number of years, it has been our plan to make a systematic study of the growth and secretory activity of the gland in correlation with successive sexual epochs in the life of the species being studied which would serve as a control for the experimental studies.

In following this program, the results of the study of the normal and experimental development of the rabbit mammary gland has been presented (Turner and Frank, 1930, 1932, and Turner and Gardner, 1931). Similar studies of the albino rat (Turner and Schultze 1931 and Schultze and Turner 1933), and the albino mouse (Turner and Gomez 1933) have been published.

The object of the present paper is to report the results of a study of the mammary glands of another common laboratory mammal, the guinea pig.

MATERIALS AND TECHNIQUE

In connection with the study of the embryonic and fetal development of the mammary apparatus, it became necessary to secure a series of embryos of known age. To accomplish this, a group of adult nulliparous females were examined each day (usually in the morning) for the opening of the vaginal orifice which has been used as a criterion of the onset of estrum. The animals in estrum were placed in the cages permanently occupied by the males for about 24 hours and then returned to individual cages. Unlike the rat and mouse, the guinea pig was not observed with a gelatinous plug indicating that copulation had occurred.

When pregnancy occurred, conception was considered as dating from the time the females were taken away from the male. While this method is considered satisfactory in securing specimens timed with reasonable accuracy, it did not appear to be the most satisfactory method as the conception rate was rather low. The

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weights of the embryos at various ages agreed very well with the weight data presented by Ibsen (1928). (Table 1.)

All embryos were obtained from females during their first pregnancy as it was planned to use the same animals for the study of the growth of the mammary gland during the course of pregnancy. At varying intervals after conception, the pregnant animals were placed under ether anaesthesia and the entire uterus containing the embryos or fetuses and one mammary gland were carefully removed. The animal was then examined daily to determine the effect of hysterectomy during pregnancy on the secretion of milk.

The embryos were fixed in Bouin's fluid or in a solution composed of a saturated aqueous solution of $HgCl_2$ ten parts and glacial acetic acid one part. The specimens were embedded in toto and sectioned from 6 to 10 microns thick. The sections were stained in Delafield's hematoxylin and eosin or Mallory's triple stain.

TABLE 1.—WEIGHT OF GUINEA PIG FETUSES AT VARIOUS INTERVALS AFTER CONCEPTION

Days After Conception	Number of Fetuses in Uterus					Ibsen (1928)		
	1	2	3	4	5	Days in Uterus	Number of Litters	Weight of Litters Ave. gms.
	gms.	gms.	gms.	gms.	gms.			
20	------	-----	0.067	-----	------	20	5	0.058
25	------	-----	-----	0.396	------	25	4	0.409
30	------	-----	1.486	-----	------	30	7	1.336
33	------	3.893	-----	-----	------	35	5	4.078
44	18.058	-----	-----	-----	------	45	5	20.390
47	37.260	-----	-----	-----	------	46	1	22.500
57	------	-----	-----	-----	59.110	55	8	49.440
64	84.900	-----	-----	-----	------	64	1	87.900

In the fetal stages from 40 days after conception until birth, the young were no longer sectioned in toto but rather the skin containing the mammary glands was removed, fixed and sectioned. The development of the mammary glands from the late fetal stages, at birth, during pregnancy, lactation, and involution was studied by means of histological sections and whole mounts of the glands. In preparing the whole mounts of the glands, the method previously described (Turner and Gomez 1933) was employed.

The sex of the fetuses was determined by an examnation of the external genitalia and further confirmed by dissection of the reproductive organs after the skin containing the mammary glands was removed. The sex could be readily determined in 25-day-old fetuses and older stages. The opening of the vaginal orifice was used as an indication of puberty.

As in previous studies, the growth and development of the mammary gland has been divided into the following stages:

I. Embryonic and fetal development
II. Prepubertal
III. Puberty
IV. Pregnancy
V. Lactation
VI. Involution

EMBRYONIC AND FETAL DEVELOPMENT

The embryonic and fetal development of the mammary glands of a large number of domestic and experimental mammals as well as man have been studied in the 60 years which have elapsed since the pioneer work of Huss (1873), Gegenbaur (1876), Rein (1881) and Klaatsch (1884). The more important studies include the papers by Rein (1881) on the rabbit, Brouha (1905) on the bat and man, Lustig (1916) on man, Myers (1917) on the rat, Gisler (1922) on the cat, Uehlinger (1922) on the horse, Turner (1930-31) on cattle, and Turner and Gomez (1933) on the mouse. In addition there are a great many studies of one or more embryos of a large number of species. In general these studies have indicated that the course of gland development is quite similar, i. e., the same typical stages of growth are observed although there are many minor variations and species and sex differences. The more important stages in the progressive development of the mammary apparatus include the mammary streak and line, the mammary bud, the primary sprout, and the proliferation of the nipple.

Only a single study of the embryology of the mammary gland of the guinea pig has come to the attention of the writers. Schickele (1899) reported the results of his observations on six early embryos. The description of his figures will be compared with the results of our study in considering the various stages of development.

The Mammary Streak and Line.—The earliest anlage of the mammary gland complex consists of the enlargement and elongation of the cells of the stratum germinativum in the general location of the future glands. Localized proliferation of these cells then occurs in the nature of a line extending in varying degrees between the anterior and posterior limb buds on either side of the ventral wall. The first of these structures has been termed the mammary band or streak and the latter the mammary line.

In the rat Henneberg (1900) observed the mammary streak in an 11-day embryo, while Turner and Gomez (1933) observed the corresponding stage in the mouse on the tenth day. In a 15-day guinea pig, the embryo was just beginning to form, whereas in the 20-day stage the mammary streak already had been passed and the mammary line was present. In a cleared preparation the mammary lines were seen on either side in the region of the dorsal limiting furrow as narrow light lines extending from the anterior limbs caudad to the posterior appendages (Fig. 1).

In sections of the embryo caudad to the anterior limbs and ventrolaterally on either side are seen thickened cellular proliferations conspicuously elevated above the surrounding ectoderm (Figs. 2 and 3). Tracing these lines caudad they gradually decrease in prominence. However, at the angle of the ventral surface with the posterior appendage the lines show greater proliferation which sink deeper into the underlying mesenchyme (Figs. 4 and 5). As will be noted later it is at this end of the line that further development takes place.

A 21-day-old embryo examined by Schickele (1899) showed approximately the same extent of development just described. However, he considered the anterior portion of the lines rudiments of supernumeraries. In several larger embryos an increasing number of supernumeraries (up to 10) were reported. At birth no sign of supernumerary teats was observed. Our observations would indicate that Schickele was calling the anterior end of the mammary line supernumerary rudiments. Possibly the uneven regression of the line appeared as separate and distinct rudiments giving the impression that they were the anlagen of supernumerary glands. The fact that no further development of these structures was observed by Schickele causes us to doubt that there was justification for calling these structures supernumeraries. It is possible that Schickele received the idea that these structures were supernumeraries from the paper by Schmidt (1896) who described similar structures in the mammary lines of human embryos. It is rather significant that Schmidt's observations were not confirmed by later observations.

The Mammary Bud.—The parallel proliferation of the ectoderm to form the mammary lines is not long continued. The line eventually regresses or is obliterated by the progressive de-

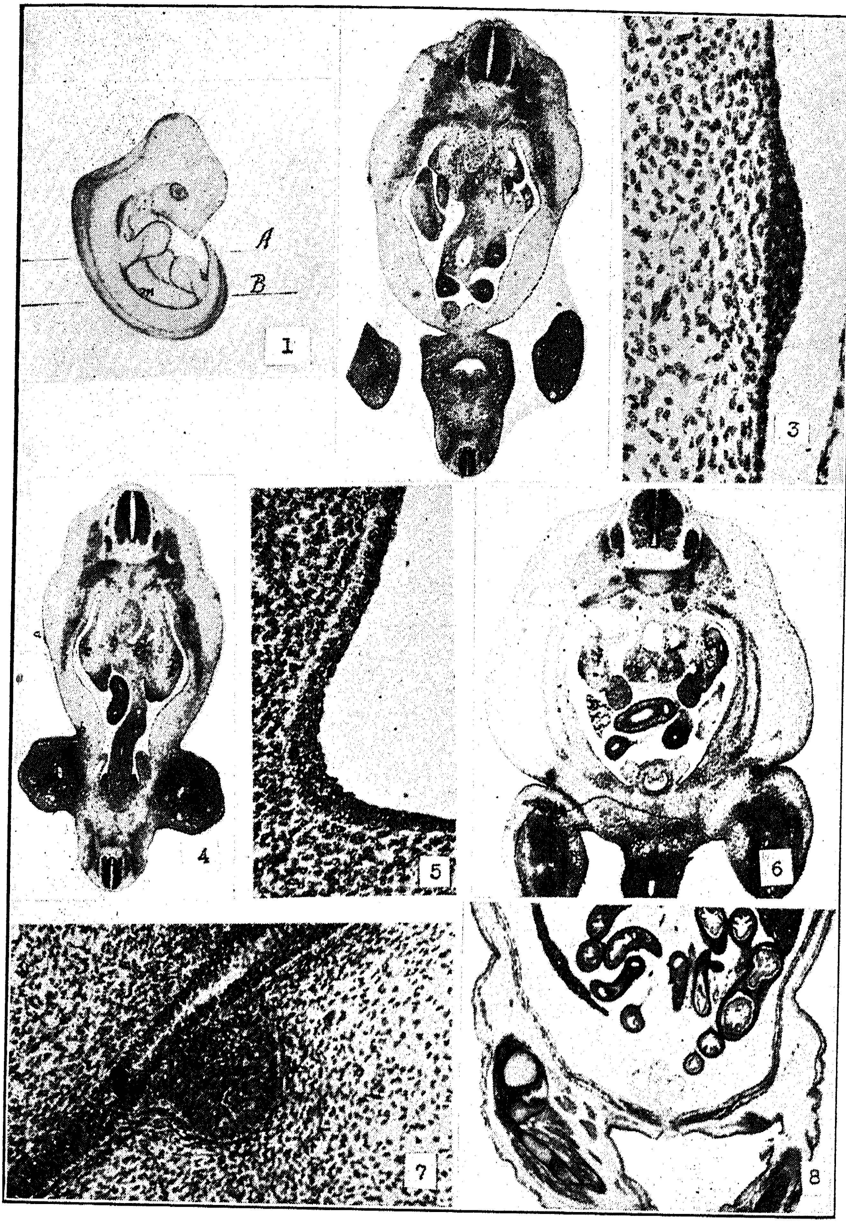

Plate I.—Explanation of Figures.

Fig. 1. Side view of a 20-day-old guinea pig embryo, showing the mammary line (m) and the levels of the section shown in figures 2 and 3. (x5)

Fig. 2. Microphotograph of a section through the mammary line of a 20-day-old guinea pig embryo at the level A shown in the figure 1. (x9).

Fig. 3. Microphotograph of the mammary line seen in figure 2. (x43).

Fig. 4. A section of the same embryo at the level **B** indicated in figure 1. Because of the flexure of the anterior-posterior portion of the body of the embryo, the mammary line at this level appears in two aspects; **a**, mid, and **b**, posterior extent of the mammary line. (x9).

Fig. 5. Microphotograph of the posterior extent of the mammary line indicated b in figure 4. (x43).

Fig. 6. Microphotograph of section of a 25-day-old male guinea pig fetus, showing the position of the mammary bud. (x10).

Fig 7. Microphotograph of the mammary bud seen in section of a 25-day-old fetus. (x43).

Fig. 8. Microphotograph of a 30-day-old female fetus showing the early development of the primary sprout. (x7).

velopment of the embryos. However, before regression of the line takes place there occurs an accentuated focus of growth in the line in the inguinal region close to the attachment of the hind leg. The Malpighian layer (stratum germinativum) gradually builds up a number of layers of cells which upon section appear as a lens-shaped thickening, in part extending above the surrounding surface and in part sinking into the mesenchyme tissue below. Soon the structure becomes entirely embedded in the mesenchyme tissue.

The successive stages of the development of the mammary bud, have been termed the mammary crest (the lens-shaped stage), the mammary hillock (the half-moon stage) and the mammary bud (spherical invagination). The development of the mammary bud was complete in the 25-day stage in both male and female. At this time the entire bud was below the surface and in some cases appeared to have continued slightly further, similar to the condition in the 10 cm. male cattle embryo (Turner 1930). The usual histological elements were present, namely, the Malpighian layer resting upon a distinct basement membrane, and a group of central cells, the intermediate cell layers, which extend centrally due to the rounding off of the bud (Figs 6 and 7).

The development of the mammary gland in the male guinea pig appears to run parallel with that of the female throughout intrauterine and prepubertal life, so the description of the successive stages of gland growth is applicable to either sex.

The Primary Sprout.—The stage of development about to be described, the mammary sprout, is of special interest because at this point the greatest species difference occurs. At this stage the number of ducts in the future teat is determined. As in the rat and mouse, only a single primary sprout is observed to develop in the guinea pig. Between the 25- and 30-day stages the cells of the distal half of the mammary bud begins to proliferate. The new development grows downward, pressing the mesenchyme tissue to either side as it proceeds. This solid cellular structure is slightly bulbous at the growing end. The 30-day stages in both male and female embryos showed that the sprout had extended a distance equal to the length of the mammary bud (Figs. 8 and 9), whereas in a 33-day stage the sprout had increased further in length. The bulbous appearance of the free ends no longer could be seen, rather the width of the sprouts was more or less uniform throughout the length of the structure (Fig. 10).

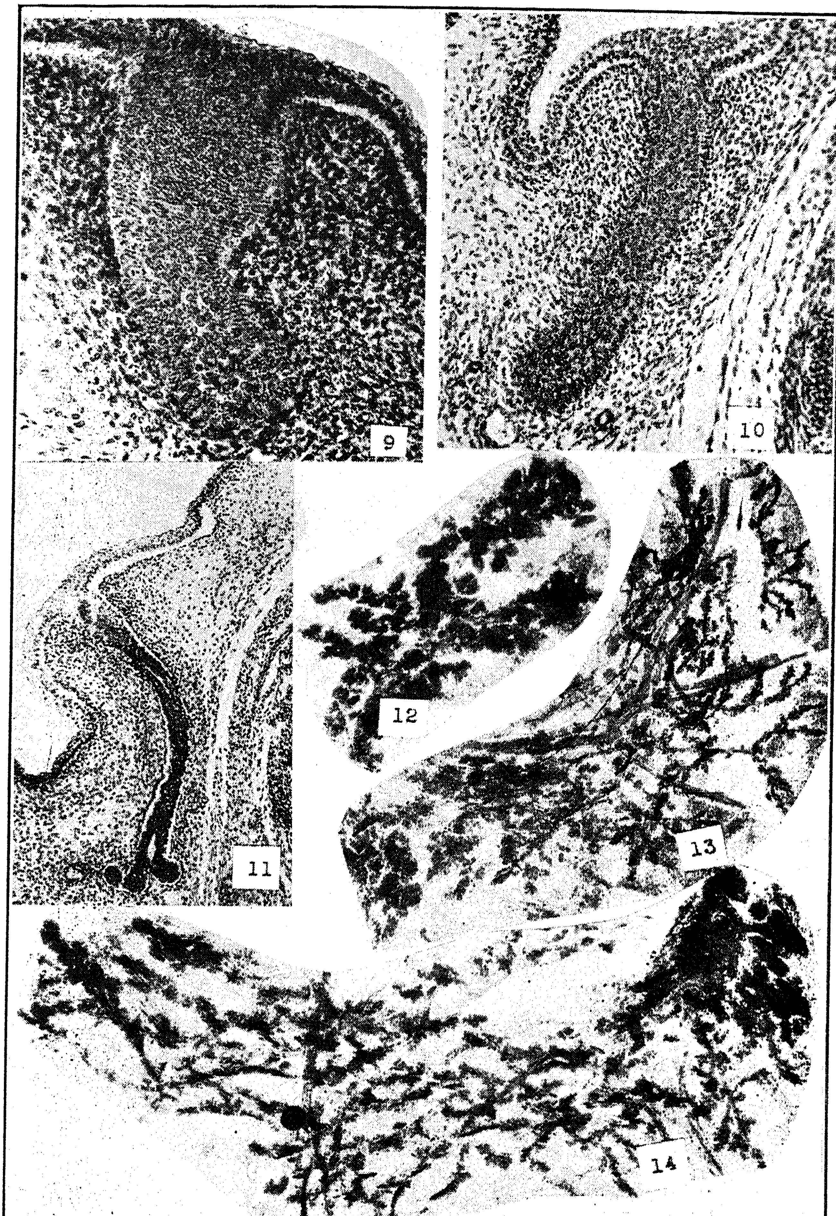

Plate II.

Fig. 9. Microphotograph of the mammary gland shown in Fig. 8. (x41).

Fig. 10. Microphotograph of the developing mammary gland of a 33-day-old female fetus. (x41).

Fig. 11. Microphotograph of the developing mammary gland of a 44-day-old female fetus. The secondary sprouts are forming. (x20).

Fig. 12. Microphotograph of a gross mount of the mammary gland of a 47-day-old female fetus. (x8).

Fig. 13. Mammary gland of a 57-day-old male fetus. (x8).

Fig. 14. Mammary gland of a 64-day-old (full term) female fetus. Compare with figures 12 and 13. (x8).

The primary sprout in 44-day-old fetuses showed considerable further lengthening, and at the distal end, the presence of two bulbous secondary sprouts (Fig. 11).

During the period of growth, the central or intermediate cell layers throughout the length of the primary sprout are loosely arranged with their axes directed toward the outside. The loose association of these cells appears to be due to the widening of the sprout coincident with its growth rather than to a degeneration of the cells as pycnotic cells were not observed.

As has been observed previously, the canalization of the primary sprout begins in the region of the distal end and proceeds toward the proximal end. Thus the sprout adjoining the rudiment of the mammary bud is last to canalize. In 44-day-old fetuses the entire length of the primary sprout has canalized up to the mammary bud which is still completely filled with cornified cells. The walls of the sprout which may now be called a duct, are lined with a two-layered epithelium (Fig. 11).

Growth of the Duct System.—The primary sprout upon canalization becomes the streak canal, the cistern or ampullary dilatation of the teat, and the cistern of the gland when such structures are present in the mature teat and gland. The secondary sprouts become transformed into the chief branches of the duct system. In the 44-day stage observed, the secondary sprouts were just forming. In a 47-day fetus the secondary sprouts showed considerable extension and canalization in the central parts so that they might properly be called ducts. The ducts in the guinea pig show numerous bud- or sprout-like outgrowths along the lateral walls (Fig. 12). In this respect the guinea pig duct system differs greatly from that observed in the mouse. In the latter the ducts showed a few dichotomous branches but were free of lateral sprouts until early pregnancy.

For this reason, upon casual observation, the guinea pig mammary gland might be thought to be sprouting lobules at a very early stage of development. Upon closer examination, however, it is observed that these numerous bud- or sprout-like ougrowths along the lateral walls are actually branches which continue to grow as integral parts of the duct system. The guinea pig appears to be unique among the animals studied, so far as the precocious growth of an extensive lateral branching of the duct system is concerned.

In seeking an explanation for the extensive side branches present it should be pointed out that the mammary duct system of the mature animal does not spread out in one plane as in the rat, mouse, and rabbit, but is restricted to a limited area and becomes relatively thick by proliferating in several planes.

In 57- and 64-day stages the duct system has become further extended and dichotomous branches of the chief ducts appear as well as further lateral sprouts of the duct walls (Figs. 13 and 14). Because of the nature of the guinea pig duct system, the whole mounts of these glands are much less satisfactory than in certain other experimental animals. By dissecting out the ducts in a single plane a fairly representative section may be obtained.

A histological study of the glands at birth indicated that the parenchyma at this time consists of ducts composed of irregular layers of cuboidal cells usually two or three cells thick. There were lateral branches of the ducts of similar construction. The growing end-buds or lateral sprouts were usually composed of solid masses of epithelial cells but the canalization of the ducts quickly follows the growth of the sprout (Fig. 16).

The most important observation that may be made as a result of the study of the sections is the firm conviction that the development of the gland at this time consists of duct growth and lateral branches and that lobule development is not yet occurring although the condensed nature of the duct system might lead one to the latter conclusion.

Nipple Development.—The development of the nipple of the guinea pig results from the proliferation of the mesenchyme cells surrounding the mammary bud. The early indication of this growth was observed in the 25-day stage at which time the mesenchyme cells are deeply stained around the bud. With the initiation of growth of the primary sprout slightly later (between 25 and 30 days) the growth of the mesenchyme cells causes a slight elevation of the bud by the 30-day stage. From this time the development of the nipple progresses rapidly so that in the 44-day stage a typical nipple is present. Simultaneously with the elevation of the nipple the growth and canalization of the primary sprout occurs. Thus in the 57-day and later stages, the nipple may be observed to be covered with a typical epidermis consisting of a germinal layer and pavement epithelium in various stages of cornification. The tunica propria no longer is composed exclusively of mesenchyme cells. Instead, connective tissue fibers are differentiating. The vascular supply is abundant in a typical zone surrounding the cistern of the nipple.

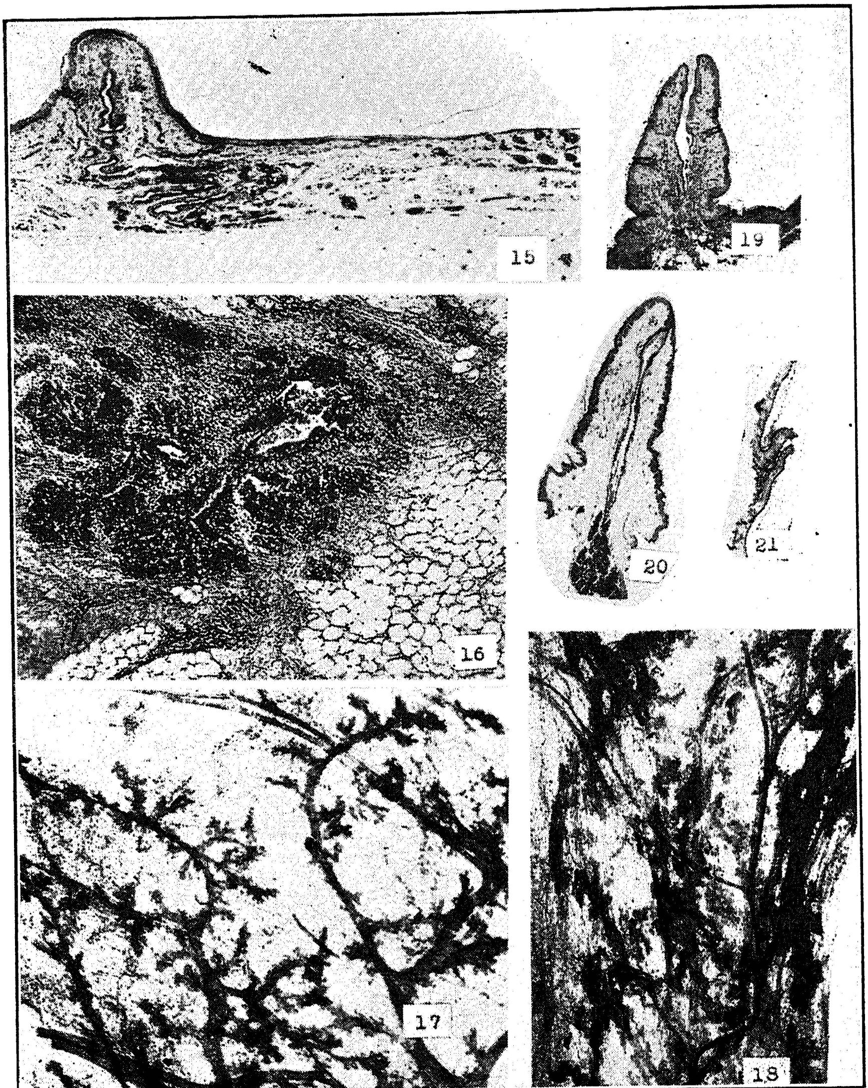

Plate III.

Fig. 15. Microphotograph of a section of the nipple and mammary gland of a 57-day-old male fetus. (x13).

Fig. 16. Microphotograph of the mammary gland of a female guinea pig at parturition, showing the gland parenchyma and adipose tissues, (x4).

Fig. 17. A portion of the mammary gland of a 30-day-old female guinea pig. (x4).

Fig. 18. A portion of the mammary gland of a 60-day-old female guinea pig. (x4).

Fig. 19. Median-longitudinal section of the nipple of a 60-day-old female guinea pig. (x5½).

Fig. 20. Median-longitudinal section of the nipple of a female guinea pig at the time of lactation. (x4).

Fig. 21. Median-longitudinal section of the nipple of an adult (90-day-old) male guinea pig. (x4½).

At the apex of the mound-like elevation of the nipple, the mammary bud residue appears as a crater with the cornified cells largely sloughed off. The future streak canal is not yet clearly differentiated but the excretory duct walls throughout their entire extent are composed of a two-celled layered epithelium (Fig. 15).

The above description of the development of the nipple in the guinea pig indicates clearly that it may be classed as a proliferation nipple such as is present in the rabbit, cow, and most other placental mammals, in contrast to the eversion nipple present in certain marsupials, and the epithelial ingrowth nipple recently described in the mouse (Turner and Gomez 1933).

During prepubertal life there is a slow progressive growth of the nipples in both sexes coincident with the growth of the duct system. However, in the male the nipples soon cease to grow, remaining rather rudimentary throughout normal adult life (Fig. 21). On the other hand, the female nipples begin to grow more rapidly at the approach of puberty. However, if pregnancy does not occur complete development is not attained (Fig. 19). The growth during recurring estrus cycles is quite insignificant.

At the onset of pregnancy, the nipples begin to grow rapidly until the latter part of the gestation period when growth is practically complete. During lactation the size of the nipples is further increased but the change probably does not represent actual growth of the nipple (Fig. 20). The actual extent of growth of the nipple may be obtained most accurately during the resting period in multiparous females. The average height of the nipples of 13 nulliparous females was 3.88 mm (range 2.5 to 4.0); of 13 multiparous females having two to four litters, 5.73 mm. (range 5 to 7) and of 10 adult males 1.8 mm. (range 1 to 2.5).

The Gland Stroma.—The supporting connective tissue surrounding the parenchyma or true gland tissue is known as the stroma. At the time of the invagination of the mammary bud, the underlying and supporting tissue is composed of condensed undifferentiated embryonic mesenchyme cells. These cells continue to surround the gland anlage during the period of primary sprout formation and the initial stage of secondary sprouting in 44-day-old fetuses. In the 47-day stage the transition of the mesenchyme cells to connective tissue cells becomes obvious. Between the 47-day and 57-day stages, there is a rapid development of adipose tissue which is divided into sections or lobes by connective tissue septa. The fatty pads which at first lie lateral and dorsal to all parts of the developing mammary gland duct system gradually extend caudally, anteriorly, and medially until the ducts are completely embedded in fat.

At parturition the gland stroma forms a cushion of fatty connective tissue extending over the abdominal wall from the groin toward the median line. The anterior-posterior extension of the stroma is limited by the inguinal region. The anterior portion of the fatty pad is free of mammary ducts. The duct system extends only posteriorly from the base of the nipples (Figs. 16 and 40).

PREPUBERTAL DEVELOPMENT

The growth of the mammary duct system from birth to the approach of the first estrus cycle in the female varies considerably in the species which have been examined. In some mammals little or no growth of the duct system occurs while in others the ducts continue to develop slowly. In the female guinea pig there appears to be a slight continuation of duct growth during this period. Glands removed at intervals during this period show considerable variation but in general the older animals have the more extended duct system. The branches of the ducts which earlier might be confused with lobules now show more distinctly in the gross mounts that they are true ducts (Fig. 17). However, the compact duct system develops in several layers in the fatty connective tissue stroma and for the ducts to be seen satisfactorily it is necessary to dissect the overlapping branches (Fig. 18). It should be made clear that the figures thus represent only isolated main ducts or branches of the system rather than the entire gland.

During this period the gland stroma composed of fatty pads of connective tissue increase considerably in size in the female and may be palpated in situ in well nourished animals.

Histologically, the mammary gland throughout this period remains the same except for the increase in the extent and number of ducts and bud-like sprouts which precede them. The larger ducts divide dichotomously but smaller ducts appear in all planes along the lateral walls which may be single or redivided into a number of further branches. The multiplicity of these small branches may appear as alveoli unless carefully examined in whole mount and section. Rather these branches are believed to be the ducts which will develop later into a lobule.

The sections of the gland show that the ducts are surrounded by a few concentric layers of connective tissue fibers and large adipose cells. The ducts are composed of two layers of cuboidal epithelium. At the free ends (growing) of the ducts and branches,

Plate IV.

Fig. 22. Microphotograph of a portion of the mammary gland of a 19-day-old male guinea pig. (x7).

Fig. 23. Portion of the mammary gland of a 47-day-old male guinea pig. (x7).

Fig. 24. Portion of the mammary gland of a 90-day-old male guinea pig. (x4).

Fig. 25. Portion of the mammary gland of a male guinea pig 10 months old. (x4).

Fig. 26. Portion of the mammary gland of a female guinea pig at estrum (at conception). (x3).

Fig. 27. Microphotograph of a section of the mammary gland of a female guinea pig at estrum. (x41).

the epithelial lining becomes more and more irregular, finally forming a solid mass of epithelial cells.

In the male, the growth of the duct system of the mammary gland during the first two or three weeks after birth is comparable to the female gland of the same age, except that in the male the ducts exhibit a distinct dichotomous system of branches with only an occasional duct or bud-like outgrowth of the lateral wall. The ducts are quite slender and usually develop in a single plane (Figs. 22 and 23).

The duct system continues to increase in length for three or four months at which time the growing sprouts have reached the posterior limits of the gland stroma (Fig. 24). Little further growth seems to occur in the male gland. In fact there appears to be some evidence of regressive changes in the duct system involving the smaller branches. Thus in a male guinea pig 10 months old the gland consisted of short wide ducts with branches tapered to a fine point which gave the appearance of having suffered involution and loss of the smaller branches. The gland stroma at this time was practically free of adipose tissue, the main support of the parenchyma consisting of mixed connective tissue fibers (Fig. 25).

In considering the male guinea pig as a subject for the study of the experimental development of the mammary gland, it may be pointed out that there develops a fairly extensive duct system beginning in late fetal life and extending for a period of several months after birth. Thus, the male guinea pig would not serve as well as the male rabbit and mouse where the duct system extends scarcely beyond the base of the nipple.

PUBERTAL DEVELOPMENT

The female guinea pigs of our stock normally reached sexual maturity, as indicated by the opening of the vagina, from about 50 to 60 days after birth. However, pregnancy has been observed to begin as early as 30 days after birth. With the approach of puberty and the gradual development of follicles in the ovaries, the mammary duct system begins to grow more rapidly, resulting in a very dense aborization of the ducts and branches. Further, bud-like outgrowths form in all planes of the larger ducts. At the free ends of these branches multiple sprouts representing the anlagen of the lobule ducts begin to form. With the occurrence of estrum followed by ovulation and the formation of corpora lutea in the ovaries, there is initiated a series of cyclic changes in the mammary gland correlated with the recurring estrus cycles.

The normal estrus cycle is approximately 15 to 17 days long (average 15.7 days) in the guinea pig according to Stockard and Papanicolaou (1917). The changes in the mammary gland during the estrus cycle have been studied by Loeb and Hesselberg (1917) in an extensive series of animals. However, no care was taken to secure young animals for examination during the first few cycles, rather animals in all conditions of development, even after pregnancy, were used. The glands were classified as proliferating, intermediate, and resting, depending chiefly upon the presence of secretion and of mitotic figures in the gland. Maximum proliferation of the gland was observed to occur at the time of heat and ovulation followed by a gradual decline until the sixth day. From this time until about the 15th day after ovulation the gland is in a resting stage. When a new ovulation is imminent the number of proliferation glands again increases.

In a second paper Loeb and Hesselberg (1917) say, "If we now correlate the cyclic changes in the mammary gland with those in the uterus and ovaries, we find proliferative changes in the mammary gland corresponding to the first phase of the ovarian and uterine cycle.. We may call this the primary growth period of the mammary gland." While no reference is made to the duct system, it is believed that the "primary growth" referred to consists of growth (proliferation) of the duct system. If this is the proper interpretation of their results, our observations may be said to offer confirmation.

In gross mounts of the glands removed at intervals during the estrus cycle it was possible to see at the approach of and during estrum evidence of the growth of the duct system, especially during the first few cycles. There appears an increasing number of bud-like outgrowths along the lateral walls of the ducts which stain deeply (Fig. 26). At this time the ducts are enlarged with fluid to the extent that a few drops of a milk-like secretion may be expressed from the teat. In contrast during the luteal phase of the cycle, the entire duct system appears shrunken and the end-buds stain less deeply.

Histological examination of sections through the longitudinal course of the ducts show that the lumina of the main ducts are continuous with the lumina of the lateral branches, which in turn are continuous with the developing lumina of the bud-like sprouts which represent the anlagen of the future ducts (Fig. 27). These ducts are lined with a double layered epithelium until close to the end buds where they gradually lose their definite arrangement.

While these ducts may be considered as a stage of lobule development, the formation of alveoli, the secretory parts of the lobule were not observed in nulliparous females. It is possible that slight lobule growth may occur at times but this is believed to be slight in extent when it occurs.

Definite evidence of secretory activity of the duct epithelium was not observed but the fact that alveoli were not present would indicate that the secretion present in the ducts was secreted by the duct epithelium. That the duct epithelium of the human breast is capable of secretion has been demonstrated in the cases of the presence of "witch's milk" in the gland of the new-born. Richter (1928) and Weatherford (1929) observed evidence of lactation in the finer ducts of cattle, cat, and rat mammary glands also.

The question of duct secretion is emphasized because many students of growth and lactation in the guinea pig have considered the presence of a secretion in the duct system and the removal of a few drops of a milk-like fluid as evidence of the complete growth of the mammary gland including the lobule-alveoli system such as is present at parturition. Our observations indicate that such is not the case. Gross mounts and sections of the gland should be made to determine with certainty that alveoli are present even when more or less secretion can be removed from the nipple.

Further evidence that growth of alveoli does not usually occur during the recurring estrus cycles may be mentioned. It has been observed that in multiparous females after a considerable interval from the last lactation, the lobules previously present have involuted completely, leaving only a duct system. It would appear that during the recurrence of estrum not only is there a lack of stimulus of alveolar growth but there actually occurs an involution of alveoli developed during a previous pregnancy.

In conclusion it may be reported that our observations would indicate that the guinea pig may be included in the class of mammals with the rat and mouse where the luteal phase of the normal estrus cycle stimulates little or no alveolar growth. In the guinea pig the lobule ducts do grow quite extensively, in this respect differing from the mouse, as a result of the stimulus imparted during the follicular phase of the cycle.

Pregnancy.—When conception occurs, there is initiated an exceedingly important phase of mammary gland growth and at the approach of parturition initiation of milk secretion. In the previous studies of the growth and development of the mammary gland of the rabbit, rat, and mouse, it was observed that two distinct phases of development occur during pregnancy. During the first half of pregnancy the growth of the lobule-alveolar system occurs. At approximately the middle of pregnancy the hyperplasia of the gland is completed. Following this period the epithelial cells of the newly formed alveoli gradually enlongate and begin the secretion of a fluid into the lumina. The lumina become enlarged rather slowly until shortly before parturition when the secretion of milk is greatly stimulated, resulting in the tremendous expansion of the alveoli as well as the entire gland.

In the study of the growth of the gland of the guinea pig during pregnancy, Loeb and Hesselberg (1917) failed to distinguish the type of proliferation occurring, neither did they mention the state of gland development at the beginning of pregnancy. If the animals had been pregnant recently the glands may be in a more advanced stage of development than in nulliparous animals. This may explain the difference observed. In one animal 6 days and in another about 11 days after copulation, the glands were described as intermediate. In three cases the pregnancy was approximately 12 to 15 days; in two cases the mammary gland was proliferating; in the third it was intermediate. From the 15th to the 19th day in three cases the glands were proliferating; in four it was intermediate. In three animals between the 20th and 23rd day the glands were intermediate. In nine animals the pregnancy was later than the 24th day after copulation and in all cases the mammary gland was proliferating. How far beyond the 24-day stage was not reported.

On the basis of the above observations they concluded that pregnancy does not induce proliferation of the mammary gland to a much higher degree than the factors active during the latter part of the normal sexual cycle unaccompanied by pregnancy, and proliferation of the mammary gland during pregnancy becomes regular only at a period of time which exceeds the duration of the normal sexual cycle unaccompanied by pregnancy. This study leaves a number of important questions unanswered.

The series of pregnant glands examined was obtained from the young females (primiparous) used as a source of the embryos and fetuses previously described. The earliest stage examined (15 days) showed that the gland still consisted of a branching duct system with deeply stained lateral branches and end-buds indicative of active proliferation of the duct system and possibly a few alveoli close to the base of the nipple where the older ducts were present (Fig. 28).

At 20 days the gland had progressed further both in the extent of the duct system around the periphery and the now clearly defined lobule-alveolar structures. It would appear that the growth of the ducts proceeds from the base of the nipple outward. The older ducts and their lateral branches consisting of lobule anlagen have reached the stage for alveoli formation while the younger ducts are still sprouting. Gradually as the duct growth is completed lobule-alveolar growth proceeds from the proximal to the distal ends of the branches.

These two types of development may still be distingushed in the 25- and 30-day stages (Figs. 29 and 30). However, the extent of lobule-alveolar development is now much more extensive with a larger proportion of the gland completely developed. By 33 days of pregnancy duct growth no longer may be distinguished. At this time the alveoli appear to be formed for the greater part although it is impossible to make a definite assertion (Fig. 31). The lumina of the alveoli are beginning to form.

In gross mounts of a 47-day gland the duct system is obscured by the enlarging lobule-alveolar development. Only at the outer margin may an occasional lobule duct be seen with the thick cluster of alveoli extending in all directions. Photographs of the whole mounts from this time forward fail to show adequately the condition of the gland which may be observed with a binocular dissecting microscope.

Upon section the gland is observed to be composed of a compact mass of tissue consisting for the most part of spherical alveoli lined with cuboidal epithelial cells. The lumina are small but filled with secretion. Sections of the larger ducts may be seen. The stroma consists of thin sheathes of connective tissue fibers surrounding the alveoli and larger divisions of the gland.

From the 33-day to the 57-day stage, the glandular parenchyma remains approximately the same anatomically except for the gradual increase in the diameter of the lumina of the alveoli coincident with the gradual accumulation of secretion. Even at the 57-day stage the lumina are not greatly distended with secretion (Fig. 32). However, at this time a milky secretion frequently may be expressed from the nipple.

The length of pregnancy in the guinea pig is variable. Ibsen (1928) stated that the full gestation period is usually 68 days. The most advanced intrauterine stage was 64 days. At this time the lumina of the alveoli had further enlarged but were considerably smaller than after parturition (Figs. 33 and 35).

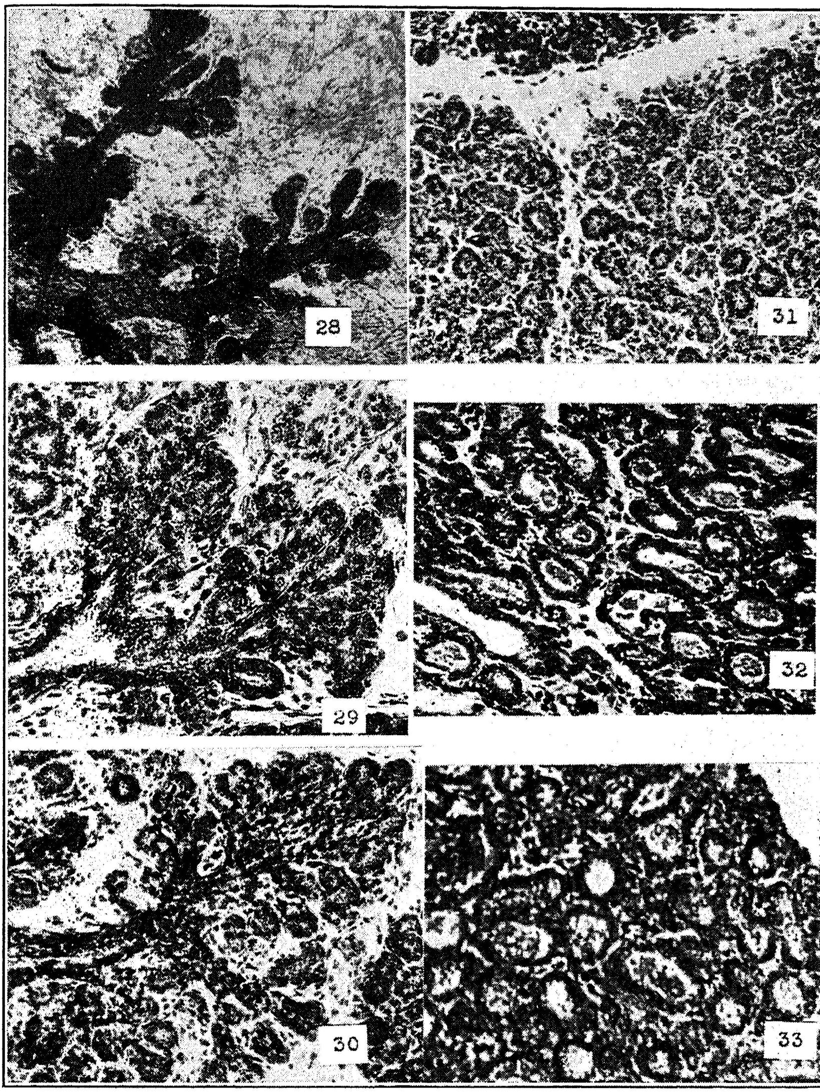

Plate V.

Fig. 28. Portion of the mammary gland of a 15-day pregnant guinea pig, showing the free ends of a rapidly growing duct. (x34).

Fig. 29. Microphotograph of a section of the mammary gland of a guinea pig pregnant 25 days. (x41).

Fig. 30. Microphotograph of a section of the mammary gland of a guinea pig pregnant 30 days.(x41).

Fig. 31. Microphotograph of a section of the mammary gland of a guinea pig pregnant 33 days. (x41).

Fig. 32. Microphotograph of a portion of a sectioned mammary gland of a guinea pig pregnant 47 days. (x41).

Fig. 33. Microphotograph of a section of the mammary gland of a guinea pig pregnant 64 days (full term). (x41).

These observations on the development of the mammary gland of the guinea pig during pregnancy indicate that in this species also the growth of the lobule-alveolar system reaches completion at about the middle of pregnancy. However, this division should be considered only an approximation. It is our desire to emphasize that the growth phase of development of the gland characterizes the first half of pregnancy while the later half of pregnancy is characterized by the gradual initiation of the functional activity of the epithelial cells of the alveoli. It is quite probable that the transition from the growth phase to the secretory phase is gradual with slight growth still continuing at the periphery of the gland while the epithelial cells of the first formed alveoli are beginning to show signs of secretory activty.

Lactation.—There is a gradual initiation of secretory activity in the epithelium of the alveoli during the latter half of pregnancy which is indicated by the enlargement of their lumina. At this time the gland is apparently prepared for lactation as indicated by the fact that abortion, hysterectomy, or the death of the fetus will result in an immediate enlargement of the gland and secretion of milk. However, the great stimulus to milk secretion observed at the time of parturition is held in check during this period.

In the guinea pig, Kuramitsu and Loeb (1921) observed that the epithelial cells had not yet reached their full size, neither were the alveoli fully distended six to 12 hours after parturition. Mitotic cells in the alveoli and ducts were occasionally observed. Two days after parturition the glands were reddish-white in appearance and had reached maximum size. Mitotic cells no longer were seen but amitosis appeared frequent. It is believed that the early proliferation depends upon the factors that determine heat and ovulation as it occurred also in the castrate animal.

Seven days after parturition they noted no further advance beyond the two-day stage. Secretion was fully established. After two weeks conditions were similar to those found after seven days. No difference between the normal and castrate animal could be observed. After three weeks some slight changes were noticed. At this period the point where the transition from intense activity to a marked diminution in the nursing occurred.

The retrogressive changes in the mammary gland were much more marked after four weeks. At this period the guinea pigs no longer nurse their young ones to the same extent as before and the mammary glands were smaller in both castrate and normal animals. The lobules and alveoli were much smaller. The connective tissue surrounding the alveoli become more prominent and lymphocytes may be seen in the stroma and migrating through the epithelium of the alveoli.

After five weeks Kuramitsu and Loeb observed in both castrated and non-castrated animals a further decrease in size of the gland. The gland was still composed of many lobules, but they were now much smaller and each lobule consisted of a smaller number of alveoli. Lymphocytes were abundant.

Our observations are in agreement with the above report. In comparing the mammary gland of an animal at parturition (64 days) with that from one a day after parturition, it was observed that the average size of the alveoli during the interval had increased considerably (Figs. 33, 34, and 35). On the second day after parturition the alveoli and ducts were greatly distended with milk. The gland stroma was relatively inconspicuous.

Involution.—The involution of the mammary gland may be accomplished rapidly by failure to remove the secretion from the mammary gland at the time of weaning or more gradually during the course of the lactation period, as evidenced by the decline in the rate of milk secretion. In small experimental animals such as the guinea pig, the latter type of involution is difficult if not impossible to study. Instead, the involution usually observed follows the gradual self-weaning of the young as other feeds are eaten or the sudden removal of the young either at birth or at any period during lactation.

Little can be added to the detailed observations of Kuramitsu and Loeb (1921) on the changes in the gland when the young do not nurse the mammary gland. Two days after parturition the lack of nursing is noticeable. At this period the mammary glands are large but rather pale. After seven days the gland is smaller than that at two days, still comprising many but much smaller lobules and fewer alveoli which have a small or medium sized lumen. The epithelium is low, cuboidal or flat in the alveoli of medium or small size, and somewhat higher and larger in some of the larger alveoli. The regression of the gland is slightly more advanced in the castrate than in the non-castrate. Degenerative processes in the gland are indicated by vacuolization, karyolysis, and pycnosis of the cells. At some places the epithelium has disappeared.

The gland at the end of two weeks shows that a steady decline in size and activity has occurred, which is continued during the fourth and fifth weeks. The gland then consists of a few lobules and a few alveoli. The cells of the alveoli are small and low. The difference between the mammary gland of the castrate and the non-castrate has almost disappeared.

In our studies it was observed that ten days after weaning the larger part of the milk had been resorbed from the alveoli leaving the gland much thinner than during lactation (Fig. 36). The alveoli were small but still distinct. After 35 days of involution the gross mounts of the gland appeared similar to the gland after 20 days of pregnancy (Fig. 37). Upon section, the lobules appear to have degenerated to a marked extent, leaving a duct system with few alveoli-like structures (Fig. 38).

Upon extended involution represented by an animal 100 days after weaning her young, the lobule-alveolar structures developed during pregnancy have degenerated, leaving intact only the larger ducts and lateral branches similar to the gland of the nulliparous animal. (Fig. 39).

The Vascular Supply to the Mammary Gland.—It is of interest to note briefly the origin and course of the blood vessels supplying the blood to the mammary gland. As the mammary glands of the guinea pig are located in the inguinal region on either side of the midline, they receive their blood supply largely from the superficial epigastric arteries arising from the femorals in the region of the inguinal canals. The superficial vessels of the thoraco-abdominal wall are probably of little if any importance, although anastomoses between the two systems may be observed on the abdominal wall. Small branches of the hypogastric arteries may pass through the subcutaneous muscles and penetrate into the gland area (Fig. 40).

The changes of the vascular system during pregnancy, lactation, and involution in the guinea pig are quite similar to those described in the mouse (Turner and Gomez 1933).

Number and Arrangement of the Nipples.—The mammary apparatus of the guinea pig normally consists of a pair of nipples and the associated glands. These are located in the inguinal region approximately half way between the anterior-posterior extent of the fatty pad on either side of the midline (Fig. 40). While the frequency distribution in the nipple number of some species show great variability, in the guinea pig little or no variability has been reported. Neither is there a sex difference as the nipple is present in both the male and female.

The observation of 150 animals consisting of 104 females and 46 males resulted in finding 148 or 98.67 per cent with two nipples and 2 or 1.33 per cent with three nipples. Of the two animals observed

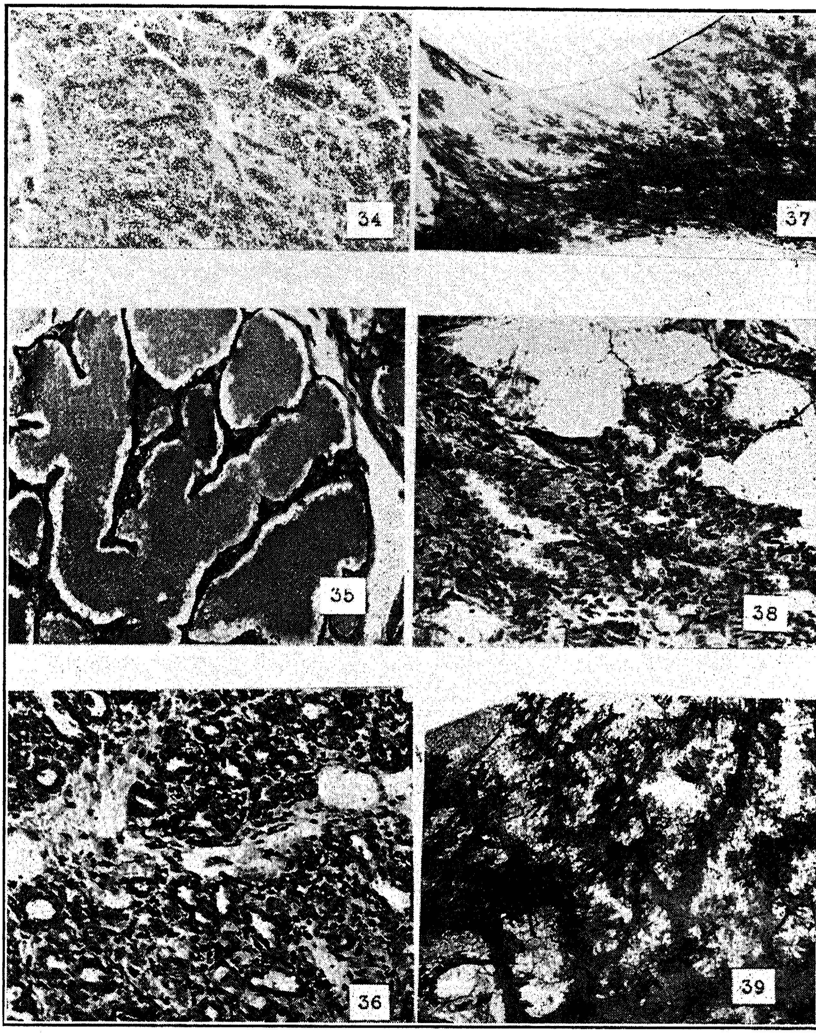

Plate VI.

Fig. 34. Portion of a frozen section of the mammary gland of the guinea pig on the second day after parturition. (x6 2/3).

Fig. 35. Microphotograph of a portion of a sectioned mammary gland of the guinea pig on the second day after parturition. (x40).

Fig. 36. Microphotograph of a portion of a sectioned mammary gland of the guinea pig, ten days after weaning. (x40).

Fig. 37. A portion of the mammary gland of the guinea pig 35 days after weaning. (x6 2/3).

Fig. 38. Microphotograph of a portion of a sectioned mammary gland of the guinea pig, 35 days after weaning. (x40).

Fig. 39. Portion of the mammary gland of the guinea pig, 100 days after weaning. (x6 2/3).

with three nipples one was a male and the other a female. In both cases the supernumerary nipples were considerably smaller than the normal nipples and the gland associated with them appeared similar to the normal except for the extent of the gland parenchyma. In the case of the male, the supernumerary nipple was observed after the nipples were grown with theelin.

The nipples are canalized by a single duct. The constricted entrance to the teat composed of pavement epithelium similar to the epidermis of the skin is called the streak canal. The duct then enlarges in the form of an ampullary dilatation corresponding to the cistern of the teat in larger mammals. At the base of the nipple the duct turns caudally and divides to form two secondary ducts.

Plate VII
(See Opposite Page)

Fig. 40. Ventral view of an adult female guinea pig with the skin removed to show the position of the glands, the lymph nodes, and the vascular system. The light shading in the inguinal region indicates the extent of the fatty pads. The deep shading shows the location of the mammary gland which extends from the nipple caudally to the posterior extent of the groin. The position of the two lymph nodes on either side are shown as dark spheroid structures.

The blood to the mammary gland comes by way of the superficial epigastric arteries (mammary arteries) arising from the femorals in the region of the inguinal canals. Large veins usually parallel these arteries. The thoraco-epigastries arise as branches of the internal thoracic arteries and supply but little if any of the blood to the mammary glands.

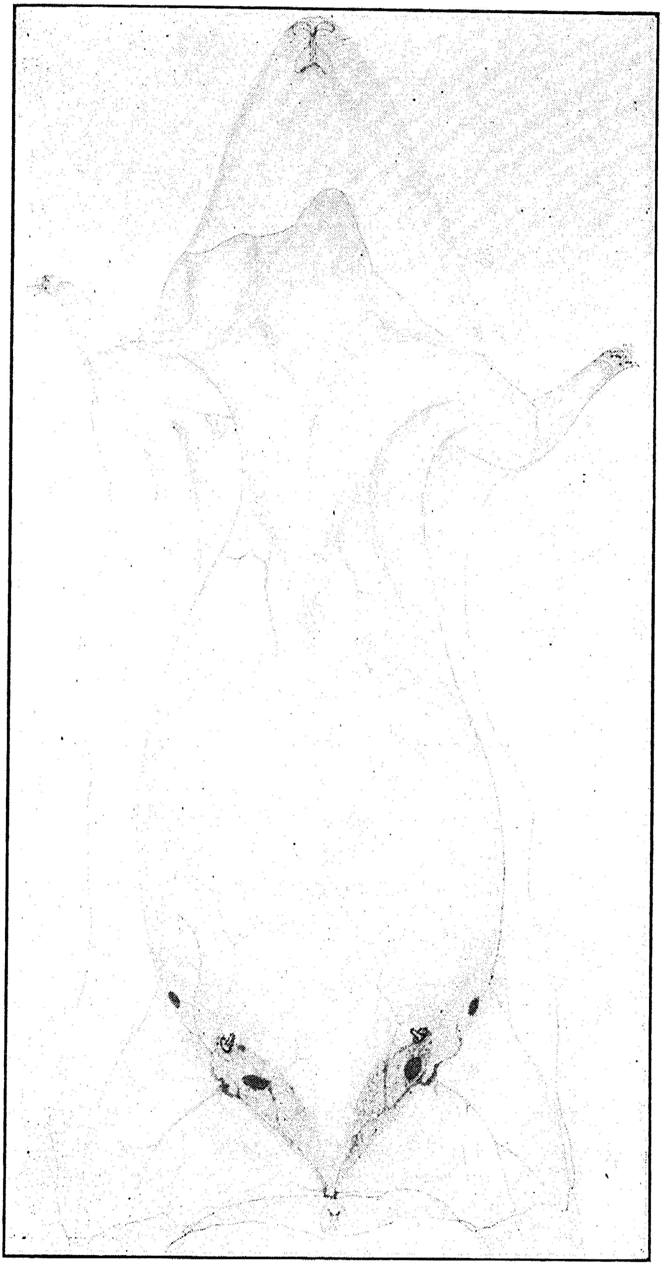

Plate VII.
(See Opposite Page for Explanation)

SUMMARY AND CONCLUSIONS

1. In a 20-day-old guinea pig the mammary lines were observed in the region of the dorsal limiting furrows appearing as narrow light lines extending from the anterior limbs caudad to the posterior appendages.

2. The next stage of development, the mammary bud, was observed completely formed in both male and female at the 25-day stage.

3. A solid cord of cells was seen sprouting from the mammary bud in the 30-day stages of both male and female embryos.This structure, called the primary sprout, showed further growth in length in the 33-day stage. Canalization of the sprout to form a duct was complete by the 44-day stage just as the secondary sprouts were beginning to form.

4. The duct system of the gland develops as a result of the growth and canalization of the secondary sprouts which showed considerable growth in the 47-day stage. In 57- and 64-day stages, the duct system had become further extended and dichotomous branches of the chief ducts appear as well as further lateral sprouts of the duct walls.

5. The growth of the mesenchyme cells surrounding the mammary bud in the 30-day stage caused the elevation of the bud and the initial stages of nipple formation. The 44- and 57-day stages showed further development reaching at the latter stage a typical papillary form.

6. The gland stroma composed of the supporting connective tissue develops in the form of fatty pads during late intrauterine life into which the duct system gradually grows.

7. In the female guinea pig there appears to be a slight continuation of duct growth from birth to the approach of estrum. The male differs from the male mouse and rabbit, while appearing more like the rat in that slight duct growth continues for several months after birth.

8. At the approach of and during estrum there appears an increasing number of bud-like outgrowths along the lateral walls of the ducts while the ducts are enlarged with fluid. In contrast during the luteal phase of the cycle, the entire duct system appears shrunken and the end-buds less deeply staining. Growth of true alveoli similar to that developing during pregnancy were stimulated only slightly if at all in the virgin female.

9. The mammary gland is a tubulo-alveolar gland. While the tubules develop extensively with recurring estrus cycles, the alveolar

or saccular ends are extensively developed only during pregnancy in preparation for lactation. These structures in the guinea pig appear to form chiefly between the 20th and 35th days of pregnancy. During the latter part of pregnancy the epithelial cells of the alveolar structures slowly initiate a secretory phase which reaches a maximum following parturition.

10. The mammary gland appears to attain maximum secretory activity within a few days after parturition. So long as all the milk is removed regularly the histologic and cytologic picture of the gland is fairly constant but as the requirements for milk become less, involutionary changes appear.

11. When the young are removed, the gland begins to undergo a steady decline in size and activity. First the milk is gradually resorbed during the first 10 days or two weeks. Then the lobules gradually degenerate, leaving eventually a duct system with few if any alveoli.

12. The mammary glands receive their blood supply largely from the superficial epigastric arteries arising from the femoral arteries. However, small branches of the hypogastrics may pass through the subcutaneous muscles and penetrate into the gland area.

13. The mammary apparatus of the guinea pig normally consists of a pair of inguinal nipples and associated glands. In only two cases out of 150 were supernumerary nipples observed. Of these one was observed in a male while the second was observed in a female.

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