

STUDIES ON THE MAMMARY GLAND

II. THE FETAL DEVELOPMENT OF THE MAMMARY GLAND IN THE FEMALE ALBINO RAT

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TWELVE FIGURES

Henneberg ('00) made a careful study of the development of the mammary glands in the albino rat from the earliest appearance of the glands through the conditions found in sixteen day fetuses. Also the postnatal (birth to ten weeks) development of these glands has been investigated (Myers, '16). Heretofore the developmental conditions between sixteen day fetuses and newborn rats have presented a gap in our knowledge of the mammary glands. The object of the present investigation is to fill up this gap, thus completing the history of the mammary glands in the albino rat (*Mus norvegicus albinus*) to ten weeks after birth. An abstract of the results has already been published (Myers, 17).

LITERATURE

No attempt is made to review all the literature pertaining to the development of the mammary gland, which is thoroughly discussed in the works of Bonnet ('97), Brouha ('05), Bresslau ('10) and Schil ('12). Henneberg's work ('00) in the early development of the mammary glands in the albino rat is here briefly reviewed, however, since the earlier stages must be kept in mind to make clear their relations with the later foetal stages described in the present paper.

Henneberg ('00) found in an albino rat embryo of eleven days, in the region of the dorsal limiting furrow (on only one side), some cubical cells in a single layer representing the anlage of the mammary streak. In an embryo of twelve days a mam-

mary streak is present on each side. Each streak consists of a single layer of cubical epithelium. The breadth of the streak has increased and now extends from a few cells dorsal to the dorsal limiting furrow ventrally to cover nearly half of the parietal zone. Its cephalic and caudal ends blend with the cubical epithelium of the limb anlagen.

At twelve days and thirteen hours, the cells of the mammary streak are larger and in the region of the dorsal limiting furrow a second layer of cells is beginning to appear superficial to the cubical cells. Immediately beneath the mammary streak the mesenchymal cells have condensed. The mammary streak shows two distinct cell layers in embryos of thirteen days and one hour. The superficial layer—stratum corneum—consists of flat cells with oval nuclei with their long axes parallel to the surface. The deep layer—stratum mucosum—is composed of large round or cubical to cylindrical cells with oblong nuclei. The streak is separated from the mesenchyma by a distinct light line—the basement membrane.

Henneberg found the first appearance of the mammary line in a rat embryo of thirteen days and fourteen hours. At this stage it is produced by a thickening of parts of the mammary streak. In some places a part of the mammary streak is converted into the mammary line by the appearance of a third layer of round cells between the superficial and deep layers. In other places the cells have slightly thickened thus producing the first appearance of the mammary line without the addition of a third layer. In other embryos of the same age the mammary line in the thoracic region is three to four layers of cells thick and its greatest breadth shows twelve to fourteen layers of cells. It disappears a short distance cephalad to the anterior extremity. In the inguinal region the line is still very indistinct and requires special technique for its study. In some embryos a complete interruption exists between the region of the future thoracic glands and the abdominal gland. This is the first intimation of the future interspace between the glands of the thoracic region and those of the abdominal and inguinal regions. From this stage, Henneberg designated the cephalic part of the line

as the pectoral portion and the caudal part as the abdominal portion.

In rat embryos of fourteen days Henneberg found that the cephalic end of the mammary line has been transformed into a structure about the shape of a biconvex lens. This is the earliest appearance of the first pectoral mammary hillock. In other embryos of the same age the second and third pectoral and the abdominal hillocks are beginning to appear. The greater convexity of each hillock lies embedded in the mesenchyma. The remaining parts of the mammary streak and line represented by the space between the hillocks are beginning to atrophy. At this stage the mammary line for the inguinal glands resembles in structure the line for the pectoral and abdominal glands in the thirteen day and fourteen hour stage.

Henneberg found in fifteen day rat embryos that the mammary gland anlagen are no longer elevated above the surface but that their deep surfaces have pressed deeper into the mesenchyma thus presenting the 'mammary point' stage. At this stage the inguinal glands are still somewhat retarded in their development. At sixteen days Henneberg states that the mammary gland anlagen correspond to the club-shaped stage which Rein ('82) found in rabbit embryos. Henneberg did not investigate the later stages in the rat.

MATERIAL AND TECHNIQUE

The fetuses for the present work were collected in the following manner. Adult males and females were placed in the same cage from six o'clock in the evening until six o'clock in the morning. As found by Danforth ('16) in case of mice, better results were obtained when the females were placed in the cage which the males occupy permanently. The females were then returned to their respective cages. In all cases of pregnancy semination was dated at the ninth hour after the females were placed in the cages with the males. The possibility of error in the age of the fetuses is plainly obvious. However, the error could only be a matter of a few hours. Sobotta and Burekhard ('11) estimated that spermatozoa of the albino rat do not

live more than nine or ten hours in the reproductive tract of the female.

During 1914-1915 a large number of observations were made on females with the hope of finding a definite way of knowing just when the animals is in heat or when copulation has taken place. No definite gelatinous plug was found closing the vaginal orifice after copulation as Sobotta ('95) observed in white mice. A yellow and somewhat viscid vaginal secretion appears at rather regular intervals. This secretion usually makes its first appearance shortly after the opening of the vagina which occurs about the eighth week. In young females it occurs thereafter at quite irregular intervals but later it may be seen about every fifth to eighth day. No definite relation has yet been established between the appearance of the vaginal secretion and the time of insemination. However, it was noticed that many of the females became pregnant while the secretion was present. The origin of the vaginal secretion and its relation to ovulation is still being studied with the hope of obtaining definite knowledge as to the time of ovulation in the white rat.

Some of the fetuses were fixed in Zenker's fluid, others in 10 per cent formalin. In the earlier (fifteen day and nine hours, sixteen day and twelve hours, and seventeen day and two hours) stages several fetuses were cut for each stage described while in the later (eighteen days and nine hours, nineteen days and six hours, and twenty days and six hours) stages only one fetus was entirely sectioned and merely the skin containing the mammary glands from several other individuals was sectioned. The mammary glands of other fetuses were studied macroscopically. In all 30 individuals were examined. A part of the material was cut at 5 μ or 7 μ and stained with iron hematoxylin; the remainder was cut at 10 μ and stained with alum hematoxylin and eosin or with Mallory's connective tissue stain. Weigert's elastic tissue stain was also applied to some of the fetuses of the latest stages. For a study of the varieties of white blood corpuscles Dominici's combination stain was used.

A few dissections and observations proved that in the late fetal stages the sex could be determined by the relative ano-genital

distance as described by Jackson ('12) in determining the sex of the newborn. In the earlier fetal stages the sex was determined by studying the developing reproductive organs.

The wax reconstructions were made according to Born's method.

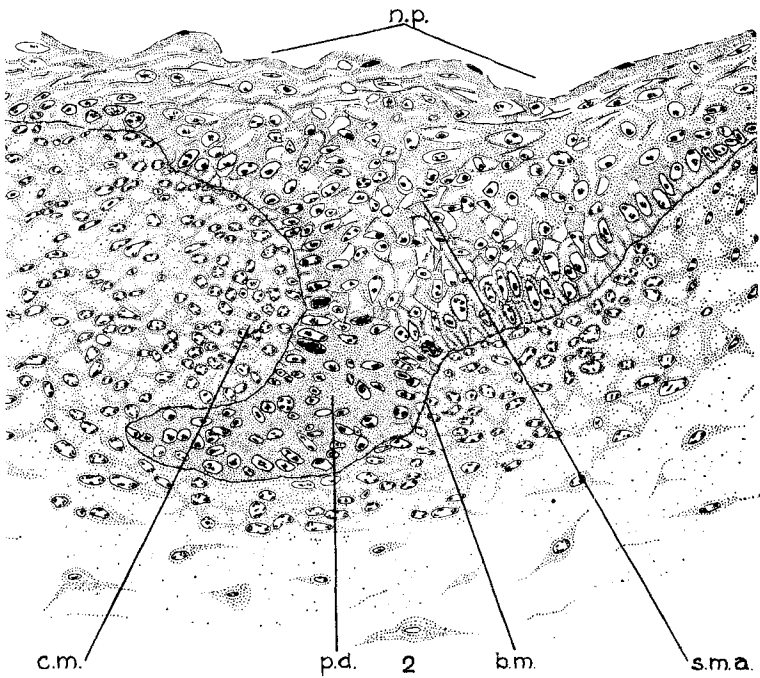
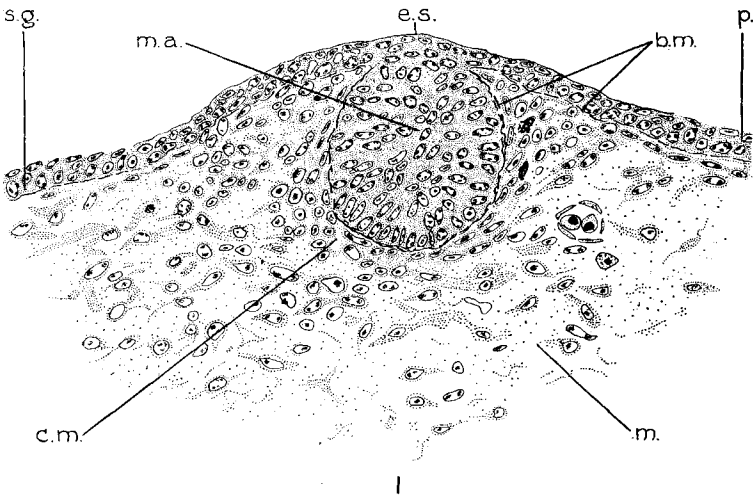
OBSERVATIONS

Henneberg states that in fifteen day and fourteen hour embryos the six pairs of mammary glands occupy their definitive positions. Since Henneberg made only a macroscopic study of the glands at this stage, a further account is here given of the condition found in embryos of nearly the same age.

Fifteen days. On the surface of the skin at this stage (fifteen days and nine hours) is a small eminence (fig. 7) over each developing gland. Such eminences are very prominent in fresh preparations. A cross section through a gland (fig. 1) shows that the epidermis in the neighborhood of the gland is composed of only two layers, a superficial layer (periderm) of flattened cells with their long axes parallel to the surface, and a deeper layer (stratum germinativum or Malpighian layer) of round or cubical cells. The nuclei of the latter layers are located toward the free end of the cells. The basal ends of the cells have a quite clear appearance and rest on a definite basement membrane (fig. 1).

The basement membrane dips down into the underlying mesenchyma to surround the spheroidal mass of epithelial cells forming the gland anlage. Likewise the stratum germinativum of the epidermis passes deep around the same circular mass of cells and forms the basal layer of the mass. The cells of the spheroidal mass are differentiated and arranged so that they possess a characteristic appearance. The cells of the basal layer appear much more elongated than those in the stratum germinativum of the adjacent epidermis. The cells occupying the center are irregular in shape and closely packed.

Superficially the gland anlage projects somewhat producing the eminence visible from the surface. Around its deep surface the mesenchyma is condensed. The mesenchymal cells lying



nearest the developing mammary gland are somewhat elongated and arranged in two or three very regular layers concentrically placed (fig. 1). Outside of the concentric layers, the condensed mesenchymal cells seem to have no definite arrangement. In the condensed mesenchyma is seen an occasional small blood vessel containing nucleated red blood corpuscles.

Wax reconstructions (fig. 10) show that the differentiated mass of cells which appears circular in cross section, forms an oblong ellipsoidal body which is attached to the epidermis by a very short, constricted neck (*nk*).

Sixteen days. In fetuses of sixteen days and twelve hours the mammary eminences still appear on the surface of the skin as slightly elevated areas which in fresh preparations have a somewhat lighter appearance than the surrounding tissue.

In microscopic sections the epidermis presents the two distinct layers of cells found in the preceding stage. In addition an intermediate layer of cells has appeared in some parts of the skin. In some places the epidermis is slightly thickened to form hair anlagen, but in no case were such anlagen observed in the epidermis adjacent to the mammary gland anlagen. The so-called basement membrane appears as a homogeneous band immediately below the stratum germinativum. Just beneath the basement membrane the mesenchymal cells are densely placed thus forming a fairly definite layer. Immediately beneath this layer the mesenchymal cells are less numerous and apparently have no regular arrangement. Mitotic figures are very com-

Fig. 1 Drawing of a section through the right second thoracic mammary gland region of an albino rat fetus of fifteen days and nine hours. $\times 300$. Zenker's fixation; hematoxylin-eosin stain. Drawn with the aid of a camera lucida. *b.m.*, basement membrane; *c.m.*, condensed mesenchyma; *e.s.*, eminence (mammary hillock) on surface of skin produced by mammary gland anlage; *m.*, loose, irregularly arranged mesenchyma; *m.a.*, mammary gland anlage; *p.*, periderm; *s.g.*, stratum germinativum.

Fig. 2 Drawing of a section through the left second thoracic developing mammary gland of a female albino rat fetus of eighteen days and nine hours. $\times 300$. Zenker's fixation; hematoxylin-eosin stain. Drawn with the aid of a camera lucida. *b.m.*, basement membrane; *c.m.*, condensed mesenchyma; *m.p.*, early appearance of mammary pit; *p.d.*, deep portion of mammary gland anlage (primary duct); *s.m.a.*, superficial part of mammary anlage, becoming cornified.

mon in the mesenchyma immediately surrounding the gland. An occasional small blood vessel is seen coursing toward the mammary gland area.

The mammary gland anlagen show about the same stage of development as in the preceding stage. A study of all six pairs shows that the inguinal mammary glands are slightly behind the others in their stage of development.

Seventeen days. At seventeen days and two hours the eminences described in the previous stages have disappeared. The gland areas instead appear as slight depressions or pits on the surface of the skin. These mammary pits represent the point of ingrowth of the epithelium. The epidermis is slightly thicker than in the preceding stage and in the regions of the mammary glands presents a very definite basement membrane. The gland anlagen now measure only about 0.05 mm. in length.

Eighteen days. Fresh preparations, sections, and wax reconstructions from fetuses of eighteen days and nine hours show a definite mammary pit on the surface of the epidermis over each future nipple area (figs. 2 and 8, *n.p.*). In cross section the stratum germinativum is now depressed so as to form a shallow funnel-shaped outline. The mouth of the funnel is directed toward the surface and is partly filled with epithelial cells which show traces of cornification and desquamation. Intercellular vacuoles are also being formed. The outlet of the funnel extends into the corium and becomes continuous with the anlage of the primary mammary duct.

At this stage the gland anlage, which in the earlier stages was an oblong, ellipsoidal mass of epithelial cells, has increased in length. Its deep part now becomes the anlage of the primary duct, while its superficial portion is undergoing vacuolization, cornification, and desquamation, thus forming the pit superficial to the primary duct. The end of the primary duct anlage directly beneath the surface pit is attached to the epidermis and throughout this paper will be designated as the attached end. The opposite end of the anlage is unattached and throughout this paper will be known as the free end. The stratum germinativum of the adjacent epidermis continues over the mammary anlage as its future primary peripheral layer of cells. The

primary duct anlage is roughly L-shaped with its attached end perpendicular and its free end parallel to the surface (figs. 2 and 8). The anlage in elongating has pushed ahead of it the above mentioned layers of condensed mesenchyma representing the corium and tela subcutanea. These layers now completely surround the free part of the anlage. In the first thoracic gland the free end of the anlage is directed cephalad. In the second inguinal gland, the free end points caudad. Likewise the free end of each of the remaining ducts is directed toward the position which the future duct and its branches will occupy.

The anlages of the ducts are longer than in the seventeen day and two hour stage. In one of the first thoracic glands of one fetus and in one of the abdominal glands of another fetus the primary duct presents two secondary ducts (fig. 17, *s.d.*). All other glands observed at this stage possess a single undivided primary duct.

When seen in cross section at this stage, the primary duct of most of the glands possesses a basal layer of cuboidal cells with large oval nuclei. The basal ends of the cells rest on a somewhat indistinct basement membrane while the opposite ends are directed toward the center of the duct. The center of the duct is filled with cells of irregular shape. Somewhat nearer the free than the attached end of some of the ducts the cells occupying the center of the duct show a tendency toward separation from each other. In other ducts some of the central cells have entirely separated, thus producing small cavities or lacunae, the first appearance of a very indefinite lumen (fig. 4). Such a condition obtains in many of the thoracic and abdominal glands examined, but is very rare in the inguinal glands of this stage. It is interesting to note that in the thoracic and abdominal glands which have already developed secondary ducts, only one of these ducts shows a slight indication of a lumen. The mesenchymal cells of the corium and tela subcutanea are somewhat condensed around the ducts. Those nearest the ducts are much elongated and are concentrically arranged.

In one abdominal gland about half way between the outlet and mouth of the funnel the cells of the stratum germinativum have slightly elongated thus forming a low ridge which projects

into the subjacent corium. The ridge extends entirely around the funnel and is the anlage of the epithelial hood, which was described in the postnatal stages of the albino rat (Myers '16).

Nineteen days. The funnel-shaped epithelial area corresponding to the mammary pit at nineteen days and six hours contains some cornified epithelium. This is apparently being cast off by the process of desquamation, thus deepening the mammary pit superficial to the attached primary duct.

The primary mammary ducts have made a rapid growth and present secondary ducts in all glands, while in most glands examined the secondary ducts present tertiary ducts. The two inguinal glands present lumina in about the same stage of development as was described in the thoracic and abdominal glands in the eighteen day and nine hour stage. The rudimentary lumina in all glands are slightly further developed toward the free ends of the ducts but are by no means confined to the free ends. Many of the cells near the developing lumina are undergoing mitotic divisions. There is no pyknosis or other evidence of cell degeneration.

The anlage of the epithelial hood is composed of elongated cells of the stratum germinativum, but a second layer of cells deep to the layer described as forming a low ridge in the preceding stage is beginning to appear. The ridge now projects deeper into the subjacent corium. Numerous mitotic figures are seen in the epithelial cells in the region of the free edge of the hood.

The developing hair follicles have grown more deeply into the corium than those described in a preceding stage. Ordinarily the follicles are located a considerable distance from the mammary pits. No follicles were observed in the mammary pits.

Twenty days. At twenty days and six hours well defined mammary pits in the epidermis represent (as in the preceding stage) the regions of the mammary glands. Wax reconstructions, however, show that at the bottom of each pit there is a rounded elevated portion of the epidermis (fig. 9, *n.a.*). This elevated part is the anlage of the nipple. In the preceding stage as noted the depression or funnel was partly filled with cells, which became cornified as age advanced, thus giving the integument

over the mammary glands a thickened appearance. Later the cornified cells were cast out and thus the funnel corresponding to the mammary pit was deepened. The anlage of the nipple in the present stage seems to have pushed from the bottom of the mammary pit toward the surface leaving a surrounding furrow or sulcus (figs. 3 and 9, s.). The superficial part of the epidermis over the nipple anlage now appears no thicker than that in adjacent regions.

The anlage of the epithelial hood has grown more deeply into the corium now encroaching upon the tela subcutanea. Cornified epithelial cells occupy the space between the inner and outer surfaces of the hood.

When the stratum germinativum of the epidermis is traced toward the region of the mammary gland it is seen to pass deeply and form the outer surface of the epithelial hood. It then covers the free edge of the hood and turning back forms the inner surface of the hood. Next it covers the deep surface of the nipple anlage. Throughout its extent in the mammary region the cells of the stratum germinativum rest on a basement membrane (fig. 3).

The corium within the epithelial hood is composed of connective tissue cells the processes of which take a deep blue stain when treated with Mallory's connective tissue stain. Small blood vessels and nerves are also included. From the surface of the nipple anlage the primary duct is seen coursing through the corium in the center of the hood on its way to the subcutaneous tela where it turns at right angles after which it lies parallel with the surface of the integument. Soon after reaching the subcutaneous tela and turning at right angles the duct divides into secondary ducts each of which in turn divides into tertiary ducts. Quaternary ducts are beginning to arise from the tertiary ducts (fig. 12). The terminal ducts present small knob-like enlargements or end-buds. Not every gland observed at this stage presents all of the above mentioned branches. For example in the second inguinal gland of one specimen the primary duct has divided into two secondary ducts which remain undivided.

The time of formation of the lumen evidently is subject to considerable variation. While its first appearance was observed in eighteen day and nine hour and nineteen day and six hour

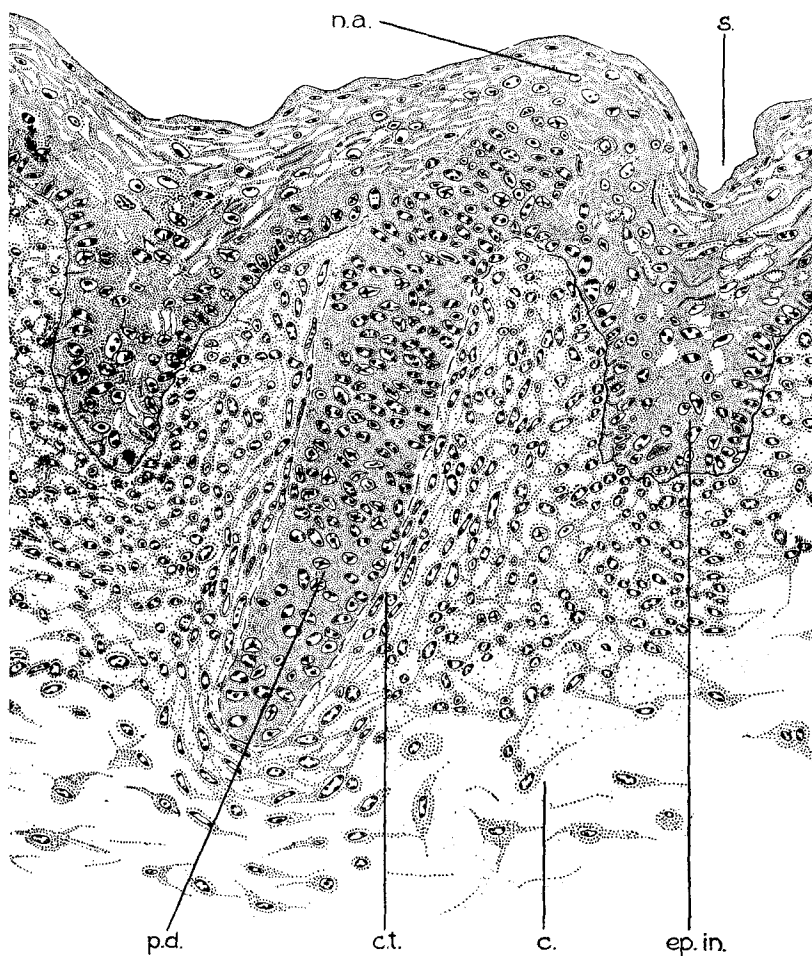


Fig. 3 Drawn from a section through right first thoracic developing mammary gland of a female albino rat fetus of twenty days and six hours. $\times 300$. Zenker's fixation; hematoxylin-eosin stain. Drawn with the aid of a camera lucida. *c.*, irregularly arranged developing connective tissue cells; *ct.*, developing connective tissue forming sheath around duct; *ep.in.*, epithelial ingrowth or hood; *n.a.*, nipple anlage; *p.d.*, primary duct; *s.*, sulcus surrounding nipple anlage.

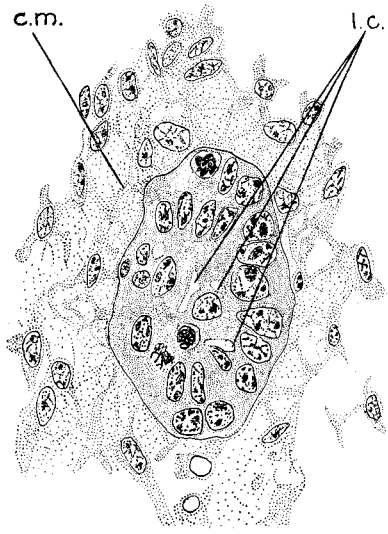
fetuses there are still systems of ducts at twenty days and six hours which show absolutely no trace of a lumen. In other glands of this stage the lumina are much larger than in the preceding stages (fig. 5). When present, the lumina are better developed in the free ends of the system of ducts, i.e., in the terminal ducts and the ones from which they arise; however, quite frequently traces of lumina are observed in the primary and secondary ducts. In no part of any system of glands observed is there a definitive lumen present. The walls of all are irregular, but have quite sharp boundaries. In no case are degenerating cells found within the lumen.

Figures 4 and 5 show that the first indication of a lumen is the appearance of a few independent lacunae. In cross section of the ducts such lacunae are usually seen located near the center of the developing ducts; however, they are not uncommonly found near the periphery, at the central ends of the peripheral layer of cells. When traced longitudinally any individual lacuna is found to extend only a very short distance; but in serial sections other lacunae are found forming more or less definite rows extending along the ducts. In some glands the lacunae are present from the end-buds well into the primary ducts.

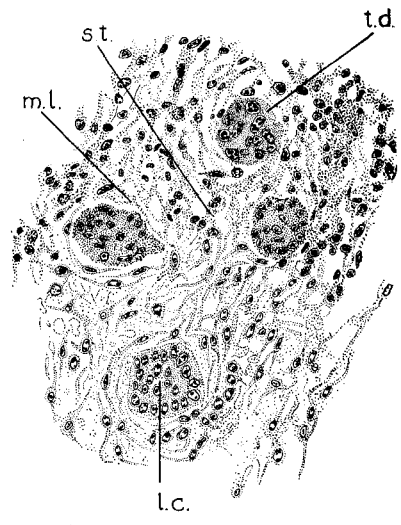
The lacunae later increase in size and apparently flow together, thus forming the lumina found in some individuals of this stage (fig. 5, l). The lumina at this stage are never continuous throughout the system of ducts. But a lumen may extend throughout a terminal duct, then with an interruption appear again in the tertiary or secondary ducts.

Owing to individual variation, it is possible to find all of the above described developmental stages of lumina in twenty day and six hour fetuses.

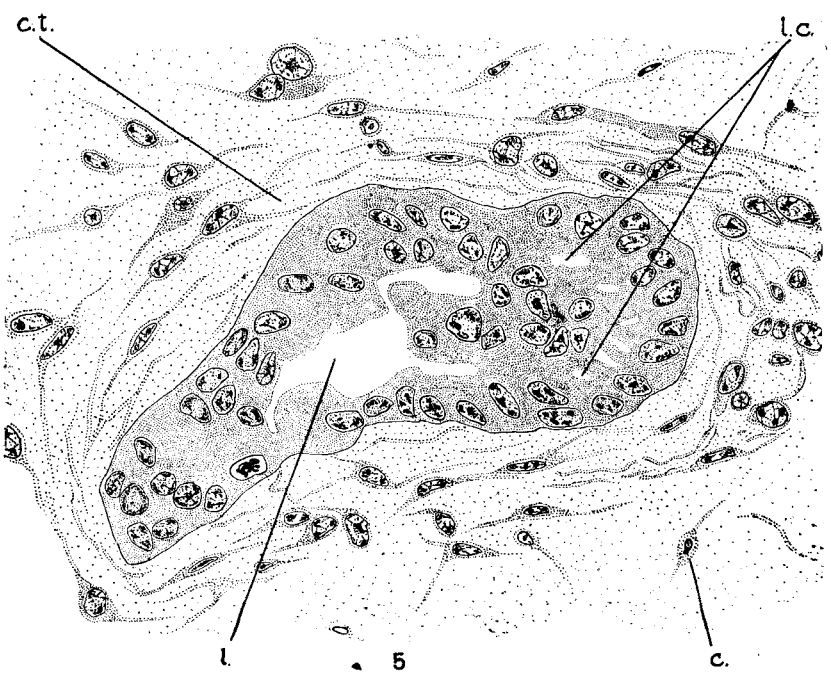
Several of the glands of this stage were stained with Dominici's combination stain. In blood vessels, the corium within the epithelial hood, the connective tissue immediately surrounding the ducts, and the ordinary connective tissue in the entire gland region were found various kinds of white blood cells including eosinophiles. In one gland a few lymphocytes were observed in the developing lumina of the ducts. None of the glands ex-



4



6



5

amed showed such an infiltration of leucocytes as Keiffer ('02) and others have described in the human newborn.

The processes of the connective tissue cells have elongated and when treated with Mallory's connective tissue stain many of them now appear as true white fibrous connective tissue fibers. Weigert's elastic tissue stain revealed no trace of elastic fibers at this stage. The developing connective tissue has so differentiated that the anlagen of two adult parts may now be recognized. That part immediately adjacent to the ducts forms a thin sheath around them. This sheath is the anlage of the mantle layer. While the connective tissue between the ducts represents the anlage of the true stroma (fig. 6, *m.l.*, *s.t.*).

Lobules have not yet formed in the mammary gland.

The masses of fat which are so conspicuous in the postnatal stages are not developed at this stage.

The foregoing stage at twenty days and nine hours brings the description up to the condition at birth which was the starting point in my previous paper (Myers, '16). In newborn rats the lumina were found to extend through the primary ducts (except the intraepidermal portion) into the secondary ducts and to terminate in the end-buds. In the primary ducts the lumina are small irregular slit-like spaces which become continuous with the more regular rounded lumina of the remaining ducts. One can

Fig. 4 Drawn from a section through the primary duct (near free end) of the left second thoracic gland of a female albino rat fetus of eighteen days and nine hours to show development of lumen. $\times 550$. Zenker's fixation; hematoxylin-eosin stain. Drawn with the aid of a camera lucida. *c.m.*, condensed mesenchyma; *l.c.*, small cavities (lacunae) which later fuse to form lumen.

Fig. 5 Drawing of a tangential section through a secondary duct of the right first inguinal gland of a female albino rat fetus of twenty days and six hours to show developing lumen. $\times 550$. Zenker's fixation; hematoxylin-eosin stain. Drawn with the aid of camera lucida. *c.*, irregularly arranged developing connective tissue cells; *c.t.*, developing connective tissue forming sheath around duct; *l.*, lumen formed by fusion of small cavities (lacunae); *l.c.*, small cavities (lacunae).

Fig. 6 Drawn from a section through four tertiary ducts of the left first thoracic gland of a female albino rat fetus of twenty days and six hours. $\times 175$. Zenker's fixation; hematoxylin-eosin stain. Drawn with the aid of a camera lucida. *t.d.*, tertiary ducts; *m.l.*, developing connective tissue to form mantle layers; *st.*, developing connective tissue forming true stroma; *l.c.*, lumen.

safely assume that the process already begun in the eighteen to twenty day fetuses continues until the time of birth, thus producing the continuous lumina found in newborn rats.

The lumina at birth have not assumed their definitive form, however. In a later study the details of the further development of the lumina in the postnatal stages of the albino rat will be described.

DISCUSSION AND CONCLUSIONS

In the following discussion, the nipple, the milk-ducts, the epithelial hood, gland stroma, variation, and cephalocaudal sequence in development will be successively considered.

The nipple

A comparison of figures 1 and 7 is sufficient to show that in the albino rat fetus slight eminences occur in the region of the future nipples. These eminences evidently correspond to the mammary hillocks described in other forms. The mammary hillocks in the rat fetus (as in other forms) are temporary eminences, each being soon replaced, as has been shown, by a shallow depression, the mammary pit. At the bottom of this pit later occurs a slight elevation representing the nipple anlage. The true nipple reaches only a very rudimentary stage of development in rat fetuses. The latest stage studied (twenty days and six hours) shows the nipple anlage as a rather slight eminence at the bottom of the mammary pit (figs. 3 and 9).

The phenomena of development in the nipple and the associated hillock and pit are rendered more intelligible by a comparison with the conditions found in lower forms.

The mammary hillocks first appear in rat embryos of fourteen days (Henneberg, '00). The present work shows that they persist through the sixteenth day at which time they are less conspicuous than at the fifteenth day. These hillocks apparently occupy the positions of the future nipples. Because of their positions and resemblance to a nipple, Schultze ('92 and '93) in the pig and other species called them primitive nipples

('primitive Zitzen'), a misleading term since, as he observed, they are merely transient structures.

Similar hillocks have been observed in human embryos by Langer ('51), Rein ('82), Brouha ('05), Lustig ('16), and others. They have been described by Rein ('82), Schultze ('92 and '93), Bonnet ('92) and Brouha ('05) in the following species: pig, sheep, dog, fox, cat, rabbit, squirrel, rat, mouse, and mole. The name mammary hillocks ('Milchhügeln') was applied to them by Bonnet ('92).

The depression or fossa (mammary pit) which forms over each developing gland resembles the pocket which contains the nipple in some marsupials and which Owen ('68), Gegenbauer ('73) and others believed to exist in Monotremes. Bresslau in 1908 proved the non-existence of such a pouch in echidna and ornithorhynchus. In an earlier work, however, Bresslau ('02) observed that a definite pocket ('Zitzentasche') developed in some marsupials in the region of the future nipple. Bresslau's findings in marsupials confirmed the work of Klaatsch ('84) and others who showed that in marsupials a fairly deep pocket is developed in the region of each mammary gland; and at the bottom of each pocket a small papilla-like eminence occurs which is believed to be the first appearance of a nipple in mammals. During the resting phases of the glands the nipples remain in the pocket, but they actually protrude from the pocket and may be drawn out to a considerable extent while the glands are active.

The ontogeny of the mammary gland nipple of the albino rat apparently repeats in most respects the above described conditions in the lower forms of mammals. In the rat we have seen the surface over the future nipple region excavated (chiefly by the processes of cornification and desquamation) so as to form a definite pocket (figs. 8 and 9), the mammary pit. At the bottom of this pit is seen in sections the proximal end of the primary duct. Later a papilla-like elevation (the nipple anlage) appears at the bottom of the pit. At this time the nipple is so small that it occupies only a part of the pocket. At birth the nipple has enlarged so as to fill the pit, with the

exception of a shallow sulcus which still surrounds the nipple. The nipple in the newborn rat thus produces a slight eminence on the surface of the skin. In an earlier paper (Myers, '16) my low power drawings do not show the sulcus around the nipple in rats at birth and one week of age. This is due to the fact that over the sulcus the epidermis is slightly thickened, and also because the sulcus contains some cornified cells. Nevertheless under high power the sulcus is still very evident in these postnatal stages.

The mammary pit which develops before the appearance of the nipple is apparently homologous with the nipple pocket which Gegenbauer ('73 and '76), Rein ('82), Klaatsch ('84), Bresslau ('02), and many others observed especially in marsupials. Bresslau ('02) believed that the mammary pit is homologous with the marsupial pouch. Later, however (Bresslau '10), he regarded it as a homologue of the nipple pocket of marsupials.

The milk ducts

In the rat fetuses the anlage of the milk duct was first observed about the seventeenth or eighteenth day. At this time the deep part of each epithelial mammary gland anlage apparently elongates or sends out a single bud-like process which is the primary duct anlage. This stage may be said to correspond to Rein's ('82) period of bud formation ('Knospenbildung') in rabbits. It differs, however, from the findings of Langer ('51), Huss ('71), Kölliker ('79), Rein ('82), Profé ('98), Hamburger ('00), Brouha ('05), Lustig ('16), and many others in that they observed a variable number of buds (primary duct anlages) in man and other animal species including the horse, pig, cat and rabbit. On the other hand it agrees with the observations of De Sinety ('77), Gegenbauer ('76), Klaatsch ('84), and Brouha ('05) who reported the existence of a single primary duct in rodents and insectivorous mammals.

Between the eighteenth and nineteenth days each primary duct in the rat fetus presents two secondary ducts. The secondary ducts later present tertiary ducts. Quaternary ducts are

present at twenty days. Very rapid growth takes place between the twenty day stage and the newborn, as my reconstructions and cleared preparations (Myers '16) show that the ducts are much elongated and several new divisions have occurred in the latter.

The first few divisions of the milk-ducts in the twenty day fetus (fig. 12) follow the true dichotomous method of branching. The divisions farther away from the primary ducts, however, do not come off so regularly, yet they present a very irregular form of dichotomy. The same condition obtains in the newborn and later postnatal stages (Myers '16). Langer ('51), Kölliker ('79), and Lustig ('16) found that for the most part the milk-ducts of human fetuses branch dichotomously. Kölliker ('79) states that the human mammary ducts branch two to eight times by the true dichotomous method after which the branching is somewhat irregular. The method of branching of the milk-ducts of the albino rat, therefore, appears to be similar to that of the human.

The terminal end of each milk-duct in all stages of the rat fetus studied presents an enlargement. Langer ('51) noticed such enlargements at the terminal ends of developing milk-ducts in the human, and they have since been reported by numerous investigators. Formerly such terminal swellings were believed to be true acini. The present work, however, as well as my previous study (Myers, '16), confirms the view that they are not true acini, but are merely growing end-buds.

The first indication of a lumen in the ducts was observed in a rat fetus of eighteen days and nine hours. The lumina appear, however, in only a part of the ducts observed at this stage, while at twenty days and six hours the majority of the ducts show lumina in an early stage of development. At birth the lumina extend from the intra-epidermal portion of the primary ducts to within 20 or 30 micra of the free extremities of the terminal ducts. Such lumina, however, have not yet reached their definitive state.

The time of development of the lumen in the mammary ducts is subject to considerable variation, not only in different species

but in individuals of the same species. In the rabbit, Rein ('82) found the first vestige of a lumen in a very late fetus. At five days after birth canalization is not entirely complete, but at fifteen days the lumen extends to the tip of the nipple. It does not open on the surface, however, owing to the presence of cornified cells in the proximal end of the primary duct. Brouha ('05) in the rabbit four days old found two of the milk-ducts with lumina throughout, other ducts at the same age showing only faint traces of lumina. At twenty-five days he found the lumina completely formed for all of the ducts. In a kitten twelve hours after birth Brouha found a part of the ducts provided with lumina. In *Vespertilio murinus* he found a trace of a lumen in the milk-ducts of 20 mm. fetuses, while at birth the lumina are quite well represented throughout the ducts. De Sinety ('75) and Lustig ('16) found the lumina begin to appear in human milk-ducts about the sixth or seventh fetal month, but are not completely developed until birth or later.

From the present work on rat fetuses and the foregoing observations of De Sinety ('75), Rein ('82), Brouha ('05), and Lustig ('16) it may be concluded that the lumina of milk-ducts usually begin to develop during the later fetal stages, but the definitive lumen does not appear until birth or later.

The earliest appearance of the lumen has been reported in different parts of the milk-ducts. In the previously published abstract of the results of the present paper (Myers, '17) it was stated that the lumina make their earliest appearance in the free ends of the milk-ducts. This statement agreed with the findings of Rein ('82), Eggeling ('04), Raubitschek ('04), and Lustig ('16). Further observations on a larger number of albino rat fetuses, however, indicate that the lumina may appear first in the excretory or external portions of the milk ducts, as observed by Kölliker ('50) and Brouha ('05) in the glands of the mouse, rabbit, cat and man. We must therefore conclude that the first appearance of the lumina of the milk-ducts is variable and may occur in various parts of the ducts. In the rat, however, in the majority of cases the lumina show slightly further progress in development toward the free ends of the ducts.

The manner in which the lumen is formed has likewise been a subject of considerable controversy. It will be recalled that in the rat fetus of about the eighteenth or nineteenth day small irregular intercellular cavities or lacunae appear in the epithelium of the milk-ducts. The lacunae are chiefly confined to the center of the developing ducts, but may occur peripherally. The cells and their nuclei in the region of the lacunae show no signs of degeneration. A little later the lacunae flow together, thus forming a lumen which is in a very incomplete stage of development at this age. The lumina are better developed at birth (Myers, '16), but are still incomplete. De Sinety ('75), Rein ('82), and Keiffer ('02) have described the formation of the lumina in human as a process of degeneration. They state that the central cells of the solid epithelial duct anlage degenerate, the débris being found in the developing lumina. My fetal stages show no such condition, but agree rather with the findings of Benda ('94) and Brouha ('05), who described the formation of the lumen in the mouse, rabbit, cat and man as a process of cell-rearrangement, rather than cell-degeneration.

The epithelial hood

The anlage of an epithelial ingrowth or hood was first observed in one of the abdominal glands of an eighteen day and nine hour rat fetus. Such anlagen are present in most of the glands in nineteen day fetuses. These anlagen were seen to bud off from the deeper epithelial surface funnel-shaped mammary pit. About the twentieth day the ingrowth forms a real hood around the proximal end of each primary duct. When examined microscopically, the part of the hood attached to the walls of the mammary pit is seen to be filled with a thin layer of cornified cells which is continuous with the mass occupying a part of the mammary pit. No cavity is yet present in the hood, although its attachment corresponds to the region of the sulcus between the nipple anlage and the wall of the mammary pit.

The epithelial hood has been observed by several investigators (Gegenbauer, '76, Rein, '82, Klaatsch, '84, in rodents and

insectivorous mammals) some of whom believed it to be homologous with the marsupial pouch or the nipple pocket of marsupials. As to the significance of the epithelial hood in the albino rat I have as yet reached no definite conclusion.

Gland stroma

The majority of the investigators have observed and described the mammary gland stroma. In the rabbit and man at the end of the period of 'Knospenbildung,' Rein ('82) found the first appearance of the gland stroma. In the albino rat according to Henneberg, the mesenchyma deep to the first anlage of the mammary gland is condensed. The present work shows that in the rat fetus at fifteen days the mesenchymal cells lying nearest the mammary gland anlage are elongated and arranged in two or three distinct rows around the anlage. At about seventeen and eighteen days, as the primary duct buds out from the main gland anlage it becomes well surrounded with developing connective tissue cells, which at this stage present long fibrous processes. As many as three or four layers of the cells and their fibers surround each duct, while farther from the ducts the connective tissue cells and fibers are arranged parallel with the surface of the skin. A short time before birth, at twenty days and six hours, the ducts are covered with a sheath of fibrous tissue. The connective tissue external to this sheath is somewhat condensed (fig. 6). The sheath which intimately surrounds each duct corresponds to the part which Berka ('12) described as the mantle layer of young girls and older virgins. The condensed tissue external to the sheath he designated as the true stroma. In the true stroma, blood vessels and nerves are found, but the blood vessels are not as abundant as one might expect.

The fatty tissue enclosed by the gland stroma, which takes an important part in the later development of the gland, was not observed in the fetal stages.

Variation

Individual variations in the development of the mammary gland are so frequent that at least mention should be made of them. Moreover, no work on the mammary gland should be regarded complete until the conditions have been studied in a sufficient number of individuals to rule out all possibility of error from individual variations.

Rein ('82) found many individual fluctuations in the developing mammary gland of human. In one pig embryo of 1.5 cm. Schultze ('93) found only the milk line while in another embryo of about the same size he found the 'primitive Zitzen.' Henneberg ('00) found in one rat embryo of eleven days no indication of a mammary streak while in another embryo of the same age a well developed streak appeared only on one side. Raubitschek ('04) states that probably no other organ is subject to such great fluctuations in its development as the mammary gland.

In the present study, it has been noted that in the eighteen day and nine hour stage of the albino rat fetus some of the glands possessed anlagen of only the primary ducts while in others there were secondary ducts. Also the lumen began to appear in one individual of this stage while in others there was no trace of a lumen present. The lumen continued to develop until at twenty days and six hours it was represented by a considerable cavity in some part of most of the ducts. Yet even at this stage an occasional individual possesses a gland without the slightest manifestation of a lumen.

The number of mammary glands of the rat likewise is subject to individual variation. Schickele ('99) found that in 6.66 per cent of the rats examined, only 11 nipples were developing. In 80 per cent of his rats 12 nipples (the normal number) were present. While in 13.33 per cent there were 13 nipples present. In no case did he find more than 13 nipples. Henneberg ('00), Myers ('16), also reported a variable number of glands in albino rats. Schultze ('93) in describing the mammary glands of a rat embryo of 1.2 cm. mentioned only two thoracic pairs of glands

but found the usual number of abdominal and inguinal pairs. In the case of the rat, some authors fail to report the species studied, which should always be stated in order to avoid errors and confusion in making comparisons. Lantz ('10) states that the female brown rat (*Mus norvegicus*) has usually 12 mammae—3 pairs of pectoral and 3 pairs of inguinal—although these numbers are not constant, one or more teats frequently being undeveloped. He also states that the black rat (*Mus rattus*) and the roof rat (*Mus alexandrinus*) have only 10 mammae—2 pairs of pectoral and 3 pairs of inguinal—with but little tendency to vary. A variable number of mammary glands has also been reported in many other forms, including man. Therefore, in all morphological and histological work as well as experimental work on the mammary gland, individual variation must be considered before drawing any definite conclusions.

Cephalo-caudal sequence in development

Henneberg's ('00) work shows that in the early stages of development of the mammary gland the more cephalic or thoracic glands are better developed than the caudal or abdominal and inguinal glands. In fact the inguinal gland anlagen remain considerably behind the thoracic anlagen. In carnivora Schultze ('93) found the more cephalic mammary gland anlagen earlier and better developed than the posterior ones at the same age. A similar condition was found in a part of the fetuses examined during the present work. However, when the twenty day and six hour stage is reached the difference is not so noticeable. The order of sequence is therefore in accordance with the general rule that those parts occupying a more cephalic position tend toward earlier development than those parts occupying a more caudal position.

SUMMARY

1. In fetuses at fifteen days and nine hours the mammary glands of the albino rat are in the club-shaped stage, the epithelial anlage forming an ellipsoidal body attached to the epidermis by a constricted neck.

2. About the seventeenth or eighteenth day the deep portion of each anlage elongates into a long solid cord of epithelium—the anlage of the primary duct. At this time each anlage is only about 0.05 mm. in length. The free end of each primary duct is directed toward the position which the future system of ducts will occupy. At eighteen or nineteen days each of the primary ducts present two secondary ducts. About the twentieth day tertiary and quaternary ducts are present. The first few divisions are usually dichotomous, while the more distal ones become somewhat irregular. Growing end-buds are present on the free ends of the terminal ducts.

3. Between the eighteenth and nineteenth days an epithelial projection grows in from the stratum germinativum around each gland area. Each projection extends entirely around the primary ducts thus forming the epithelial hood.

4. The mammary pit first appears on the surface as a slight depression over each developing gland. It is apparently formed by the processes of cornification and desquamation of the thickened epithelium. The pit is well developed at nineteen or twenty days.

5. The nipple anlage was first observed in twenty day and six hour fetuses. At this stage it is a small papilla-like eminence lying at the bottom of the mammary pit. The nipple reaches only a rudimentary stage of development in the prenatal stages of the albino rat.

6. The lumina of the ducts were first observed in eighteen day and nine hour fetuses. They were not confined to any definite part of the system of ducts, but usually appeared slightly better developed toward the free ends of the ducts. The lumina do not reach their definitive stage in the fetal state. In the fetuses examined, the lumina are apparently formed by a rearrangement of the cells, thus producing numerous lacunae which later flow together to form the incomplete lumina of the latest stage studied. No traces of cell degeneration were observed.

7. In the earliest stages studied the mesenchymal cells are condensed around the mammary gland anlage. Later these cells elongate and develop long fibrous processes. At twenty

days and six hours these cells and fibers constitute the greater part of the gland stroma which may be divided into two parts: (1) the mantle layer which is a thin layer immediately surrounding the ducts; (2) the true stroma which lies between the ducts and outside of the mantle layer. The true stroma contains the larger blood vessels and nerves of the glands.

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PLATE 1

EXPLANATION OF FIGURES

7 External view of a wax model reconstructed from the right first thoracic gland of an albino rat fetus of fifteen days and nine hours. $\times 100$. *e.s.*, eminence (mammary hillock) on surface of skin produced by developing mammary gland.

8 External and part of internal view of a wax model reconstructed from the left first inguinal gland of a female albino rat fetus of eighteen days and nine hours. $\times 50$. *n.p.*, depression representing mammary pit; *p.d.*, primary duct anlage.

9 External view of a wax model reconstructed from the left second inguinal gland and surrounding region of a female albino rat fetus of twenty days and six hours. $\times 50$. *n.a.*, nipple anlage; *n.p.*, mammary pit; *s.*, sulcus surrounding nipple anlage.

10 Internal view of a wax model reconstructed from the right first thoracic gland of an albino rat fetus of fifteen days and nine hours. $\times 100$. *m.a.*, ellipsoidal mass of cells (mammary gland anlage) connected to epidermis through a constricted neck (*nk*).

11 Internal view of a wax model reconstructed from the right abdominal gland of a female albino rat fetus of eighteen days and nine hours. $\times 50$. *e.b.*, end-bud; *p.d.*, primary duct; *s.d.*, secondary ducts.

12 Internal view of a wax model reconstructed from the left first inguinal gland of a female albino rat fetus of twenty days and six hours. $\times 50$. *e.b.*, end-bud; *ep.in.*, epithelial ingrowth (hood); *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct.

