

STUDIES ON THE MAMMARY GLAND

I. THE GROWTH AND DISTRIBUTION OF THE MILK-DUCTS AND THE DEVELOPMENT OF THE NIPPLE IN THE ALBINO RAT FROM BIRTH TO TEN WEEKS OF AGE

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SIX TEXT FIGURES AND FOUR PLATES

The mammary gland undergoes many important changes from birth to old age. Numerous details in the normal structure of the gland at various stages of its history are still imperfectly known. Because of the lack of anatomical knowledge, pathologists are often unable to determine whether a certain condition of the mammary gland is due to a physiological or a pathological change. Moreover, a knowledge of the normal course of development is necessary as a basis for various lines of experimental work upon the mammary gland. In view of these facts further investigation of the developmental changes in the mammary gland during its life history seems desirable. Hence a series of studies upon this subject has been undertaken. The present paper, which is the first of the series, deals only with the growth and gross relations of the ducts, and the gross development of the nipples from the first day to ten weeks after birth. In later papers, the prenatal condition as well as various changes involved during pregnancy, lactation, and involution will be considered.

MATERIAL AND TECHNIQUE

The present study is confined to the mammary gland of the albino rat (*Mus norvegicus albinus*). This form is easily reared in the laboratory and is thus available at all times. Its life cycle is short, so that any desired stage in the history of the mammary

gland can be obtained in a comparatively short time. The various developmental stages are therefore easily obtained and controlled.

In the present study three methods were used. (1) Microscopic sections were studied; (2) wax reconstructions (of the newborn) were made according to Born's method; (3) the ducts were studied from cleared preparations. These preparations were made according to the method employed by Lane-Claypon and Starling ('06). The skin of the entire ventral part of the body was removed, spread out on a sheet of cork, and fixed in a mercuric chloride-formalin solution (10 per cent formalin in a saturated aqueous solution of mercuric chloride). The corium and tela subcutanea containing the gland were then removed in a single sheet. In the older specimens it was usually necessary to dissolve out the fat with alcohol and ether before staining. The preparations were then placed in a very dilute solution of alum-hematoxylin or carmalum until they were sufficiently stained. When necessary, the excess stain was washed out with acid-alcohol. After dehydration, beechwood creosote and cedar oil were used as clearing agents, the specimens being first placed in creosote for a few hours and then transferred to cedar oil. After being thoroughly cleared they were mounted in damar on glass slides.

Frank and Unger ('11) state that Starling's technique of staining and clearing the breasts, as in the rabbit, could not be employed in the rat. No explanation is given as to why Starling's method could not be employed. It is true that this method is rendered quite difficult owing to the development of the panniculus carnosus muscle in the thoracic region. Being very closely related to the milk-ducts, it is almost impossible to dissect this muscle off without destroying some of the mammary gland. However, good cleared preparations can be studied to advantage even when the muscle is intact. The fact that the panniculus carnosus muscle is lacking in the abdominal and inguinal regions makes the study of the glands in these regions comparatively easy. Fat is quite easily removed in cleared preparations, hence the considerable quantities of it deposited in the

region of the abdominal and inguinal glands do not add many difficulties in the technique.

Sections for microscopic study were prepared by fixation in Zenker's fluid, and embedding in paraffin. The sections were cut 10 micra thick, mounted serially and stained with Mallory's connective tissue stain or with alum-hematoxylin.

The albino rats used were in good health. After weaning, at the age of three weeks, they were fed upon whole wheat (Gramham) bread, soaked in whole milk. They were in general of average weight or above, as indicated by the following gross body weights of the rats from which the specimens represented in the figures were obtained: newborn, about 4.5 grams; 1 week, 8.5 grams; 2 weeks, 15.5 grams; 3 weeks, 30 grams; 4 weeks, 53 grams; 5 weeks, 54 grams; 7 weeks, 75 grams; 9 weeks, 114.5 grams.

OBSERVATIONS

1. General arrangement of glands and ducts

As shown in figure 1, the mammary glands of the albino rat may be grouped according to the regions which they occupy. The three most cephalic pairs of glands lie in the thoracic region, hence are designated thoracic mammary glands. Passing caudad, the next pair lies in the abdominal region at about the level of the umbilicus. Henneberg ('00) speaks of this pair as the abdominal mammary glands. The two remaining pairs, known as the inguinal mammary glands, lie in the inguinal region.

Each of the first pair of thoracic glands lies slightly cephalad to, or at the level of, the fore limb. Each adult nipple is located about 10 to 12 mm. from the mid-line. In the case of the second pair of thoracic gland as a nipple is found immediately behind each forelimb. Here the distance from the nipple to the mid-line is somewhat greater, being about 15 to 16 mm. The distance between this and the first pair of thoracic glands is normally about 30 mm. The last pair of thoracic glands is very closely associated with the second pair, the nipples being only about 12 to 15 mm. apart. The distance from each nipple to

the mid-line is approximately 35 mm. The abdominal pair of glands stands somewhat isolated from the others, lying about 50 mm. caudad to the last thoracic and 25 mm. cephalad to the first inguinal. As stated above, these glands lie near the level of the umbilicus. Their distance from the mid-line is about the same as, or slightly greater than, that of the last thoracic glands. Each nipple of the first pairs of the inguinal glands lies in the

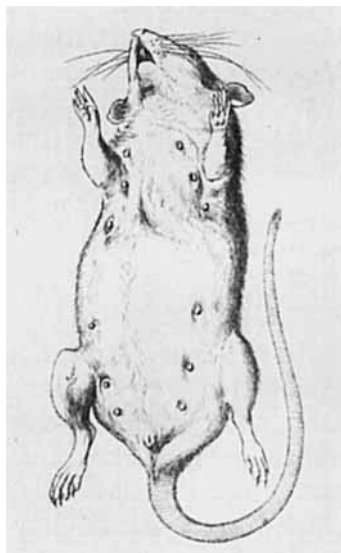


Fig. 1 Ventral aspect of adult female rat during lactation, to show location and arrangement of the nipples (from Henneberg '00).

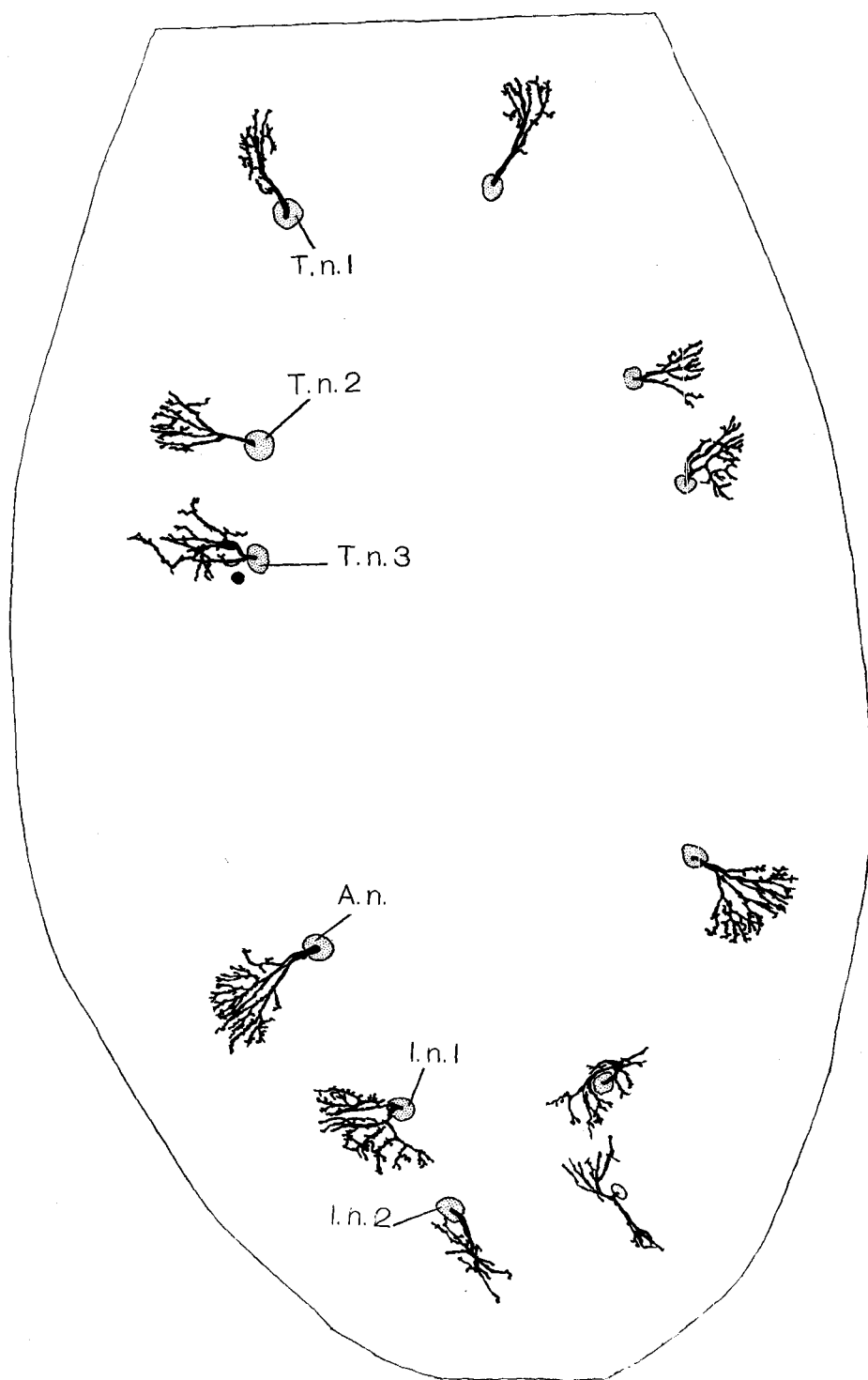
inguinal region immediately medial to the thigh. The distance from the nipple to the mid-line is approximately 12.5 mm. The nipples of the second pair of inguinal glands lie in the most caudal part of the inguinal region. They are located latero-cephalad to the urethral orifice. Like the first thoracic glands, the nipples of the last inguinal glands approach the mid-line, the distance being only about 10 to 12 mm. It should be observed that in passing caudad from the first thoracic glands the distance from the nipples to the mid-line gradually increases until the last

thoracic pair is reached, remains about the same for the abdominal pair, then decreases to the last inguinal pair.

From figure 1 and the above measurements it will be observed that the abdominal pair of glands is more closely associated with the inguinal than with the thoracic glands. This arrangement points toward a localization of the glands in only two regions. So far as the distribution of the mammary glands is concerned, the rat may be regarded as occupying a position between those forms which possess a continuous row of mammary glands on each side from the thoracic through the inguinal regions and those in which the mammary gland is confined to a single region.

The average number of mammary glands in the albino rat is 12 (6 pairs), but Henneberg ('00) and Frank and Unger ('11) have called attention to the fact that the number varies between 10 and 14. Henneberg examined 28 embryos from the age of 14 days and 20 hours to 15 days and found in 5 cases that supernumerary mammary hillocks appeared. All were in the pectoral region. The accessory hillocks, which in all cases appeared smaller than the normal, were located in 4 cases between the second and third normal hillock, and in one case caudal to the third. In the adult animals, however, he found that hypermastia rarely occurred, as only one case was reported from 150 observations. In this one case the individual possessed a pair of apparently functional glands just caudad to the last pair of thoracic glands. In the 150 adult rats only one case of hypomastia was found. In this case the individual lacked the first pair of inguinal glands.

In the present study, the number of glands present was noted in all animals available. From observations made on 100 individuals ranging in age from 10 days to adults, 80 were found to possess the normal number of mammary glands. Only one supernumerary gland was observed. It was located just caudad to the third thoracic gland on the left side, the right side presenting the normal number. In 12 cases the second thoracic gland was apparently lacking on the right side only, while in 7 other cases the second thoracic gland was lacking on both sides.



A very good time to make these observations on the albino rat is about the tenth to the fourteenth day of life. As pointed out by Jackson ('12), the mammary glands (nipples) are very conspicuous at this time. After the first two weeks have passed, it is very difficult to make accurate observations as the glands are well covered with dense hair. During pregnancy and the period of lactation the glands again become very conspicuous.

Just medial to the apex of each nipple is a single opening which leads into one large duct. This duct after reaching the tela subcutanea turns almost at right angles and courses through this layer parallel to the surface. Instead of receiving a large number of tributaries from all directions, this single duct at first receives only a small number of tributaries usually from a single direction. Figure 2, drawn from a cleared preparation of the integument of a rat two weeks old, will serve to show the general direction taken by the ducts of each gland. Here it may be observed that the main duct of the first thoracic gland extends cephalad, then breaks up into numerous branches. No ducts are seen to pass out in any other direction from the nipple. The general direction of the ducts of the second thoracic gland is somewhat different as they pass almost directly laterad from the nipple. In a number of cases, however, these ducts were found to extend somewhat latero-cephalad. A larger number of branches lead from the third duct than from either of the other thoracic nipples. The ducts of the third take the same direction as do those of the second thoracic gland. The abdominal gland, whose duct shows a greater amount of branching than any of the others, sends its branches in a caudo-lateral direction. The main duct of the first inguinal gland, after passing a short distance caudo-laterad, breaks up into ramification some branches of which take a cephalic while others take a caudal direction. The duct of the last or second inguinal gland usually sends all of its branches directly caudad. In some specimens, however, a few branches

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

pass cephalad. It is evident from the drawing of the surface of the abdomen (fig. 1) that the nipples are arranged on each side so as to form somewhat of an arch. It should be noticed in figure 2 that the arch effect is also carried out by the direction and distribution of the ducts.

The milk-ducts of late human and rabbit fetuses were divided by Rein ('82) into several subdivisions as follows: (1) the inflated terminal branches of the 1st, 2d, and 3d orders which together form the outline of the secretory part of the gland; (2) that part extending from these ramifications to the mammillary zone which represents the future lactiferous sinus; (3) the intramammillary portion which constitutes the proper excretory duct; (4) the infundibuliform part which passes through the epidermis. Brouha ('05) was unable to adopt the subdivisions proposed by Rein, because neither at birth nor even in the course of the first month of postnatal life was he able to distinguish any part which was differentiated into a lactiferous sinus. Owing to this fact Brouha suggested and followed a somewhat different subdivision. He considers each milk-duct composed of three segments; (1) the intra-epidermal infundibuliform segment; (2) the excretory segment which extends from the preceding to the place of the first bifurcation; (3) the secretory segment, which is composed of the succeeding branches. This subdivision Brouha bases upon the later histological characters of the different portions of the lactiferous arborization.

A somewhat different classification of the ducts is used in this paper. That part of the duct passing through the epidermis is called the intra-epidermal portion of the primary duct. That part extending from the epidermis to the first division is designated the primary duct. The ducts resulting from the divisions of the primary ducts are spoken of as the secondary ducts. The secondary ducts divide into the tertiary ducts. The collateral ducts are those given off from the sides of the main ducts. All those ducts which end blindly are called terminal ducts.

When the various pairs of glands are examined it will be observed that for certain features a single general description can be applied to the ducts of all. However, each varies more or

less from a general type; and it thus becomes necessary in some places to make the description somewhat detailed. This description is given later under "Growth of the ducts."

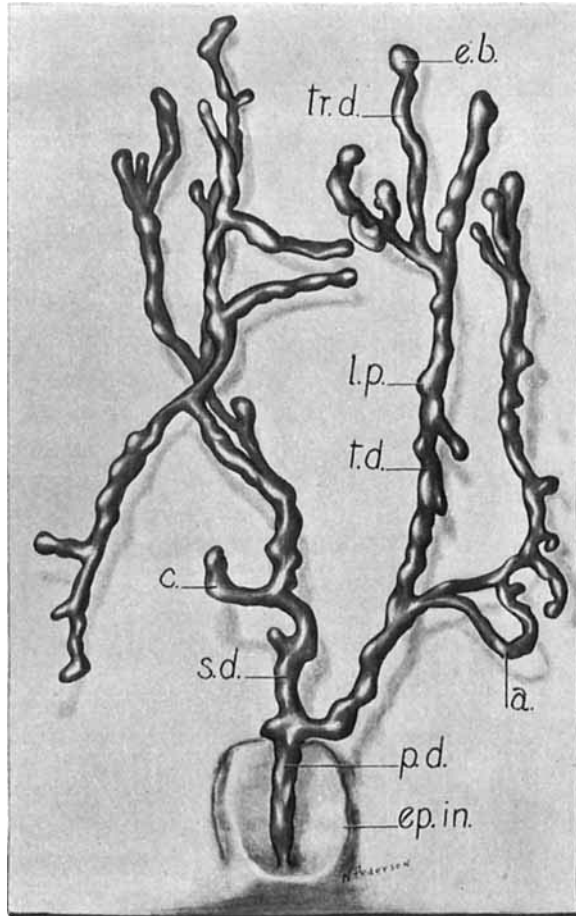


Fig. 3 Internal view of a wax model reconstructed from the left second thoracic gland of a newborn albino rat. $\times 40$. *a*, anastomosis; *c*, collateral duct; *ep.in.*, epithelial ingrowth of nipple; *e.b.*, end-bud; *l.p.*, lateral process; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct.

In figures 3 to 6 (from wax reconstructions of the newborn) the intra-epidermal portion of the primary duct is not visible,

but the primary duct is seen to emerge from the inner surface of the epidermal layer of the skin, pass deeply into the tela subcutanea and suddenly turn at right angles after which it lies paral-

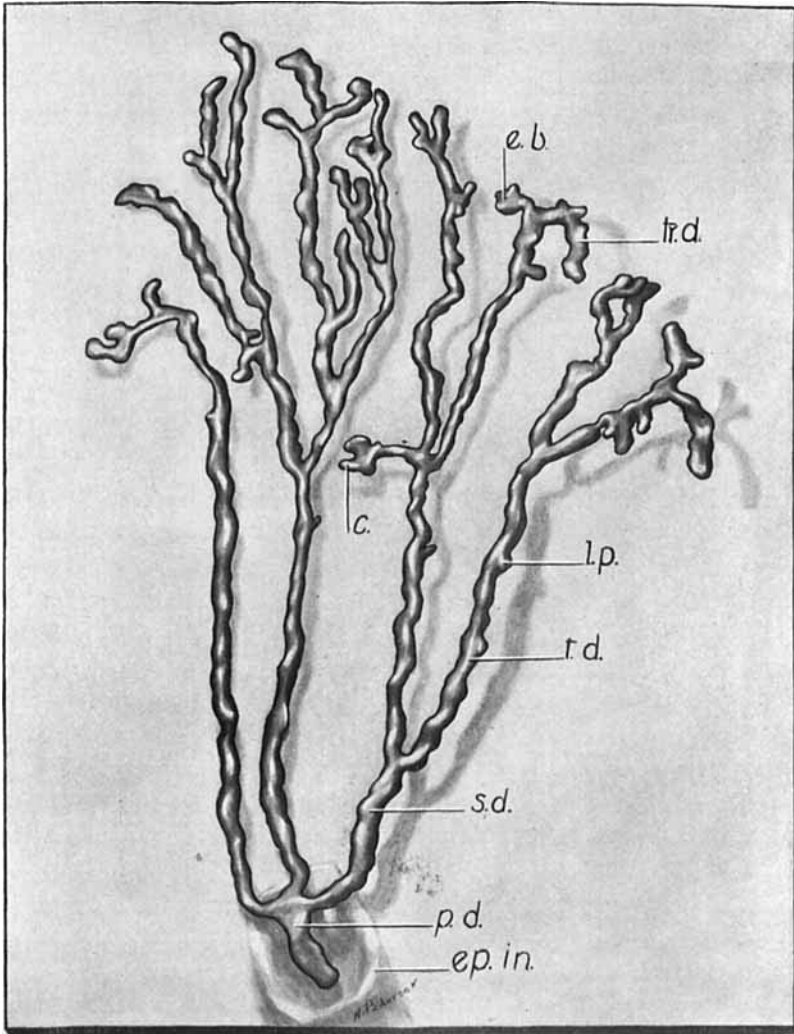


Fig. 4 Internal view of a wax model reconstructed from the right third thoracic gland of a newborn albino rat. $\times 40$. (For lettering, see fig. 3.)

lel to the surface of the skin. In most cases observed the primary duct extends only a short distance until it divides into two branches (secondary ducts) nearly equal in size.

The extent of the primary duct varies considerably. For instance, in the first thoracic and the last inguinal glands the pri-

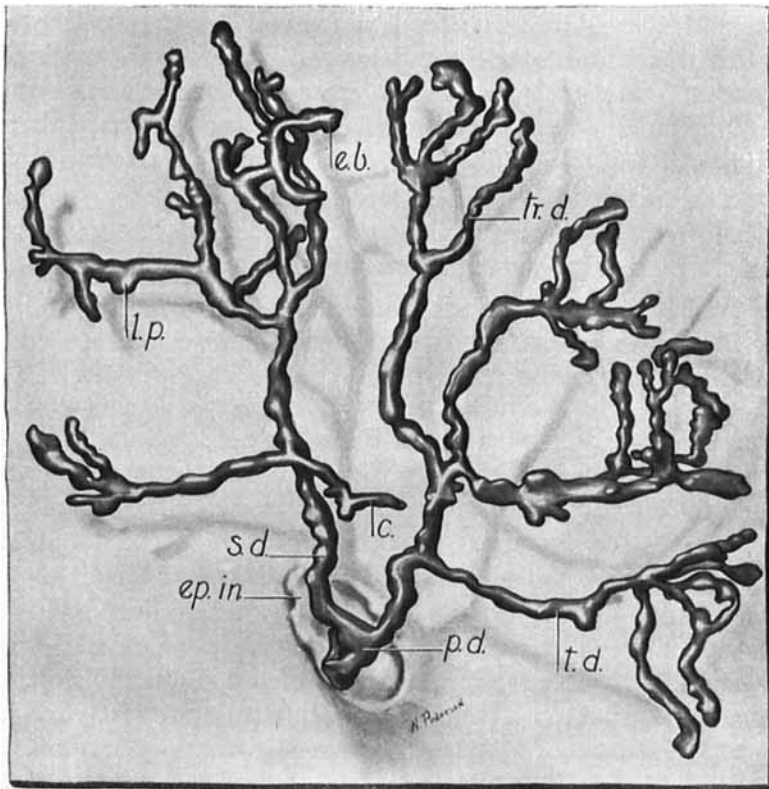


Fig. 5 Internal view of a wax model reconstructed from the left first inguinal gland of a newborn albino rat. $\times 40$. (For lettering, see fig. 3.)

mary ducts present a rather extensive course before dividing, while in the remaining glands they divide almost immediately after making a sharp turn in the tela subcutanea. In figure 4 (last thoracic gland) the primary duct is seen to divide into three branches. This is an exception to the general rule that the primary duct divides into two branches.

As compared with the primary duct, the secondary ducts present a rather extensive course, after which they break up each into two or more branches (tertiary ducts). It will be noticed that at birth (figs. 3 to 6) the terminal branches of each tertiary duct vary from one to three in number. On the end of most terminal branches is a small bud-like enlargement. These enlargements were described as true alveoli by earlier investigators, but this was found later to be incorrect. Billroth (according to Berka '11) doubts whether completely formed end-vesicles occur in young human virgins. While he called the terminal enlargements 'real end-vesicles,' yet he adds that they later develop into '*true* end-vesicles' and further multiply during pregnancy. Berka ('11) states that true alveoli do not occur in young (human) virgins. Similarly the terminal enlargements found on the milk-ducts of young rats are not true alveoli, but are only enlarged growing processes corresponding to the end-buds found in other developing glands. The microscopic structure of these enlargements and the development of true alveoli will be discussed in a later paper dealing with the histology of the mammary gland.

The question often arises as to whether the ducts of glands branch dichotomously or otherwise. From the various figures it will be seen that the more proximal parts of the terminal segments usually follow the dichotomous method, but the distal portions, as stated above, may terminate as a single duct or divide into two or three branches. In the last thoracic gland (fig. 4) the secondary branches approach true dichotomous division.

Anastomoses occur between ducts, but they are not very frequent in the newborn rat. In the reconstructions made from glands at birth, only two distinct anastomoses occur (fig. 3). However, others have been observed in cleared preparation at the same stage.

It will be noted that along the secondary and tertiary ducts numerous lateral buds occur (figs. 3 to 6). Many more of them are present on the distal than on the proximal ducts. Such buds later form collateral branches destined to develop into ducts similar to those already present. This point will be more clearly brought out in the older stages.

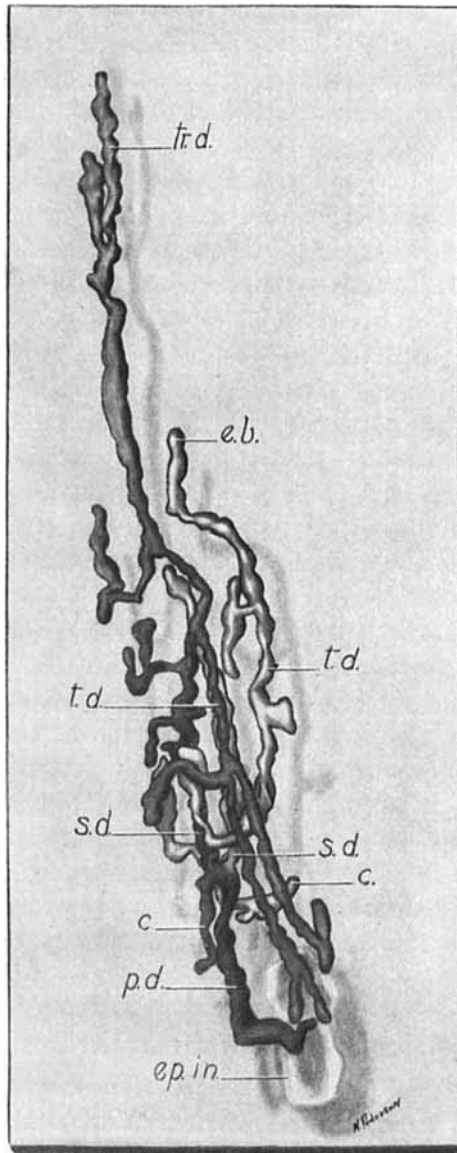


Fig. 6 Internal view of a wax model reconstructed from the left second inguinal gland of a newborn albino rat. $\times 40$. (For lettering, see fig. 3.)

A point which has been discussed at some length recently and one which has proved to be of considerable importance in experimental work is that of the variation in the relative size and development of the various glands in the same individual, and of glands from different individuals of the same age. Lane-Claypon and Starling ('06) in working on the growth and activity of the mammary gland concluded that breast hyperplasia of pregnancy is caused by chemical substances formed in the embryo. Such substances passing through the placenta into the maternal blood-stream cause growth of the mammary gland. To decide definitely as to just what tissues cause this growth Lane-Claypon and Starling injected extracts of placenta, placenta and uterus, ovaries, fetus, fetus together with the placenta and membranes, and mucous membrane of the uterus into virgin rabbits. Some of the extracts when injected caused very little apparent change in the size of the mammary gland of virgins, while others (fetus extract, for example) seemed to cause a marked development of the glands.

Frank and Unger ('11) in repeating certain of Lane-Claypon and Starling's experiments obtained different results, and furthermore found that their own series of experiments did not show uniform results. Thus they concluded that some disturbing factor remained to be accounted for, so they decided to study more carefully the anatomy and the physiology of the normal mammary glands of the rabbit. For such study they selected a number of apparently virgin adult female rabbits and under the necessary precautions removed a mammary gland from each. At various intervals of time other mammary glands from the same animal were removed and studied. From these experiments Frank and Unger were able to demonstrate in virgin rabbits changes which were indistinguishable from those seen at the end of the first third of pregnancy. Thus some physiological factor must be involved. Frank and Unger found a partial explanation for this condition in an article by Bouin and Ancel ('09) who describe variation in the size and appearance of the rabbit's mammary gland corresponding to the development of the corpus luteum. A little later O'Donoghue ('12) showed that

there is a decided change in the structure and size of the mammary glands of *Dasyurus viverrinus* when ovulation is not succeeded by pregnancy.

A comparison of the individual glands of the rat at birth and at two weeks (figs. 2 to 6) will show that there is considerable difference in the size and development of the various glands in the same rat, sometimes even in the same pair of glands (fig. 2). It has also been observed that corresponding glands from different rats of the same age and approximately equal weights show considerable variation in size and complexity of structure. The differences in size and development observed by me in the rat are not so marked as those described by Frank and Unger, Bouin and Ancel, and O'Donoghue. Yet they are worthy of mention and are certainly sufficient to prove that the normal structure and variability under different conditions of any part of the animal body should be thoroughly investigated before conclusions are drawn from experimental work. It is quite possible that such knowledge of the mammary gland of the rabbit would have changed decidedly the conclusions of Lane-Claypon and Starling.

2. Growth of the ducts

In the newborn rat, models were reconstructed showing one gland of each of the six pairs (figs. 3 to 6). At two weeks, all the glands are represented in figure 2, to show the general topography of the ducts. At the other stages (1 week, 2, 3, 4, 5, 7 and 9 weeks, figs. 7 to 13) it is found unnecessary to reproduce all the glands, so only the abdominal and inguinal glands of the left side are shown. A general description of all glands at each stage will be given, however.

Newborn. The figures drawn from wax reconstructions of the glands at birth (figs. 3 to 6) show that with few exceptions the ducts of each gland in these stages all lie approximately in the same plane, parallel to the surface of the skin. It was noticed that in regions where there are no obstructions the ducts spread very freely and cover a considerable area. The unobstructed ducts almost invariably lie in a single plane at this stage of

growth. The last thoracic and the abdominal glands (fig. 4) are good examples. Here, as is readily seen, the integument is free from appendages or anything that would tend to limit the uniform spreading of the ducts. It was also noticed that in regions where there are obstructions the branching of the ducts is more irregular and that the ducts arrange themselves so as to lie in more than one plane. The best example of this condition is seen in the last inguinal mammary gland (fig. 6). Here as previously stated the nipple lies in the caudal part of the inguinal region. To the lateral side of this gland the area available for ramification of ducts is obstructed by the hind-limb while to the medial side the external urinary and genital organs limit the area. This leaves only a very narrow region free for the distribution of ducts. Consequently, instead of spreading freely and occupying a single plane, the ducts branch so as to lie in three or four planes, each of which is parallel to the surface. Small areas where the growth of the mammary gland is obstructed by lymphatic glands are shown in several of the figures of later stages. Thus the course, branching, and spreading of the ducts depend largely upon the available space.

The reconstructions show that the ducts of the abdominal and inguinal glands lie much deeper from the surface than those of the thoracic glands. This is probably due to the absence of the panniculus carnosus muscle which may to some extent prevent the ducts from passing deeply in the thoracic region. Also considerable fat is present in the abdominal and inguinal region, which is apparently a very favorable substance for the ramification of ducts.

First week. At the end of the first week the ducts are not much different from those at birth except that they are slightly more branched. The ducts of the first thoracic glands give off a few branches which take a caudal direction; all the other branches of this pair of glands pass cephalad. The second pair of glands sends the ducts in a latero-cephalic direction, but many collaterals are given off some of which take a cephalic while others take a caudal direction. The third thoracic glands send their ducts in the same general direction as the second. The

greater number of the collateral ducts of this gland pass so far cephalad that only a comparatively small space exists between them and the caudal collaterals of the second thoracic gland. The caudal collaterals are few in number, yet quite long.

The ducts of the abdominal glands (fig. 7), which are most branched of all, send their branches in a latero-caudal direction. Many collaterals are given off, more of which take a caudal than a cephalic direction. In the case of the first inguinal gland, the number of collateral branches taking the cephalic direction is about equal to the number taking the caudal direction. The ducts of the last inguinal glands send the majority of their branches directly caudad; however, a few branches may be seen passing cephalad toward the first inguinal gland. Terminal end-buds are very prominent on all the glands at this stage. Also the small lateral buds are numerous but they are largely confined to the more distal ducts.

Two weeks. Figure 2 represents all of the mammary glands of a rat at the end of the second week of life. Figures 2 and 8, together with the description given in an earlier part of this paper, render further description unnecessary.

Three weeks. The three weeks' specimen from which figure 9 was drawn shows less branching than the one-week stage, but the ducts are greater in diameter. This specimen greatly emphasizes the marked variation in the development of the glands in different individuals, since in this instance the glands of an individual one week old show greater development than those of another individual three weeks old. Also, as pointed out above, the glands of one side may show more advanced stages of development than corresponding glands of the opposite side.

Four weeks. At the end of four weeks the first pair of mammary glands does not show a marked increase in development over the two-weeks stage. One very noticeable difference is that the proximal parts of the secondary ducts bear a large number of lateral buds similar to those appearing on the distal parts of the same segment of the earlier stages. Such processes are much less numerous on the corresponding parts of the second and third thoracic glands. The second glands in some of the speci-

mens show a very slight increase in development over the two-weeks and three-weeks stages. The ducts of the third thoracic glands show a much greater development than has been observed in any of the earlier stages. The ramifications are so numerous that some are seen to pass superficially while others take a deep course. This arrangement necessarily takes them out of the plane of the main ducts. Thus from this stage the ducts become so crowded that it is impossible for them all to occupy a single plane, as found at birth in all except the last inguinal glands. Large numbers of branches from the ducts in the third thoracic gland also take a cephalic course, which is so extensive on the right side that they very nearly come in contact with the ducts of the second thoracic gland. On the left side the interval between the ducts of the two glands is greater. Figure 10 shows that the abdominal and inguinal glands present much richer arborizations than corresponding glands of earlier stages. Some lateral buds are developed on the secondary ducts yet they are not so numerous as in the first thoracic gland of the same stage. The general course taken by the ducts is the same as that given in previous descriptions. A well defined interval exists between the ducts of the abdominal and the first inguinal glands. There is also a considerable space existing between the ducts of the first and second inguinal glands. However, the second inguinal has sent numerous ramifications in the direction of the first inguinal gland.

Five weeks. During the fifth week the ducts increase very rapidly in length. Also a great many new branches spring from the more distal ducts of the glands. The first thoracic gland is much more complicated than in any of the previous stages studied. Between the ducts of this and those of the second thoracic gland a wide interval still exists. The interval between the ducts of the second and those of the third thoracic glands has largely disappeared on both sides, there being a slight overlapping of a few of the ducts from each gland. In the specimens observed, however, there are no anastomoses present between the ducts of the two glands.

During this week the abdominal and inguinal glands (fig. 11) also undergo very rapid development. This is especially noteworthy in view of the fact that the body weight of the rat from which the gland at five weeks was drawn was practically the same as that of the rat used at four weeks (fig. 10). The ducts of the abdominal and first inguinal glands at five weeks (fig. 11) interlace very intricately. With the aid of the microscope (especially the binocular) one can be reasonably sure that many of the ducts simply overlap, there being no anastomoses. However, there are areas in which it is impossible to decide definitely as to whether true anastomoses occur. This holds true in all the later stages. Further investigations are necessary to determine this point concerning anastomosis. In this same week the ducts of the second inguinal glands on each side have grown cephalad to meet, and in some places even overlap, the ducts of the first inguinal. Here the overlapping is not very complicated and one can see distinctly that no anastomoses occur. The ducts of the second inguinal gland taking a caudal direction branch very profusely.

Six weeks. At the end of the sixth week the glands do not differ greatly from the five-weeks stage. Some of the glands, the abdominal for example, show greater development. Others, as the second inguinal, reveal no increase; in fact, in some specimens they are less developed than those of the five-weeks stage. The terminations of the ducts of the first and second inguinal glands are separated from each other by considerable space.

Seven weeks. In the seventh week stage, the caudally directed ducts of the first thoracic gland have made considerable advance toward the cephalically directed ducts of the second thoracic gland. However, they are still separated by a space of five to eight millimeters in width. As in some of the previously described stages the ducts of the second and third thoracic glands overlap. The branching of the ducts of these glands is somewhat more complicated at this stage. The abdominal and inguinal glands (fig. 12) present very complicated systems of ducts; also the overlapping between the ducts of the first and second inguinal glands is very marked at this time.

Eight weeks. In the eight-weeks specimens, the first and second thoracic glands have increased to such an extent that their ducts overlap. This overlapping may occur on one side only, there being an interval between the corresponding ducts of the opposite side. Concerning the other glands of this stage, very little need be said except that the ducts have increased in length and new branches have been added. It should be pointed out that at this stage the rat possesses only four distinct masses of mammary gland tissue. All of the thoracic glands of each side have their ducts so interlaced as to form one apparently solid mass of ducts. Also the abdominal and inguinal glands of each side are so matted together that no dividing line exists between them.

Nine and ten weeks. The nine and the ten-weeks stages (see fig. 13) show a tremendous increase in development over the previously described stages. The ducts have spread out to cover a much larger area. Not only is the overlapping of ducts more complicated but each gland has produced a large number of medial and lateral branches. For example, some of the medial ducts of the first thoracic glands of each side have grown so near to the mid-line that only a narrow space separates them. In the case of the first inguinal glands the medial ducts actually reach the mid-line, and a slight overlapping of the ducts of opposite sides occurs. The medial ducts of the second inguinal glands almost surround the vagina, the ducts from the opposite sides very nearly meeting in the mid-line both cephalad and caudad to the vagina. At this stage the proximal or secondary ducts also bear large numbers of short collateral ducts which have developed from the lateral buds mentioned in the earlier stages.

It is frequently stated that from birth to puberty the human milk-ducts undergo very little development, merely keeping pace with the general body-growth. At puberty an abrupt change like that affecting the entire organism is said to occur. So far as the rat is concerned, an examination of figures 2 and 7 to 13 will show that in the virgin, from birth to the age of ten weeks, there are apparently two periods when the increase in the mammary gland ducts is somewhat marked. The first period occurs

about the fourth and fifth weeks. Whether there are definite factors causing this first increase has not been determined. It is possible that it is due to individual variation. It does not appear to be due to any greater relative increase in the body weight at this period, and is of doubtful significance.

The second and more important period of increase in the mammary gland occurs about the ninth week. Donaldson ('15) states that the female rat arrives at the age of puberty about 60 to 70 days after birth, the gonads indicating sexual maturity at the age of two months or less. Jackson (12) however, found pregnancy to occur in one case at the age of seven weeks. Lantz ('10) cites from Buckland a case where a white rat is said to have given birth to 11 young at the age of eight weeks (and which accordingly must have become pregnant at the age of five weeks). These are very exceptional cases, however.

Jackson ('12) states that the vaginal aperture does not appear until the middle or end of the second month. There is considerable individual variation on this point. The average taken from fifteen observations by me is 8.3 weeks. Therefore, the marked increase in the development of the mammary glands of the rat between the eighth and ninth weeks evidently corresponds closely with the age of puberty. Thus the second marked increase in the size of the mammary glands is readily accounted for.

3. *Lumen of the ducts*

The time of appearance and method of formation of the lumina of the milk-ducts in various animals have been described by various authors. In the albino rat at birth a small irregular slit-like lumen is present in the primary duct (fig. 14). This lumen when traced proximally disappears in the intra-epidermal portion of the primary duct, but when traced distally becomes continuous with the more regular rounded lumen of the secondary ducts. The tertiary and in fact all of the remaining ducts at birth possess lumina throughout their entire extent. The terminal buds also present distinct lumina to within 20 or 30 micra of their distal extremities. The lumen of each bud does not

appear greater in diameter than that of other parts of the ducts but the walls of the bud are usually considerably thickened. In some places the lumina of the ducts at birth are partially filled with substance which resembles colostrum.

At the end of the first week, the lumen is considerably larger and extends farther into the intra-epidermal segment. Considerable quantities of the substance mentioned above are present in the lumina of all segments of the ducts.

Figure 16 shows that the lumen opens on the surface of the nipple at two weeks. At this stage it is larger and more oval throughout the primary duct than in the previously described stages.

In other stages up to the tenth week no marked differences were observed except that the lumina gradually become larger as the ducts increase in size (fig. 17). No true alveoli were observed, yet the end buds in the later stages are changed somewhat in appearance so as to approach, or at least suggest, beginning alveoli. In all the stages more or less of the substance mentioned in the earlier stages was observed. This agrees with findings of Berka ('11), who describes particles of fat in the human ducts even in the later virgin stages. He thinks the source of this fat is the colostrum secretion of the newborn and that it lies in the ducts for years.

4. Growth of the nipple

Owing perhaps to the fact that the rat is born in a somewhat immature state, the nipples show only slight development at the time of birth. In the newborn the nipple can be recognized by the naked eye only with difficulty, the nipple areas appearing slightly lighter than the surrounding tissue. A section through the nipple area at this stage reveals the fact that it is only very slightly elevated above the surface of the skin (fig. 14). The epithelium of this area is considerably thickened and the figure reveals an epithelial ingrowth on each side of the thickened portion. Reconstructions show that these epithelial projections are continuous around the nipple area and that through them a

definite epithelial hood is formed, as shown in figures 3 to 6. Through this hood, which is filled with loose connective tissue, the primary duct passes on its way toward the surface.

Very little change takes place in the appearance of the nipple during the first week of postnatal life. From the surface the nipple areas appear slightly more elevated than at birth. Figure 15, drawn from a section cut very obliquely, shows that the thickened area of epithelium has become more extensive, also that the epithelial projections (ingrowth) are slightly longer.

During the second week the nipple develops very rapidly. It is no longer necessary to speak of it as the nipple area, since from its size and shape it now resembles a true nipple. Figure 16 shows the nipple at the age of two weeks to be a very prominent structure with the epithelial projections (ingrowth) correspondingly well developed. At this stage the primary duct actually pierces the surface. As previously stated, the nipples are very conspicuous externally toward the end of the second week of life, and from the tenth to the fourteenth days it was found easier to observe the glands externally than at any other time during the virgin state. During the third week, the nipples become hidden by the development of the hair coat.

Figure 16 also shows that at the age of two weeks a sulcus is beginning to appear between the nipple and the skin of the surrounding region. This sulcus is immediately superficial to the epithelial ingrowth mentioned above. Beyond the two-weeks stage (up to ten weeks) no changes have been observed to take place in the nipple, except that it slowly increases in size. This is shown in a nine-weeks stage (fig. 17) in which the structure is similar to that in figure 16, the chief difference being one of size.

SUMMARY

The results of the present investigation of the growth and gross relations of the ducts and the nipples of the albino rat from birth to the tenth week may be summarized as follows:

1. Observations made on 100 rats show the number of glands varies between 10 and 13, the normal number being 12 (6 pairs).

Only one supernumerary gland was observed. The second thoracic glands are those most often absent.

2. Only one primary duct is present in each gland. This duct after reaching the tela subcutanea turns at right angles and pursues a course parallel to the surface of the skin. The majority of the branches of ducts belonging to the first thoracic gland lie cephalad to the nipple, while those from the second and third thoracic glands lie latero-cephalad to the nipple. In case of the abdominal and first inguinal glands the greater number of the ducts lie latero-caudad to the nipples, while in the last inguinal the ducts lie caudad to the nipples. The dichotomous method of branching frequently occurs, especially in the proximal branches.

3. Reconstructions and cleared preparations show that anastomoses sometimes occur between the ducts of a single gland. It is uncertain whether anastomoses occur between the ducts of different glands.

4. End-buds are present on a large number of terminal ducts at all stages studied. No true alveoli were observed. Large numbers of lateral buds are present on the sides of all ducts distal to the primary duct during the earlier stages. Such lateral buds later develop into branches of the ducts.

5. Considerable individual variation in the development of the glands was noticed. Not only do the corresponding glands of opposite sides differ in their degree of development, but also the glands of one individual may be better developed than those of another even several days older.

6. The characteristic distribution and ramification of the ducts apparently depend upon the space available for their growth.

7. The growth and branching of the ducts goes on at an unusually rapid rate about the ninth week, probably corresponding to the age of puberty.

8. A distinct lumen is present at birth in all the ducts distal to the intra-epidermal portion of the primary duct. At the end of the second week the lumen extends to the surface of the nipple.

9. The periphery of the nipple area is marked by a hoodlike epithelial ingrowth. The nipples make the most rapid growth during the second week, toward the end of which time they are very conspicuous.

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PLATE 1

EXPLANATION OF FIGURES

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

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

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

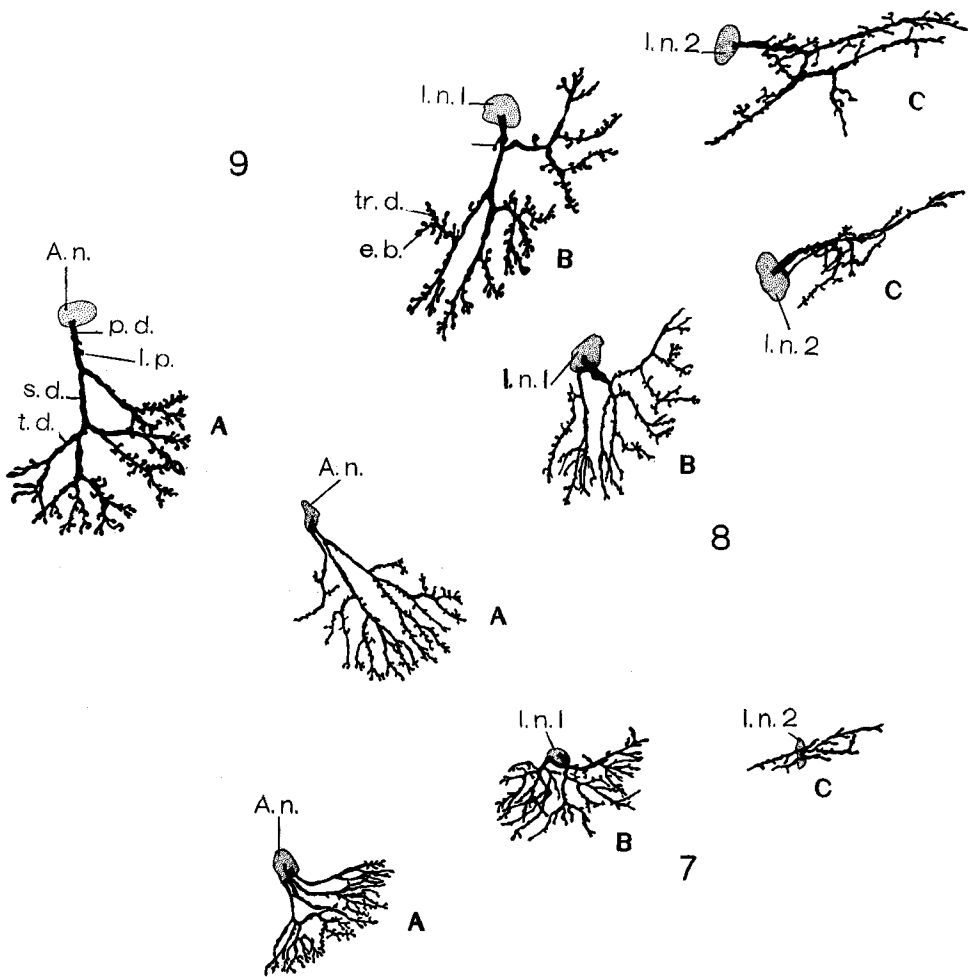


PLATE 2

EXPLANATION OF FIGURES

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

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

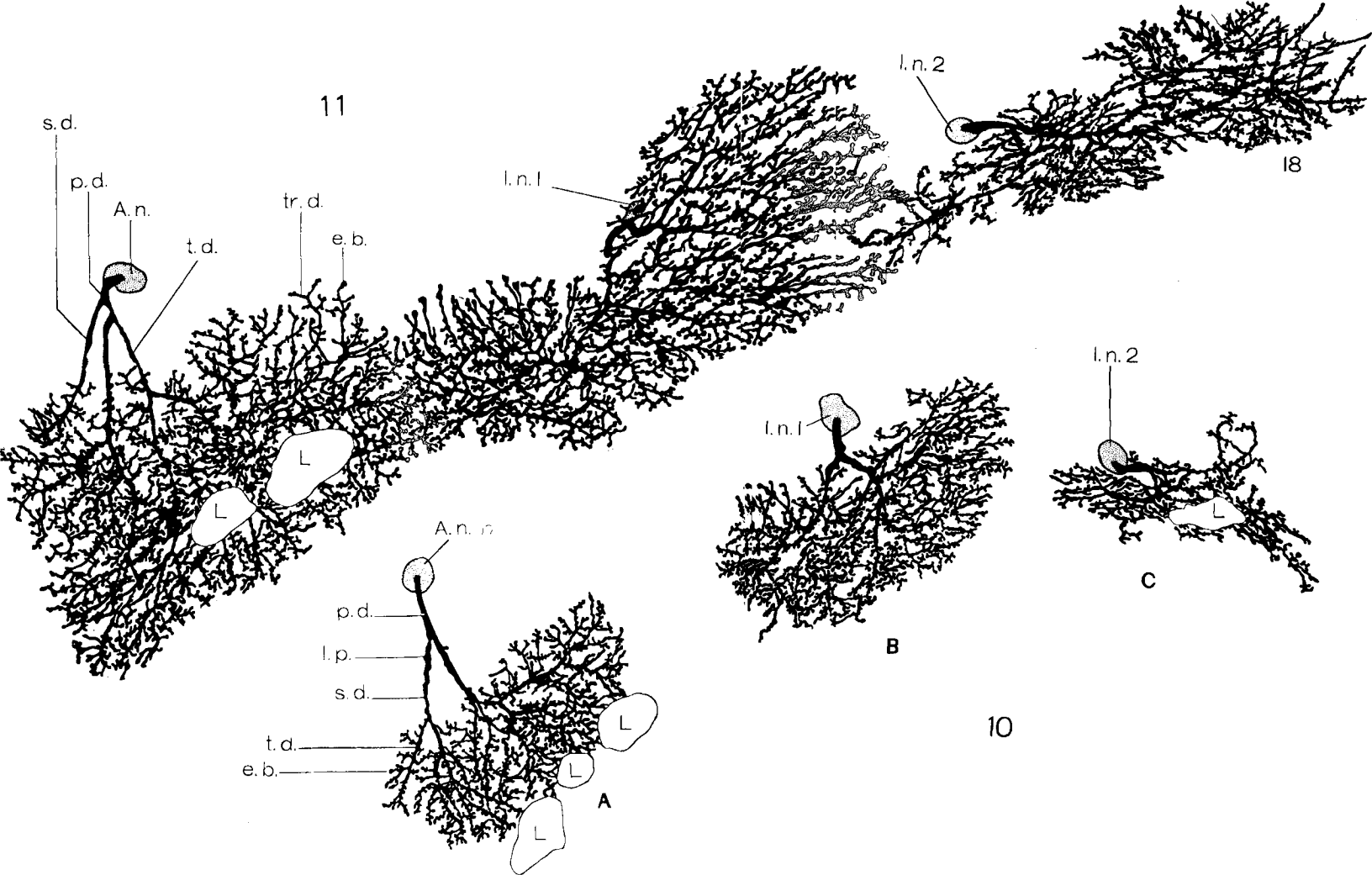


PLATE 3

EXPLANATION OF FIGURES

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

13 Same as figure 12, but from an albino rat nine weeks old. (Weight 114.5 grams.) $\times 5$.

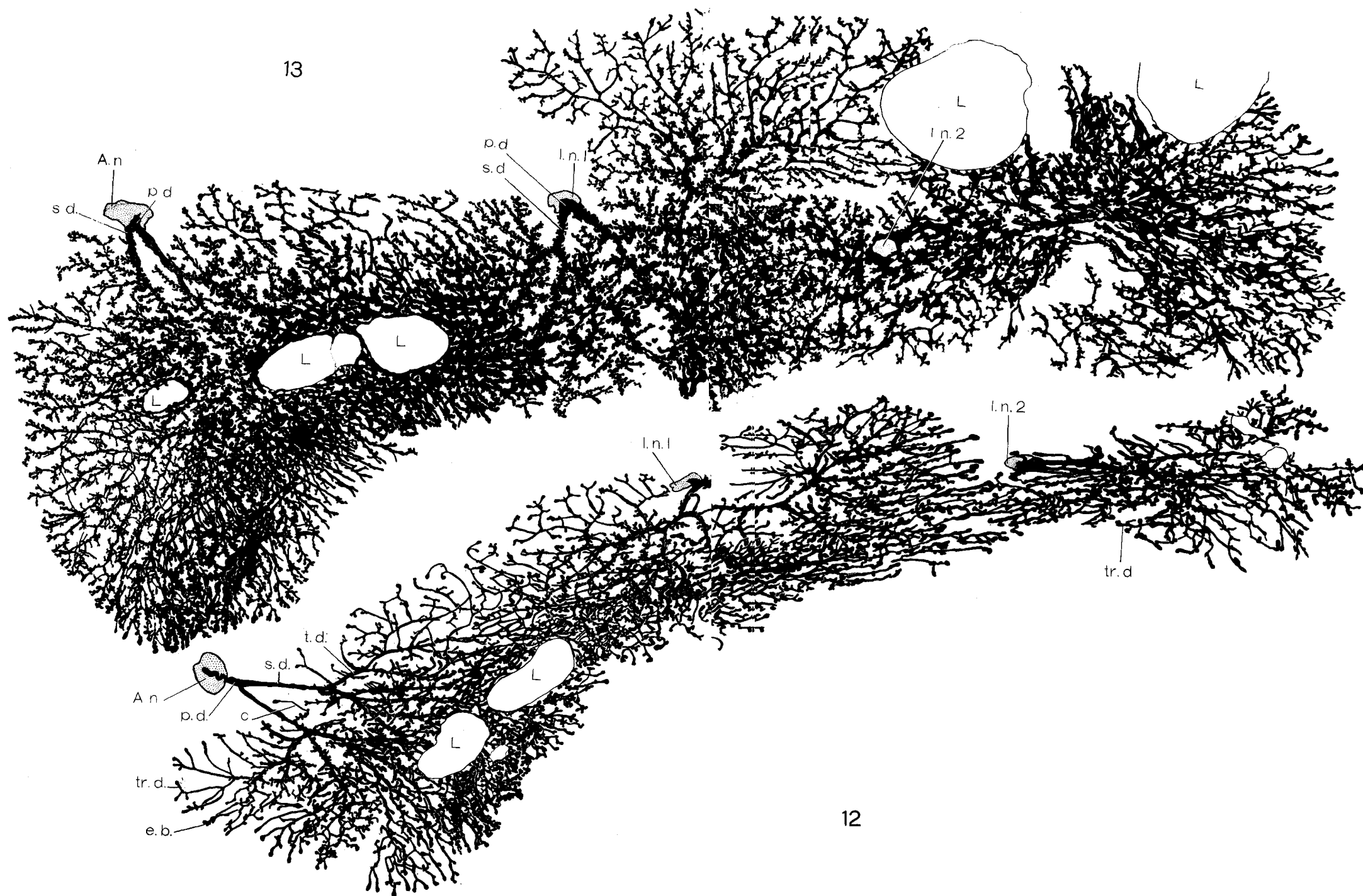


PLATE 4

EXPLANATION OF FIGURES

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

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

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

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

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

