The Lactation Cycle of Elephantulus myurus jamesoni (Chubb)

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Phil. Trans. R. Soc. Lond. B 1954 **238**, 62-98
doi: 10.1098/rstb.1954.0006

THE LACTATION CYCLE OF *ELEPHANTULUS MYURUS JAMESONI* (CHUBB)

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(*Communicated by F. W. R. Brambell, F.R.S.—Received* 17 *April* 1952—
Revised 11 *February* 1954)

[Plate 1]

CONTENTS

The development of the mammary glands follows the same course during all first pregnancies, regardless of the time of the breeding season. When a second or third pregnancy occurs at the beginning of the breeding season, the development of the glands during the pregnancy resembles the development during the first pregnancy once the embryos have reached the primary amniotic cavity stage.

In all other pregnancies the development is complicated by a concurrent lactation and later involution. However, the structure of the glands during lactation is essentially the same, regardless of which pregnancy it follows, and of whether the lactation overlaps with a subsequent pregnancy, or occurs during anoestrus. Therefore it seems that the presence of developing embryos does not influence the course of lactation in any way. Owing to this similarity, the structure of the glands is the same in both the second and third pregnancies until the developing embryos are approximately 5 mm long, if the pregnancy occurs during the middle or at the end of the breeding season. It appears that the young are weaned when the developing embryos reach this size, i.e. 5 mm long.

However, the changes in the glands during involution are affected not only by the time of the breeding season, but also by the pregnancy of the individual animal. When involution occurs during anoestrus it proceeds until a final state, only slightly more developed than the virgin condition, is reached. When involution occurs concurrent with the next pregnancy it differs according to the pregnancy of the individual animal. After a third pregnancy, which commenced either at the beginning or during the middle of the breeding season, the animal usually dies of old age before involution is established, but after a first or second pregnancy commencing at these times, involution takes place during the subsequent pregnancy. In these animals the extent to which the involution proceeds is governed by the size attained by the developing embryos at that particular time. When the involution occurs during a second pregnancy, i.e. after the first period of lactation, the most involuted condition is found when the embryos are 32·6 mm long. When it occurs during a third pregnancy, i.e. after the second period of lactation, involution is completed earlier, when the embryos are only 13·7 mm long, and this involution is more pronounced than that occurring during the second pregnancy.

It is difficult to explain why this first period of involution should be more gradual, and take so much longer than the second. Moreover, in the latter there is a definite point whereafter involution is striking and rapid; this does not occur during the first period of involution. The only feasible explanation I can offer is that possibly, during the first period of pregnancy, lactation, and then involution, the regular cycle has not yet been established in the mammary glands. After this stage they may be fully mature, and the cycle stabilized.

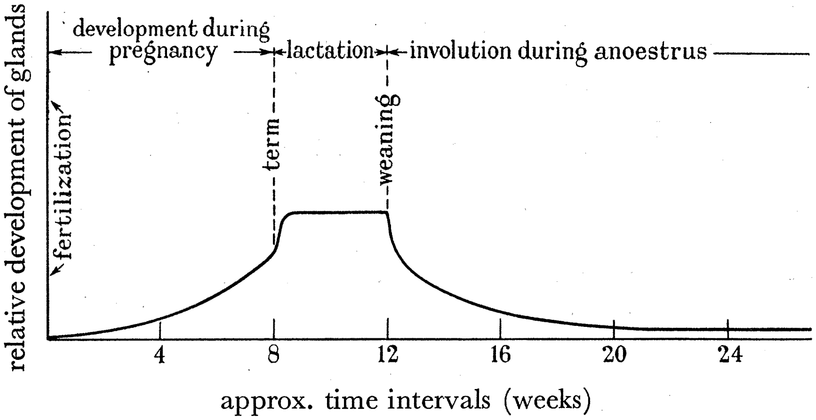
I. Introduction

In recent years the structure of the mammary glands of various mammals has been fairly thoroughly investigated during different phases of their development, i.e. during pregnancy, lactation and involution, and also under experimental conditions. Jeffers worked on the cytology of the mammary gland of the bat, *Myotis grisescens*, during pregnancy, lactation and involution (1940) and similarly on that of the albino rat (1935). In the latter case she also noted the effects of experimentally induced conditions. Changes occurring in the mammary gland of the rat during the reproductive cycle have been reported on by Astwood, Geschickter & Rausch (1937) and also by Maeder (1922); those in the guinea-pig by Hesselberg & Loeb (1937) and in the mouse by Cole (1933). Lane Williams (1942), also working on the mouse, only considered the changes occurring in the mammary glands during involution.

Besides these, and other works, devoted wholly to the structure of the mammary glands during the lactation cycle, many works include a note on the mammary-gland structure, as related to the oestrous and reproductive cycles, of various animals. This was done by Deanesly in her work on the reproductive cycle of the female hedgehog, *Erinaceus europaeus*

(1934), and in the female weasel (1944), and by Deanesly & Parkes (1933) working on the grey squirrel, *Sciurus carolinensis*.

No work has yet been done on the lactation cycle of *Elephantulus myurus jamesoni* (Chubb). Van der Horst & Gillman (1941) and van der Horst (1946, 1950) have investigated and reported on the embryology, menstrual and reproductive cycles of this animal, so it seems worth while to follow up this work with an investigation into the changes occurring in the mammary glands of *Elephantulus* during pregnancy, lactation and involution.

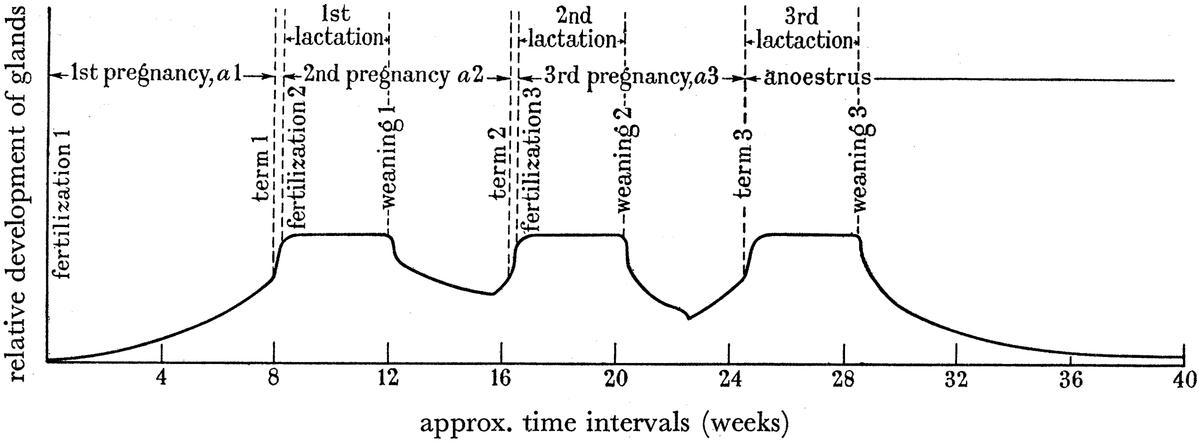


Graph 1. Graphic representation of the development of the mammary glands of *Elephantulus myurus jamesoni*, when the first pregnancy occurs at the end of the breeding season (case *c* 1). Note that the development of the glands commences from the undeveloped, virgin condition, and progresses steadily throughout the pregnancy. The development is accelerated for a short period immediately after the birth of the young, while a state of full lactation is established. The condition of the lactating glands remains at a fairly constant level until weaning, when there is a very rapid drop in the rate of secretory activity. Then involution proceeds steadily until a state, only slightly more developed than the virgin condition, is achieved, and this state persists throughout the period of anoestrus.

In studying the lactation cycle of *E. myurus jamesoni* it must be realized that, if the animal is a mature virgin at the beginning of the breeding season it will have three pregnancies during this breeding season. The subsequent pregnancies occur within about 2 days after parturition, when post-partum ovulation takes place. Thus there is no lactation anoestrus. The duration of the gestation period is 8 weeks; therefore, the first pregnancy of the season, setting in at the beginning of August, results in parturition at the end of September; the second pregnancy lasts from the end of September or beginning of October, until the end of November or beginning of December, and the third pregnancy, starting at the beginning of December, terminates at the end of January. After this the animal either goes into anoestrus until the next August, or dies. On the whole there are only three pregnancies during the animal's lifetime, occurring in one or two seasons (van der Horst 1946).

Due to the fact that there is no lactation anoestrus, it is only in the first pregnancy of the season that the developing embryos influence the growth of the mammary glands without any interference due to lactation. In the second and third pregnancies of the season embryos are developing at the same time as the young from the previous pregnancy are being suckled. However, the young are weaned before the developing embryos reach full term, so there is a retrogression of the mammary tissue during the gestation period, and thereafter a further development due to the stimulus of the growing embryos. Another result of this overlapping of pregnancy and lactation is that complete involution of the mammary glands, following the weaning of the young, only occurs after the third pregnancy of the season (graph 2).

This only applies to animals that are mature virgins at the beginning of the breeding season, and therefore were born at the end of the previous season, and went straight into anoestrus.



GRAPH 2. Graphic representation of the development of the mammary glands of *Elephantulus myurus jamesoni*, when the animal has its three pregnancies during the one breeding season (animal *a*, pregnancies *a* 1, *a* 2 and *a* 3). In this case the effect of the overlap of the pregnancies with the periods of lactation and involution is apparent. Only the development during the first pregnancy, and the lactation and involution following the third pregnancy, are uncomplicated, and resemble those occurring in case *c* 1. After the first and second periods of lactation, involution is not complete; in the former case the most involuted condition is found when the embryos of the second pregnancy are approximately 32 mm long, whilst after the second period of lactation the most involuted condition occurs when the embryos of the third pregnancy are approximately 13 mm long. Note that during the second period of involution the process is accelerated in its final stages.

In addition, animals may also be born during the breeding season. Since young animals become sexually mature in four or five weeks after birth (van der Horst 1946), these animals still have either one or two pregnancies during the season in which they were born, depending on whether they are the issue of a first or second pregnancy of that season. In these cases the animals go into anoestrus at the end of the breeding season, and have their remaining one or two pregnancies during the next season. The result of this is that at any time of the season some animals are in their first pregnancy, others in their second, and still others in their third or even fourth pregnancy.

A diagrammatic representation of this is given below:

first pregnancy			anoestrus	second pregnancy	
a 1	*a* 2	*a* 3			
	b 1	*b* 2		*b* 3	
		c 1		*c* 2	*c* 3

Each block represents the whole breeding season, and each line a pregnancy. This scheme indicates how the three pregnancies of animals *a*, *b* and *c* may be divided over one or two breeding seasons.

The main difficulty in this work is that the animals were not bred in captivity, and so it was not possible to ascertain the length of the period of lactation, nor could I obtain glands immediately the young were weaned and at definite time intervals thereafter. Throughout there were no data available as to the time lapse between one stage and another.

II. Material and methods

Sixty specimens were studied for this work on the mammary glands of *Elephantulus*. The majority of the animals were killed as soon as they were caught, and the whole specimen fixed immediately, using either of the following two fixatives, Bouin or 4 % formalin. Those fixed in Bouin were then stored in 70 % ethyl alcohol.

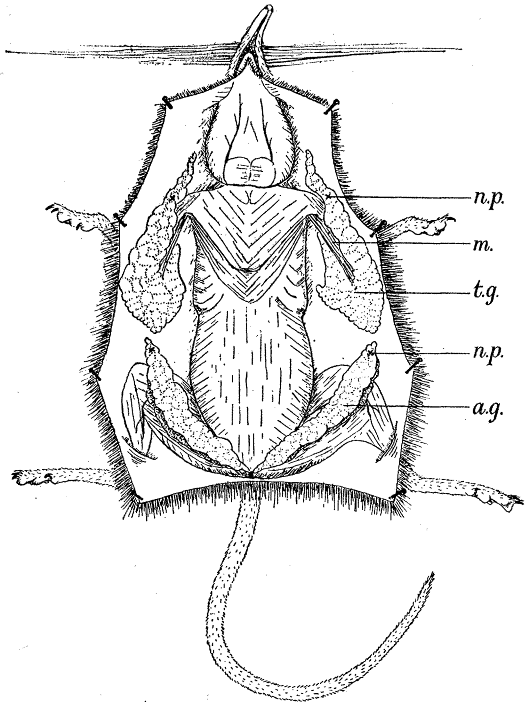


Figure 1. Ventral view of an entire animal to show the position and extent of the mammary glands during lactation. Half-natural size. *a.g.* abdominal gland; *m.* muscle; *n.p.* nipple position; *t.g.* thoracic gland.

I was fortunate in obtaining two living specimens that had given birth recently, and so was able to fix some of their glands in 2 % osmic acid, and the others in Bouin.

In *Elephantulus* there are two pairs of mammary glands, a thoracic pair and an abdominal pair. The nipples of the latter are situated in the abdominal region, but during lactation the glands extend to the inguinal region of the body (figure 1). In each case the right member of the thoracic pair was studied. The gland was embedded in paraffin wax and sections of a thickness of 10 μ were used. Of the following staining techniques, van Gieson's stain and trioxyhaematin, Heidenhain's haematoxylin and eosin, Mallory's triple stain and Hansen's haematoxylin counter-stained with eosin, the latter two yielded the best results.

The Mallory's triple stain demonstrated the septa of collagenous fibres between the lobules of the gland, and also between the alveoli, extremely well, staining them blue. Connective and reticular tissue also showed up blue, while the nuclei stained red and the cytoplasm of the cells pink.

With Hansen's haematoxylin and eosin the nuclear structure was clear, but the septa of collagenous fibres were not as distinct as with Mallory's stain. The presence of cell inclusions was well demonstrated with haematoxylin, as also was the composition of the alveolar and ductal contents.

Unfortunately, in glands fixed in Bouin, fat globules and pseudo-yolk spheres were dissolved out in the acetic acid used in the fixative. Therefore the presence of fat droplets is only indicated by clear spaces in the cytoplasm of the cells. Similarly, when fat droplets are present in the secretion, the fat is dissolved out, and all that remains to denote their occurrence are crescents of cytoplasm, the indented region having contained a fat globule, or fine rings of cytoplasm enclosing a clear area which was previously occupied by fat. Moreover, the fat was not preserved in specimens fixed in formalin, since the fat was not fixed and so dissolved out in the alcohol used in the staining process.

In the two previously mentioned specimens fixed in osmic acid, the fat was clearly demonstrated and showed up as dense black globules. These were found to correspond closely to the clear, vacuole-like areas, presumed to be regions where fat was present, in the other glands taken from the same animals and fixed in Bouin. Therefore it seems justifiable to presume that clear areas in the cells and secretion represent fat droplets, which were dissolved out in the fixative, and I shall proceed to do so in the following work.

III. Observations

(1) *The structure of the mammary gland of a virgin* Elephantulus

In *Elephantulus* no development of mammary tissue, involving alveolar formation, occurs until the onset of the first pregnancy. This condition was also found in *Erinaceus europaeus* (Deanesly 1934), and in the weasel, *Mustela nivalis* (Deanesly 1944).

In animals up till the beginning of the first oestrous cycle the gland is represented merely by a thickened area of adipose connective tissue. However, in some specimens this area is still in a transitory stage, showing that fat is in the process of being laid down in the areolar connective tissue. Many specimens contain a lymph node enclosed in a thin, but definite connective tissue capsule, the whole being embedded in the adipose tissue.

Glands taken from animals at the beginning of their first oestrus are still essentially represented by a thickening of adipose connective tissue, but there may be signs of an accumulation of cells in various regions. These cells multiply later to form alveoli. However, there are as yet no alveoli nor indications thereof. The branching and lengthening of the main lactiferous duct has started, but this formation of the duct tree has not extended very far beyond the nipple region. A transverse section of the main duct presents an irregular outline showing the initial stages of the development of side branches. The duct is lined with stratified epithelium, and has an investment of fibrous connective tissue comprised mainly of collagenous fibres (figure 11, plate 1). This fibrous tissue is not as dense as normal white fibrous connective tissue, and, moreover, it contains more cellular elements. Maeder (1922), working on *Mus norvegicus albinus*, reports the presence of a sheath of elastic tissue fibres around the ducts of lactating glands, while Lane Williams (1942) states that in the mouse, using Masson preparations, he could distinguish a fairly thick layer of fibro-elastic and muscular tissue around the larger ducts. In these glands of *Elephantulus*, although elastic elements may be present, they are very difficult to detect, and are definitely in the minority, collagenous fibres being the main constituents of the ductal sheath.

(2) *First pregnancy at the end of the season* (*case* c 1)

In this section I shall consider mammary glands from those animals which are pregnant for the first time at the end of the season and thereafter go into anoestrus. In these specimens the development, lactation and involution of the gland are not masked, nor complicated, by a concurrent lactation in the case of the development, nor by a concurrent pregnancy in the case of the involution. Therefore the true course of the development, lactation and involution can be studied.

Actually the glands from any animals in their first pregnancy, regardless of the time of the breeding season, show the development without any complications, i.e. the development is the same in cases *a* 1, *b* 1 and *c* 1. The glands of all these animals show the course of the development from the virgin condition to the stage when they are in full lactational activity. During the early part of the pregnancy, growth and differentiation of the glands are extremely gradual, and it is some time before they acquire a structure characteristic of mammary-gland tissue.

A. *The development of the mammary glands during the pregnancy*

When the embryos are in the primary amniotic cavity stage the structure of the mammary glands shows very little advance as compared with the virgin condition, only the ducts exhibit a further development. At this stage the main duct is very definite and longer than in the virgin gland. It has an irregular outline with lateral outgrowths (figure 2) which indicate the formation and presence of side branches. Clusters of cells often occur close to the tip of a duct (figure 2) showing that cells, just in advance of the developing duct system, multiply, and aggregate in this region in preparation for the extension of the duct, or for the formation of alveoli. The ducts are lined with stratified epithelium, two cells in depth, and have a thick investment of connective tissue, comprised mainly of collagenous fibres. This collagenous-sheath frequently encloses more than one duct branch where the branches are lying fairly close together.

From this stage onwards there is a steady development of the duct system. By the time the embryos have attained a length of 8 mm the branching is fairly extensive, and there may be as many as ten ductal branches enclosed in the same area of collagenous tissue (figure 3). Moreover, the sheath of collagenous tissue surrounding the ducts has increased in thickness and extended, so that in some regions there are fairly large areas of this tissue in which no ducts have developed yet (figure 3). However, it appears that, as more ductal branches, and also alveoli, develop, they do so in these areas of collagenous tissue. As a result these areas are reduced to moderately thin sheaths, each surrounding a single duct. This reduction has occurred by the time the developing embryos are 10 mm long.

These areas of collagenous tissue seem comparable to the intralobular connective tissue described in the resting stage of the human mammary gland by Maximow & Bloom (1930) (*Textbook of histology*, chap. XXVI), in that the tissue is very cellular, and contains practically no fat as compared with the remaining, or interlobular connective tissue occurring in the rest of the gland. However, the other distinctions between the inter- and intralobular connective tissue drawn by Maximow & Bloom, that the intralobular tissue is less dense, and contains fewer collagenous fibres than does the interlobular connective tissue, do not apply in the case of *Elephantulus*, since exactly the opposite condition is found. This dissimilarity may be due rather to a difference in the structure of the interlobular tissue than to one in the area enclosing the ducts. The interlobular connective tissue in *Elephantulus* is either areolar or adipose connective tissue, while Maximow & Bloom describe it as a rather compact tissue comprised of collagenous and elastic fibres forming a dense network.

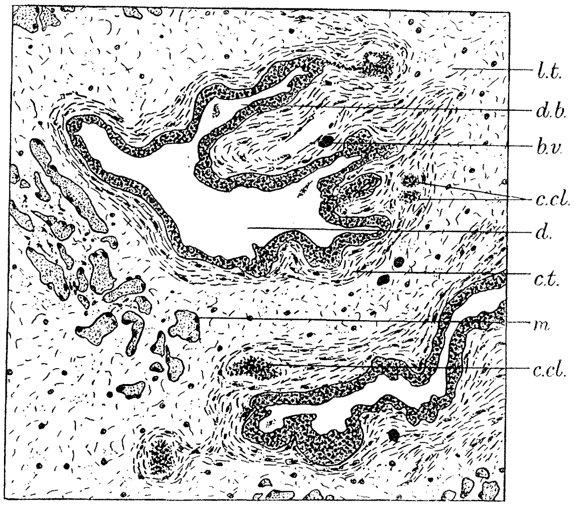


FIGURE 2. Shows the structure of a gland from an animal in its first pregnancy, when the embryos are in the primary amniotic cavity stage. Note the lateral outgrowths of the main ducts; these are the early stages in the formation of side branches. There are also clusters of cells lying in close proximity to the ducts. A wide sheath of collagenous tissue surrounds each duct. (Magn. ×96.) *b.v.* blood vessel; *c.cl.* cell cluster; *c.t.* collagenous tissue; *d.* duct; *d.b.* duct branch; *l.t.* loose connective tissue; *m.* muscle.

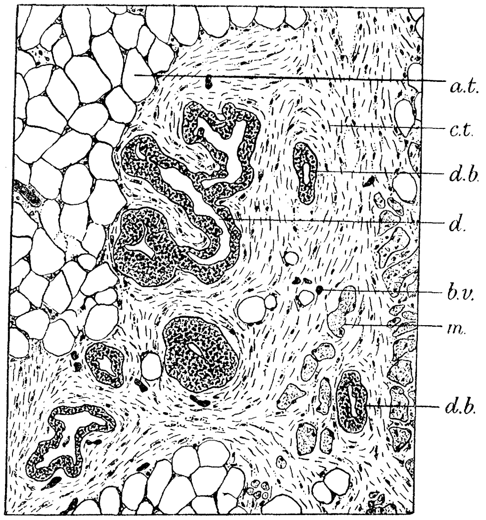


FIGURE 3. Shows the extent of development of the ducts when the embryos of the first pregnancy are 8 mm long. Note that many duct branches are enclosed in the same area of collagenous tissue, and that there are comparatively large areas of this tissue in which no alveoli nor ducts have yet developed. (Magn. ×96.) *a.t.* adipose tissue; *b.v.* blood vessel; *c.t.* collagenous tissue; *d.* duct; *d.b.* duct branch; *m.* muscle.

The condition of the ducts does not change other than a further enlargement and branching of the duct tree, until the embryos have attained a length of 30 mm. By this stage the glands, although not measuring more than 0·6 mm in thickness, present a structure typical of mammary tissue. This has been achieved by the development of alveoli. The first definite alveoli occur when the crown-rump length of the embryo is 8 mm. At this stage small clusters, frequently consisting of only two or three alveoli, are found forming alongside the ducts, in the areas of collagenous tissue. These alveoli have no lumen yet, but by the time the embryos are approximately 10 mm long, there is a small but distinct lumen in many of the alveoli. These alveoli are lined with a single layer of columnar epithelium.

From this stage onwards there is a steady development of alveoli, and the size of the clusters increases so that, by the time the embryos have attained a length of 30 mm, the alveoli are arranged in definite lobules. Nevertheless, there are still numerous epithelial cells lying scattered in the stromal tissue of the glands. However, practically all these cells soon become organized to form definite alveoli.

As a result of this development of glandular tissue the adipose tissue is replaced by glandular elements, so that by the time the embryos are 30 mm long, and definite lobules have formed in the mammary glands, the proportion of glandular tissue to adipose tissue is in the ratio of 6:1.

Very soon after this, when the crown-rump length of the embryo is 30·7 mm, the initial stage in the formation and storage of products in preparation for lactation occurs. At this stage small fat droplets are found forming in the alveolar epithelium. Nevertheless, there are no signs of secretory activity, and the lumina of the alveoli are quite empty. Concurrent with this development the epithelium lining the ducts loses its stratified condition, and the ducts are now lined with a single layer of either cuboidal or columnar epithelium.

Secretory activity does not commence until shortly before term. When the embryos are 39·8 mm long there is slight secretory activity in the glands, which, although only 1 mm in thickness, have a very compact gross structure, and adipose tissue only occurs as thin strips separating the lobules from each other. At this stage the alveoli are lined with either low columnar or cuboidal epithelium, and the majority of these cells contain three or four small fat droplets. In some cases the fat droplet is larger and situated apically in the cell, causing the tip of the cell to protrude into the lumen of the alveolus. In many alveoli it appears that there is a slight indentation at the base of the protrusion as though the fat droplet, with its surrounding cytoplasm, is in the process of being constricted off into the lumen. Occasionally the material protruded consists merely of cytoplasm, thus giving rise to the formation of cytoplasmic buds.

In his work on glands Eggeling (1931) states that mammary glands are recognized as being apocrine glands. In describing the method of the formation of secretion in apocrine glands he says that irregular outgrowths, consisting of cytoplasm and fat, form on the inner surface of the epithelium of the gland. These are constricted off and accumulate in the lumen. In *Elephantulus* the fat droplets and cytoplasmic buds are secreted by this method. When working on the rat (1935) and the bat (1940), Jeffers describes the method as being one of decapitation, but it is essentially the same as that outlined above.

At the stage which shows the commencement of secretory activity the alveolar lumina are moderately large, having an average diameter of 30·8 μ, but they contain only a small amount of secretion, which generally consists of a narrow area of fine granular material around the circumference of the lumen. This condition resembles that described by Jeffers in the bat, *Myotis grisescens* (1940). The amorphous granular material constitutes the main component of the secretion, and is most likely formed by the disintegration of cytoplasmic buds. There are also larger bodies, of which fat droplets, surrounded by cytoplasm, are most common. Owing to the fact that the material was originally stored in alcohol the fat itself has dissolved out, and all that remains is the surrounding ring or crescent of cytoplasm. Spheres, consisting entirely of cytoplasm, are more rare. Occasional lymphocytes and disintegrating nuclei also occur, but entire epithelial cells are not present in the secretion.

In this work, when describing the composition of the secretion, I could only note the solid components, and fat droplets suspended in the secretion. Water is, of course, the chief constituent, but it, and substances dissolved therein, is not shown in the material available for this work.

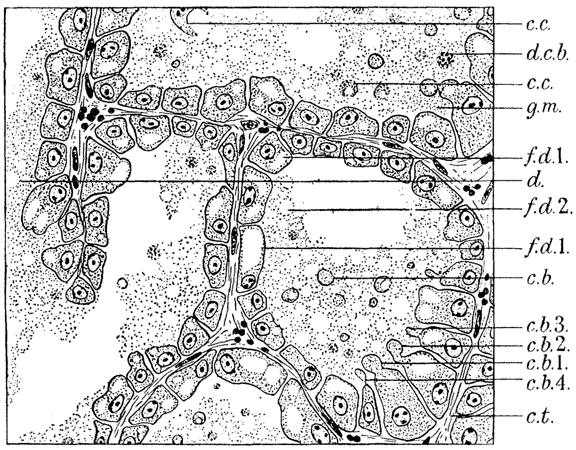


FIGURE 4. Shows typical alveoli of a gland from an animal in its first pregnancy, when the embryos are 46·2 mm long, i.e. near term. Note the large fat droplets in the cells of the alveolar epithelium, and the successive stages in the formation of cytoplasmic buds. The composition of the secretion is also shown. On the left of the drawing there is an alveolus opening into a duct. (Magn. ×420.) *c.b.* cytoplasmic buds; *c.b.* 1, 2, 3, etc., successive stages in the formation of cytoplasmic buds; *c.c.* cytoplasmic crescent; *c.t.* collagenous tissue; *d.* duct; *d.c.b.* disintegrating cytoplasmic bud; *f.d.* 1, fat droplet in the cytoplasm of a cell; *f.d.* 2, fat droplet in the secretion; *g.m.* granular material.

Concurrent with the commencement of secretional activity in the gland, the main branches of the duct system become enlarged, and each lobule of the gland is drained by one of these large branches. Although the ducts are enlarged, they are not congested. In fact many of them are devoid of secretion, while at the most they are filled to only three-quarters of their capacity. The single layer of epithelium lining the ducts is now very flattened.

Associated with the increased activity of the gland there is a considerable enlargement of the blood vessels supplying this region.

The rate of secretory activity increases rapidly, so that by the end of pregnancy when the embryos have attained a length of 46·2 mm, the glands are in readiness to assume a state of full lactation once parturition occurs. Glands taken from animals at this stage show a definite enlargment of the alveolar lumina. This enlargement is due to a flattening of the alveolar epithelium rather than to a marked increase in the size of the actual alveoli. The alveoli are now lined with cuboidal epithelium, the height of the cells generally being about the same as the diameter of their nuclei. Fat droplets have become more plentiful in these epithelial cells, and there may be either one large droplet or several smaller ones per cell (figure 4). The rate of secretory activity in the alveoli has increased, and all stages in the formation and secretion of fat droplets and cytoplasmic buds occur (figure 4).

The alveoli are, on the whole, moderately full, with granular material still forming the ground substance of the secretion. There are now, in addition, numerous other bodies, most common of which are fat droplets and cytoplasmic buds. Lymphocytes and degenerating nuclei are rare (figure 4).

The epithelium lining the ducts now also exhibits a secretory activity, in particular that of the small ducts at the periphery of the gland rather than that of the large main ducts. However, it is never as vigorous as in the alveoli. Despite the increased activity of the glands the ducts are by no means congested.

B. *Lactation*

Thus far there has been a steady development of the glands, involving a lengthening and branching of the ducts, and a differentiation of alveoli, so that, at full term, the gland is a compact organ with a structure typical of mammary tissue. Secretory activity is first apparent shortly before term, but until the gland is stimulated by suckling it is not very vigorous, and the secretion is retained in the gland.

Suckling commences soon after the birth of the young, and so the alveoli and ducts are then drained of their secretion. This stimulates the gland to a higher rate of secretory activity. The resulting development of the gland is more rapid than that occurring at any time during the pregnancy. This highly developed condition is maintained for some time.

After pregnancies which take place at any other time of the season, i.e. near the beginning or during the middle of the season, the animal again becomes pregnant at the end of the post-partum period. In these cases the duration of the total post-partum period is approximately 2 days. However, after this pregnancy, which occurs at the end of the season, the animal goes into anoestrus, and therefore lactation continues uncomplicated by a concurrent pregnancy.

The post-partum period is arbitrarily divided as follows: early, intermediate and late post-partum. A state of full lactation has been established by the time the animals are in the late post-partum stage. In this case (case *c* 1) glands were also obtained from animals 4 and 7 days post-partum, and in these a state of full lactation was still maintained.

Unfortunately, there was no material indicating the stage at which lactation ceases when the animal is in anoestrus.

(*a*) *Early post-partum*. During this stage the glands become far thicker than those from an animal near term, being 2·8 mm in thickness as compared with 1·8 mm. Moreover, a transverse section of a gland has a more dense appearance than hitherto, since the gland now consists almost exclusively of glandular elements, adipose tissue occurring only as narrow strips between some of the lobules. In many places the interlobular region is reduced to a thin collagenous septum, while in the more solid regions of the gland the lobules have merged together, so that the intervening septum is indistinguishable from that of the individual alveoli. In the grey squirrel, *Sciurus carolinensis* (Deanesly & Parkes 1933), and also in the hedgehog, *Erinaceus europaeus* (Deanesly 1934), the lobules of the mammary gland grow together to form solid strips during lactation. Cole (1933), working on the mouse, found that, at full term, the individual lobules are indistinguishable at the periphery of the gland. Thus the coalescence of the lobules of the mammary gland at the beginning of lactation, as found in *Elephantulus*, is a widespread and anticipated phenomenon.

During early post-partum the size of the alveoli does not increase as compared with that at full term, but the lumina are fuller. Due to the increase in the rate of secretory activity there are generally somewhat fewer fat droplets retained in the cytoplasm of the alveolar cells, while in occasional alveoli practically all of the cells have discharged their fat droplets. Moreover, cytoplasmic buds and fat droplets become more plentiful in the secretion.

The formation of cytoplasmic buds only occurs in localized lobules of the glands. In addition, the discharge of large fat droplets from the alveolar cells is more common in these lobules than in other regions of the gland.

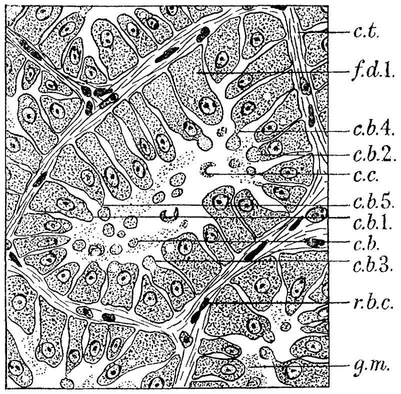


FIGURE 5. Type 1 alveoli from a gland in a state of full lactation. Note the small lumina, the height of the epithelium, and the vigorous formation of cytoplasmic buds. (Magn. × 420.) *c.b.* cytoplasmic bud; *c.b.* 1, 2, 3, etc., successive stages in the formation of cytoplasmic buds; *c.c.* cytoplasmic crescent; *c.t.* collagenous tissue; *f.d.* 1, fat droplet in the cytoplasm of a cell; *g.m.* granular material; *r.b.c.* red blood corpuscle.

The only change in the ducts is that they too become filled with secretion.

(*b*) *Late post-partum*. During early post-partum there are indications of the differentiation of two types of alveoli. By late post-partum the alveoli can be divided into two definite main types (figures 12 and 13, plate 1).

Type 1 *alveoli*. The majority of the gland is comprised of alveoli in which the lumen is the same size as that of an alveolus from an early post-partum gland, i.e. the average luminal diameter is 27·4μ. These alveoli are lined with tall columnar epithelium, the luminal borders of the alveoli being irregular (figure 5). The height of adjoining cells varies, and the free edges of the cells project unevenly into the lumen, or they may be curved. In the albino rat, *Mus norvegicus albinus*, the alveolar epithelium from a lactating gland showed an irregular border on the luminal side of the cells, due to the number of fat droplets in the process of secretion and discharge (Maeder 1922). Similarly in this gland from *Elephantulus* these alveoli are actively secreting. Fat droplets, and cytoplasmic outgrowths, projecting from the luminal border of the cells are common (figure 5). In a few instances the nucleus is being constricted off with the bud of cytoplasm. This is the first gland in which nuclei may be included in the buds. The fat droplets are small, and there are never more than one or two in a single cell.

All these alveoli contain secretion, some are congested. This secretion consists of a granular base containing numerous cytoplasmic buds and fat droplets (figure 5). In addition, lymphocytes and disintegrating nuclei may be present.

Type 2 *alveoli*. The second main type of alveoli is comprised of those with a very large lumen, and lined with flattened epithelium (figure 6). These alveoli are situated in lobules at the tip of the gland, and constitute roughly a quarter of the total glandular area. The epithelial cells are so flattened that they may be up to four times as wide as they are tall, and the nucleus causes a bulge in the cell. There is very little active secretion taking place in these alveoli. The fat droplets are small, and no large apical ones are present (figure 6). Occasional cytoplasmic buds are in the process of formation.

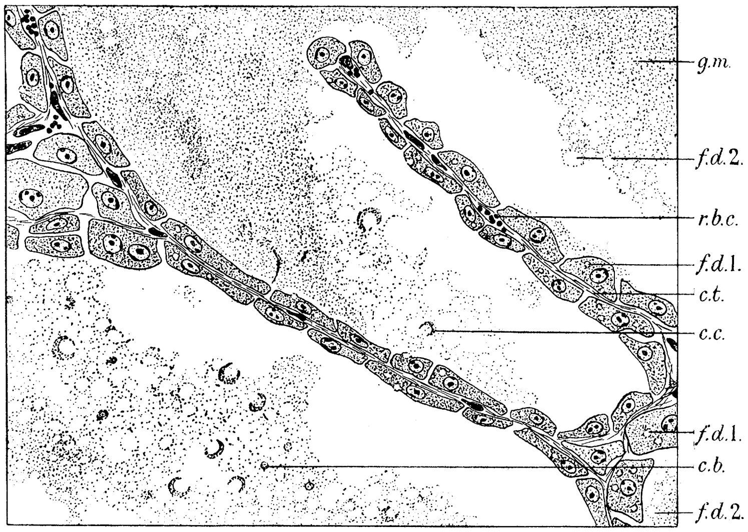


FIGURE 6. Shows portions of type 2 alveoli from a gland in a state of full lactation. Note the low epithelium, and the very large lumina (in each case only a part of the lumen is shown). (Magn. × 420.) *c.b.* cytoplasmic bud; *c.c.* cytoplasmic crescent; *c.t.* collagenous tissue; *f.d.* 1, fat droplet in the cytoplasm of a cell; *f.d.* 2, fat droplet in the secretion; *g.m.* granular material; *r.b.c.* red blood corpuscle.

The lumen of these alveoli is two to three times as large as that of the type 1 alveoli occurring in the gland, and there is less secretion therein, some of the alveoli being filled to only half of their capacity. Compared with the secretion in the type 1 alveoli, the ratio of fat droplets and cytoplasmic buds to the granular basic material in these alveoli is lower, the decrease involving the cytoplasmic buds in particular (figure 6). Lymphocytes are also more rare.

Ducts. During this period the ducts are large and filled with secretion, but secretory activity is still restricted to the smaller branches. Very few fat droplets occur in the cells of the ductal epithelium, and all of them are small. The composition of the ductal contents is the same as that of the secretion in the second main type of alveoli.

(*c*) *Four and seven days post-partum.* At these stages the state of full lactation, found during late post-partum, is maintained. The lumina of the alveoli and ducts are all filled with secretion, giving the glands an extremely solid appearance.

The most notable development is that the alveoli are now all of the type with large lumina, the average luminal diameter being $75 \cdot 1\,\mu$. The epithelium becomes so flattened that the cells may be up to four times as wide as tall (figure 7). There is a decided increase in the fat content of the alveolar cells, and by 7 days post-partum the amount of fat present is excessive. By this stage the epithelial cells of the ducts as well as the alveoli are frequently distended by large fat droplets.

The secretory activity, both in the ducts and alveoli, has increased since late post-partum, and the rate of discharge of fat droplets is very high. However, cytoplasmic bud formation is comparatively rare.

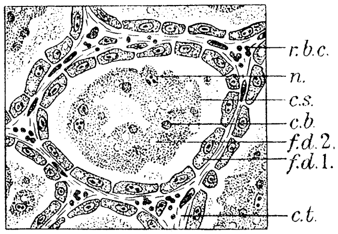


FIGURE 7. Shows the type of secretion occurring in the alveoli at 4 days post-partum. Note the conglomerates of secretion. The alveolar epithelium is very flattened. (Magn. ×420.) *c.b.* cytoplasmic buds; *c.s.* conglomerate of secretion; *c.t.* collagenous tissue; *f.d.* 1, fat droplet in the cytoplasm of a cell; *f.d.* 2, fat droplet in the secretion; *n.* nucleus; *r.b.c.* red blood corpuscle.

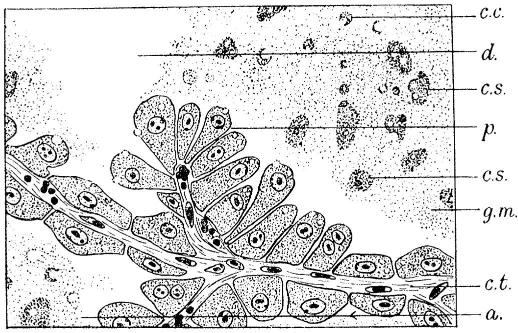


FIGURE 8. Shows a 'finger-like' papilla in the duct wall of a gland from an animal at 4 days post-partum. Note the taller epithelium on the papilla, the secretory activity of this epithelium, and the conglomerates occurring in the secretion. (Magn. ×420.) *a.* alveolus; *c.c.* cytoplasmic crescent; *c.s.* conglomerate of secretion; *c.t.* collagenous tissue; *d.* duct; *g.m.* granular material; *p.* 'finger-like' papilla.

The ducts have now developed outgrowths of their collagenous septa, which project into the lumen. They are covered with columnar epithelium (figure 8). These outgrowths occur in both the main ducts as well as in the smaller branches, but are more plentiful in the latter. This is a feature not present in earlier stages of the development of the mammary glands.

Cole (1933) noted that in the mouse, as from the seventh day of lactation, the ducts, although showing less secretory activity than do the alveoli, secrete fairly actively. To facilitate this the duct epithelium develops finger-like papillae projecting into the lumina.

These outgrowths of the duct epithelium in *Elephantulus* must be homologous with the finger-like papillae reported by Cole in the mouse, although they appear slightly earlier in *Elephantulus*, i.e. on the fourth day post-partum as compared with the seventh day of lactation in the mouse. In *Elephantulus* they also increase the secretory activity of the ducts, seeing that the epithelial cells thereof contain more fat droplets than do other cells of the duct epithelium. Many of these droplets protrude into the lumen in the process of discharge (figure 8), and cytoplasmic bud formation, although less active than in many of the alveoli, is more common here than in other regions of the ducts.

In conjunction with the high rate of secretory activity the lumina of the alveoli and ducts are congested with secretion, much of which is in the form of dark staining masses. These masses are either dense, compressed granular material, or conglomerates of cytoplasmic buds and fat droplets (figure 7). There are also free fat droplets, cytoplasmic buds and disintegrating nuclei, in this order of frequency, suspended in granular material, which has not the dark, condensed appearance of the masses. The number of lymphocytes present in the secretion increases so that, by 7 days post-partum, they have become as common as the disintegrating nuclei. A few polymorphonuclear leucocytes also appear in the secretion.

C. *Involution* (*case* c1).

There is no definite data for the animals showing involutionary stages as to what period of time elapsed between the birth of the last pair of young, and the slaughter of these animals. Therefore in glands from animals caught within a few days after each other, one can merely gauge the extent of involution, and so place them in sequence on this basis. Animals caught on the same date do not necessarily show the same extent of involution.

(*a*) *Early stages of involution.* Successive stages of early involution are shown best by glands from three animals caught on 10 February 1949, at Bronkhorstspruit.

The early stages of involution are marked by a rapid decrease in the thickness of the gland, and an invasion of adipose tissue, which is soon far in excess of glandular tissue. This invasion is first apparent between the lobules, but later islands of adipose tissue appear within the actual lobules, which are thus reduced to small, irregular clusters, consisting of a few alveoli, scattered in the adipose tissue. The adipose tissue appears to be divided into lobules by septa of collagenous tissue. A similar feature was noted by Maeder (1922) in the involuting mammary glands of the albino rat.

An increase also occurs in the amount of collagenous tissue between and around the alveoli, so that there is a sheath of dense collagenous tissue around each alveolus. This sheath becomes one to two times as wide as the alveolar epithelium. Then outside this there is a loose collagenous stroma, wherein are scattered numerous epithelial cells, which are the remnants of retrogressing alveoli.

Another striking characteristic of the early involution is the decrease in the size of the lumina of the alveoli. This progresses steadily throughout the three stages, and it ultimately results in the alveoli becoming reduced to either solid clusters of epithelial cells, or there may be an extremely small lumen present.

At the same time as the lumina of the alveoli decrease in size, the epithelium lining them also undergoes changes. First it becomes uniformly cuboidal. Thereafter many of the cells show signs of disintegration and, moreover, some of them slough off into the lumina. In addition, the amount of fat present in the epithelium lining the alveoli, and also the ducts, changes during involution. It appears that initially there is an increase in the fat content of the epithelial cells. Later there is a decrease, finally resulting in the complete absence of fat droplets from the epithelium of both the ducts and alveoli.

The secretory activity must cease very rapidly once the young are weaned and suckling ceases. If this did not occur the glands would become so distended with secretion that they would eventually burst. There is no formation of cytoplasmic buds in any of these involuting glands, but in the earliest stage occasional fat droplets are still being discharged from the alveolar epithelium. At this stage, although the alveolar lumina are reduced in size, they nearly all contain some material; on an average they are filled to half their capacity. This secretion contains approximately equal proportions of granular material and epithelial cells, many of which contain fairly large fat droplets. There are only a few free fat droplets in the secretion, and lymphocytes are comparatively rare.

After this earliest stage the only material discharged into the lumina consists of cells sloughed off from the alveolar epithelium. This results in a further decrease in the amount of secretion present in the alveoli, with disintegrating epithelial cells predominating. There is also a little granular debris, which is possibly formed as a result of the disintegration of these epithelial cells.

The involution of the ducts is, in some respects, less rapid than that of the alveoli. The reduction in size becomes marked at a slightly later stage in the ducts, but once it occurs the duct lumina become small and convoluted. In some of the ducts the epithelium resumes a stratified condition, although in the majority there is still a single layer of cuboidal epithelium. As in the mouse (Cole 1933), so also in *Elephantulus*, there is an accumulation of collagenous tissue around the ducts during involution.

However, changes in the secretory activity and in the composition of the secretion in the ducts are more rapid and resemble those in the alveoli. 'Finger-like' papillae are not present in the duct walls of any of these involuting mammary glands. This is correlated with the reduction and cessation of the secretory activity. Nevertheless, the extent to which the ducts are filled with secretion does not decrease quite as rapidly as does that of the alveoli. It is only when there is a striking reduction in the size of the duct lumina that the amount of secretion becomes greatly reduced, and the ducts are practically empty.

(*b*) *Late stages of involution*. In this section glands from three animals caught during the months of June, July and August are considered. In all cases the animals had been pregnant at the end of the previous breeding season.

There is a very close resemblance between the three glands, and since the animal killed in August was at the end of anoestrus, one can be certain that there are no further stages of involution.

The glandular tissue is reduced to small islands scattered within a comparatively vast area of adipose tissue (figure 14, plate 1). These islands, which measure $50 \times 240\mu$ on an average, are comprised of fairly dense collagenous tissue in which numerous epithelial cells are scattered. In a few cases these cells appear to be organized into clusters, but there are no longer any definite alveoli with lumina. The cytoplasm and cell boundaries of the epithelial cells are indistinct. There is no fat present either in these epithelial cells or in those lining the ducts.

The main ducts have remained definite and all have a small lumen, which is either a narrow, elongated slit, or else very irregular and convoluted in shape (figure 14). Some of the largest of these ducts are lined with a single layer of cuboidal epithelium, but in all the others the epithelium is stratified. The sheaths of collagenous tissue surrounding the ducts have not increased in width as compared with the last stage of early involution. The duct lumina are without contents.

(3) *The cycle in the mammary glands when pregnancy and lactation overlap*

Since young animals become sexually mature in 5, or at the most 6 weeks after birth, the young may still have one or two pregnancies during the season, in which they were born. In the animals considered so far, which are the issue of a second pregnancy of the season and have their first pregnancy at the end of the breeding season (case *c* 1), the development of the mammary glands during the pregnancy is straightforward and uncomplicated, as also are the periods of lactation and involution (graph 1). However, in other cases the changes in the glands are complicated. When the young are the issue of a pregnancy occurring at the beginning of the breeding season, the animals will have their first pregnancy during the middle of the season (case *b* 1). Animals which are the issue of a final pregnancy of the season, go straight into anoestrus, and do not have their first pregnancy until the beginning of the following breeding season (case *a* 1).

In cases *a* 1 and *b* 1 the lactation and period of involution of the mammary glands following this pregnancy, occur concurrently with the next pregnancy. This complicates these two processes. In fact, the cycle in the glands is complicated at one or other stage during all pregnancies except for the previously described case *c* 1, where the first pregnancy occurs at the end of the season (compare graphs 1 and 2).

In considering cases *a* 1 and *b* 1 it is found that the development, lactation and involution of the mammary glands follow the same course regardless of whether the first pregnancy occurs at the beginning or during the middle of the season.

A. *Cases* a 1 *and* b 1 *when the first pregnancy occurs at the beginning, or during the middle of the season.*

(*a*) *The development of the glands during pregnancy*. The development of the glands of these animals is the same as that previously described, when the first pregnancy occurs at the end of the season.

(*b*) *Post-partum period.* During the short interval which elapses between parturition and the onset of the second pregnancy, a period of 2 days at the most, a state of lactation is established. The changes occurring during this period are the same as those found in the early and intermediate post-partum glands after a first pregnancy at the end of the breeding season (case *c* 1). These have already been described.

(*c*) *Lactation and involution.* Although a state of lactation is established during the post-partum period, most of the lactation occurs after the onset of the next pregnancy. Thus the greater part of the lactation, and all the subsequent involution following these two pregnancies (*a* 1 and *b* 1) occur concurrently with the next pregnancies (*a* 2 and *b* 2). Therefore the changes taking place during these periods will be considered in the section dealing with the second pregnancy.

B. *Second and third pregnancies*

B 1. *Second and third pregnancies occurring at the beginning of the breeding season* (*cases* c 2 *and* b 3). Second or third pregnancies may also occur at the beginning of the season (cases *c* 2 and *b* 3).

This is in the case of older animals which were pregnant at the end of the previous season, and then went into anoestrus during the winter months.

(*a*) *The development of the mammary glands during the pregnancy.* The development of the mammary glands of animals in their second or third pregnancy at the beginning of the breeding season differs initially from that occurring in the glands of animals in their first pregnancy at the beginning of the season. This is due to the fact that, at the end of the previous breeding season, the glands did not involute right back to the virgin condition found at the time of the first oestrus (compare figures 11 and 14, plate 1). Actually the involuted glands are very similar to those showing the slight development which occurs by the time the embryos of the first pregnancy are in the primary amniotic cavity stage.

As a result of this slight development no further development is discernible until after the embryos of these second or third pregnancies have reached the primary amniotic cavity stage. Thenceforth the development is the same as that occurring in the glands of animals in their first pregnancy.

(*b*) *Post-partum period.* After partus at the end of a second or third pregnancy which occurred at the beginning of the season (cases *c* 2 and *b* 3), a state of full lactation is soon established. The changes leading up to this during the post-partum period are the same as those occurring in the mammary glands after a first pregnancy at the end of the breeding season (case *c* 1).

(*c*) *Lactation and involution.* As in the case of a first pregnancy occurring at the beginning of the season (case *a* 1), so also when a second or third pregnancy occurs at the beginning of the season (cases *c* 2 and *b* 3), the greater part of the period of lactation, and all of the subsequent involution following the pregnancy, occur concurrently with the next pregnancy of the season. Nevertheless, the stages during the lactation and subsequent involution following pregnancies *c* 2 and *b* 3 differ somewhat from those occurring after a first pregnancy at the beginning of the season (case *a* 1), but resemble those following a second or third pregnancy occurring during the middle of the season, i.e. the stages of lactation and involution following *c* 2 are the same as those following *a* 2, and the stages of lactation and involution following *b* 3 are the same as those following *c* 3. Therefore these stages will be discussed in the next section.

B 2. *Second and third pregnancies occurring during the middle and at the end of the breeding season* (*cases* a 2, b 2, a 3 *and* c 3). Besides the second and third pregnancies which occur at the beginning of the breeding season (cases *c* 2 and *b* 3), it is possible for animals to have their second and third pregnancies later in the season. These are the pregnancies denoted as *a* 2, *b* 2, *a* 3 and *c* 3. No difference could be observed between the mammary glands of parous animals *a* 2 and *b* 2, or *a* 3 and *c* 3 during their pregnancy, although the pregnancies of *a* 2 and *c* 3 occur during the middle of the breeding season, and those of *a* 3 and *b* 2 occur at the end of the season. Therefore the changes that occur in the glands during these second and third pregnancies are determined by the pregnancy of the individual animal, and not by the time of the breeding season.

When the animals go into anoestrus after parturition (cases *a* 3, *b* 2 and *c* 1) there are no indications at all of the length of the period of lactation, nor of that of the involution of the mammary glands. In the cases of the animals *a* 2, *b* 2, *a* 3 and *c* 3 the size of the embryos gives at least a vague indication of the duration of lactation and involution of the glands related to the previous pregnancy. These cases will be considered in this section.

B2 (i). *Second pregnancy* (*cases* a2 *and* b2). During the first part of this pregnancy the young from the first pregnancy (cases *a*1 or *b*1) are being suckled, and therefore a state of full lactation is maintained in the mammary glands. Once these young are weaned the glands involute. This is later followed by another period of development, stimulated by the growth of the embryos of the second pregnancy. Therefore, during lactation and the period of involution, I shall consider the form of the glands as related to the size of the embryos of the subsequent pregnancy.

A state of full lactation has already been established during the short post-partum period following the first pregnancy (case *a*1 or *b*1). This is maintained during the first half of the second pregnancy, and appears to reach a peak when there are one to four cell stages in the embryo chamber. At this stage the gland shows a structure typical of full lactation and is very similar to that of an animal in the late post-partum stage of a first pregnancy at the end of the season, except that the secretional activity seems to be more vigorous. Moreover, the gland has a solid, compact appearance, but the fusion of the lobules has not progressed further than in the post-partum stages. The gland is 3·2 mm thick, an increase of 0·4 mm as compared with those of the post-partum stages. This is the maximum thickness found during this period of lactation.

From studying the mammary glands from animals in their second pregnancy with embryos of the following sizes—embryos in the blastula and blastocyst stages, and embryos of a length of 4·9 mm (41 somites)—it is found that a state of full lactation is maintained unchanged until the embryos are approximately 5 mm long. The main features observed during this period of lactation are as follows.

Throughout lactation the two types of alveoli, which first appear in post-partum glands after the first pregnancy, are present. The alveoli attain their maximum diameters when the embryos are in the blastula stage, the average luminal diameter of the type 1 alveoli being 75μ, and that of the type 2 alveoli 164μ. Concurrent with the enlargement of the lumina, there is a flattening of the epithelium of the alveoli, resulting in the epithelium of the type 2 alveoli becoming so flattened that the cells may be as much as five times as wide as they are tall.

When this period of lactation was first initiated during the post-partum period following either pregnancy *a*1 or *b*1, the type 1 alveoli were in excess of the type 2 alveoli, but by the time the embryos of the second pregnancy are in the blastula stage the alveoli with large lumina are in excess of the smaller type 1 alveoli. Once this occurs, the type 1 alveoli are found to occur mainly down the inner edge of the gland, although in some instances a few of these alveoli may be interspersed within the body of the gland. The type 2 alveoli are predominant throughout this range of glands showing a state of lactation.

During the whole period of lactation secretional activity is more vigorous in the type 1 alveoli, in particular, the secretion of cytoplasmic buds which reaches a maximum when there are one to four cell stages in the embryo chamber. In the type 2 alveoli the activity consists almost exclusively of the extrusion of fat droplets. There is a reduction in the fat content of the alveolar epithelium as compared with the late stages of the first pregnancy;

the size of the droplets is not affected, only the number thereof. This compares with the condition in the period of lactation following pregnancy *c* 1.

Throughout this period the type 2 alveoli are filled with secretion, while the type 1 alveoli usually contain a small amount of newly formed material, the composition of which is dependent on the rate of cytoplasmic bud formation. When this reaches a maximum, i.e. when there are one to four cell stages in the embryo chamber, cytoplasmic buds are the predominant constituents. In the other stages cytoplasmic buds are not strikingly in excess of the fat droplets. In the type 2 alveoli the composition of the secretion is fairly constant, and the bulk of the secretion always consists of fat droplets and granular material. One notable fact is that the cytoplasm in the buds and crescents has a more granular, disintegrated appearance in the secretion contained in the type 2 alveoli than in the type 1 alveoli.

Meanwhile the ducts become distended. The height of the duct epithelium is never constant throughout a gland, both cuboidal and columnar epithelium frequently occurring in the ducts of the same gland. Once a state of full lactation is established in the alveoli, the epithelium of the ducts also exhibits a secretory activity, although never to the same degree as in the alveoli. This first appears very soon after the establishment of the second pregnancy, therefore within about 3 days after the commencement of lactation. 'Finger-like' papillae, which increase the secretory surface and are centres of secretory activity, appear in the duct walls, in particular those of the smaller branches by the time the embryos are in the blastula stage. After pregnancy *c* 1, when lactation occurred while the animal was in anoestrus, 'finger-like' papillae were first apparent in the glands of an animal killed on the fourth day post-partum. This may give an indication of the time lapse between parturition and the time when the embryos of the next pregnancy are in the blastula stage.

This state of full lactation is maintained until the embryos of the second pregnancy are about 5 mm long, after which the glands become thinner, and the diameters of the lumina of the alveoli are reduced somewhat. The formation of cytoplasmic buds in the type 1 alveoli is less vigorous, but a new feature, i.e. the sloughing off of whole epithelial cells, is now exhibited by these alveoli. The amount of secretion in the lumina of these alveoli is reduced, in fact many of the alveoli are almost devoid thereof. However, the secretion in the type 2 alveoli and ducts has become very dense and there are occasional lymphocytes therein.

There are distinct signs of involution by the time the embryos are nearly 8 mm long, and the mammary glands show progressive stages of involution from this time until the embryos of the second pregnancy are 32·6 mm long, when the mammary gland measures only 1 mm in thickness. An increase in the amount of adipose tissue present in the gland is first apparent when the embryos are about 8 mm long. The invasion of adipose tissue proceeds along the same lines as after pregnancy *c* 1, but it does not progress as far in this case, i.e. during the second pregnancy. When the invasion of adipose tissue is first apparent strips of this tissue separate the individual lobules. By the time a length of 32·6 mm is attained, the invasion of adipose tissue is complete, comprising at least a third of the gland. The lobules no longer have a definite, compact form. Besides the wide strips of adipose tissue between the lobules, there are small islands of this tissue interspersed amongst the alveoli.

The two types of alveoli persist in the involuting mammary glands until the embryos are 29 mm long, but alveoli intermediate between the two main types appear before this stage. By the time the embryos are 21 mm long at least half of the total number of alveoli are of this intermediate type. At this same stage a few of the alveoli are without a lumen, and there are signs of an equalization of the epithelial heights in both main types of alveoli; the cells lining the type 1 alveoli are somewhat lower, whilst there is an increase in the height of the cells in the type 2 alveoli. By the time the length of the embryos is 29 mm the alveoli are all of the same type, and have a very reduced lumen. This reduction in luminal size continues, so that, by the end of this period of involution, i.e. when the embryos measure 32·6 mm, the lumen is reduced either to a mere slit, or else remains circular, but has an average diameter of only 3·4μ. By this stage at least half of the alveoli have no lumen at all. Similar changes were noted after pregnancy *c* 1.

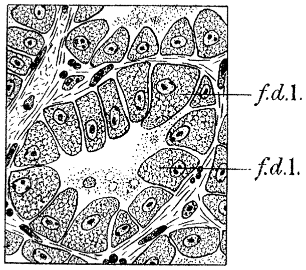


FIGURE 9. Alveoli of a gland from an animal in the second pregnancy with embryos 29 mm long. The epithelium is tall, and the cytoplasm riddled with small fat droplets. (Magn. × 420.) *f.d.* 1, fat droplet in the cytoplasm of a cell.

As involution proceeds there is a steady increase in the size and number of fat droplets in the alveolar epithelium, in particular in the type 1 alveoli. This is first apparent when the embryos are about 8 mm long. A similar increase occurred after pregnancy *c* 1. There is also an increase in the proportion of fat in the secretion at this stage, and it forms a predominant constituent.

The size of the fat droplets in the epithelium increases until the embryos are 17 mm long, when many of the droplets are three times as large as the nucleus. In these cells there appears to be as much, if not more, fat than cytoplasm. After this stage the droplets become smaller, but the fat content does not decrease immediately. Shortly before the end of this involution when the embryos are 29 mm long, the cytoplasm of the alveolar epithelium is riddled with small fat droplets, giving the cells a highly vacuolated appearance in these preparations (figure 9).

Meanwhile the boundaries of the cells lining the alveoli and ducts become very indistinct, and the cells show signs of disintegration after the embryos reach a length of 17 mm.

As involution proceeds there is a definite decrease in the rate of cytoplasmic bud formation, and it has ceased completely by the time the embryos are 17 mm long. The sloughing off of epithelial cells, a feature of involuting mammary glands, continues until the alveolar lumina have become very small. This, however, is not a true secretory process.

As previously mentioned, fat is the predominant constituent of the secretion when the embryos are 8 mm long. At this stage the type 2 alveoli are still more or less filled with secretion. There is a distinct decrease in the amount of secretion in these alveoli once the embryos are 20 mm long. Soon after this the secretion becomes very scanty. Meanwhile the composition of the secretion is also changing. Disintegrating nuclei from the sloughed off epithelial cells are most plentiful when the embryos are about 17 mm long. Lymphocytes, which first appear in significant numbers at the beginning of involution, are also fairly common at this stage. The number of cytoplasmic buds rapidly decreases, and by the time the embryos are 21 mm long practically all the cytoplasmic buds have disintegrated. Once the alveolar lumina become very small the secretion therein is reduced, and consists merely of granular material.

The ducts decrease in size throughout involution, and by the time the embryos are 29 mm long the duct lumen is very small, and either slit-like or convoluted. Meanwhile the thickness of their collagenous sheath increases, finally becoming two to four times as wide as the epithelial layer of the ducts. By this stage the epithelium of some of the ducts has become stratified. Changes in the ducts as regards increase in fat content of the epithelium and the cessation of secretory activity resemble those in the alveoli, as also does the rate of decrease in the amount of secretion present. ‘Finger-like’ papillae have disappeared by the time the embryos are 17 mm long.

The final state of involution is found in the glands of an animal with embryos 32·6 mm long (figure 15, plate 1), and it results in a condition closely resembling the state of development found in glands from animals with embryos of this size in the first pregnancy.

The mammary glands of *Elephantulus* have started developing again in preparation for lactation by the time the embryos of the second pregnancy are 36 mm long. These glands resemble those at the same stage of the first pregnancy very closely. The only notable changes in these glands from animals in their second pregnancy are that the total thickness of the gland is somewhat greater, the alveolar lumina are slightly larger, and the ducts already show the first signs of the development of ‘finger-like’ papillae. This slightly more developed condition in the second pregnancy is possibly due to the fact that, having recently passed through one period of development and lactation, the glands are more predisposed to development.

From the 36 mm embryo stage until partus the glands follow the same main course of development as did those at the corresponding stages of the first pregnancy.

B2 (ii). *Lactation and involution following a second pregnancy.* After the second pregnancy the sequence of events taking place during lactation and involution is determined by the time of the breeding season. If the second pregnancy has occurred at the end of the season (case *b* 2) the animal then goes into anoestrus, and the periods of lactation and involution proceed without any complications and resemble those occurring after pregnancy *c* 1.

If, on the other hand, the second pregnancy has occurred either at the beginning or during the middle of the breeding season (cases *c* 2 and *a* 2), a third pregnancy follows. Under these conditions a state of full lactation is established during the 2-day post-partum period between the two pregnancies. The stages in the establishment of lactation are the same as those following a first pregnancy. The remainder of the period of lactation, and all of the involution occur during the third pregnancy. Therefore changes taking place during these stages are shown by the glands from animals in their third pregnancy.

B2 (iii). *Third pregnancy* (*cases* a3 *and* c3). During the first part of this pregnancy the young from the second pregnancy are being suckled. The structure of these lactating glands is similar to that of the corresponding stages of the second pregnancy when the young from the first pregnancy were being suckled. The slight variations which occur are of no real significance. It is not until the young are weaned, and involution sets in, that any notable deviations are observed between the glands of the second and third pregnancies.

Involution is completed sooner after the second period of lactation than after the first. Until the embryos of the third pregnancy are 12 mm long the involution is a gradual process, then between this stage and that when the embryos are 13·7 mm long it is extremely rapid.

The initial stages of involution are first apparent when the embryos are 4·63 mm long. This gland closely resembles that from an animal with embryos of a similar size in the second pregnancy. The latter was the gland which first showed involuting conditions after the first period of lactation. Therefore involution, and so weaning, occurs at the same stage after the first and second periods of lactation.

After the second period of lactation the most advanced state of involution is found when the embryos are 13·7 mm long. This is very much earlier than after the first period of lactation, when the most advanced involution was attained only by the time the embryos of the second pregnancy measured 32·6 mm. Actually this involution follows the same general course as that occurring during the second pregnancy except that, first, certain changes are accelerated, and secondly, there are some which are retarded.

The most notable of these exceptions falling in the first category, i.e. showing acceleration, are the changes in the alveolar types. Alveoli, intermediate between the two main types, make their appearance almost immediately. They are definitely present by the time the embryos are 5·5 mm long, whereas, after the first period of lactation, they did not appear until the next pair of embryos was nearly 20 mm long. Similarly, the equalization of the epithelial heights of the two main types of alveoli is initiated earlier, and starts when the embryos are 12 mm long, as opposed to 21 mm after the first period of lactation. The decrease in size of the luminal diameters is also more rapid, and lumenless alveoli occur by the time the length of the embryos is 13·7 mm; after the first lactation they did not occur until the embryos had attained a length of 21 mm.

The decrease in the rate of secretory processes, in particular cytoplasmic bud formation, commences at the very beginning of involution after both the first and second periods of lactation, but in the latter cytoplasmic bud formation ceases completely at an earlier stage than in the former, i.e. at an embryonic length of 13·7 mm as compared with 17 mm. The sloughing off of whole or portions of epithelial cells is a feature common to both periods of involution.

The sequence of changes in the composition of the secretion, retained in the alveoli and ducts, does not differ notably. In the second period of involution the type 2 alveoli are most congested and distended when the embryos are 10 mm long; thereafter there is a decrease in the amount of retained secretion. In the first period of involution this decrease only occurs when the embryos are about 20 mm long.

Besides these changes which show a definite acceleration there are two processes which are apparently retarded. Both concern the fat content of the gland. First, the increase in the amount of fat present in the alveolar epithelium is not striking until involution is complete. After the first period of lactation it was apparent as from the start of involution. Secondly, the invasion of adipose tissue is not obvious before the final stage of involution, whereas it progressed steadily from the beginning of the first period of involution.

As previously stated, the most involuted mammary glands occurring in this pregnancy are found when the embryos are 13·7 mm long. This is very precocious as compared with the second pregnancy. To achieve this the changes taking place between the stages with embryos measuring 12 and 13·7 mm are extremely rapid. Most notable of these is the invasion of adipose tissue, which is first apparent when the embryos are 12 mm long, and progresses so that half of the total area of the gland is comprised of adipose tissue by the time the embryos are 13·7 mm. In the second pregnancy, at the end of the first period of involution, only a third of the total area consisted of adipose tissue. Therefore, although this invasion of adipose tissue is retarded initially during the third pregnancy, once it occurs it proceeds further and more rapidly.

Besides this invasion of adipose tissue being more pronounced, the whole structure of the gland shows a more involuted condition than is achieved at the end of the first period of involution (compare figures 15 and 16, plate 1). The amount of collagenous stroma around and between the alveoli shows a marked increase. When present the alveolar lumina are smaller, and there is also a higher proportion of lumenless alveoli, many of which are reduced to a mere cluster of epithelial cells (figure 16). Moreover, epithelial cells scattered haphazardly in the stromal tissue are more common. The fat content of all these epithelial cells, regardless of whether they are organized into alveoli or not, is lower.

The structure of the ducts does not exhibit such marked differences.

By the time the embryos of the third pregnancy are 16 mm long the glands are developing once more. Development proceeds along the normal lines until partus.

B2 (iv). *Lactation and involution following the third pregnancy*. *Elephantulus* usually only has three pregnancies during its lifetime. At the end of the third pregnancy the ovary may not be completely exhausted, but still retain a few follicles. These may be sufficient for the animal to survive a second period of anoestrus. Therefore, if the third pregnancy occurs at the end of the breeding season, the animal then goes into anoestrus. However, if the third pregnancy occurs at any other time of the breeding season the few follicles left after another ovulation are only sufficient for the early stages of the fourth pregnancy. It appears that *Elephantulus* does not live beyond the completion of its sexual life. Therefore, if a fourth pregnancy occurs, the animal will die of old age during this pregnancy (van der Horst 1946).

In the mammary glands studied from animals going into anoestrus after the third pregnancy (case *a*3), the periods of lactation and involution are the same as those after any other pregnancy which occurred at the end of the breeding season.

Very little material was obtained to show the sequence of changes in the mammary glands following a third pregnancy which occurred at the beginning or during the middle of the breeding season (cases *b*3 and *c*3). The few glands studied from animals in their fourth pregnancy indicate that the period of lactation overlapping with the fourth pregnancy is the same as those overlapping with either a second or a third pregnancy.

There was no material which showed the stages of involution following the third period of lactation when there was a concurrent pregnancy. This lack of material may be attributed to the fact that, if a fourth pregnancy occurs, the animal usually dies of old age after the early stages of the pregnancy, before involution of the mammary glands is established. Moreover, many animals succumb to the dangers of their environment before they reach this age.

IV. Discussion

The lactation cycle of *E. myurus jamesoni* is more complicated than in most mammals. This is due to the fact that an animal may have three pregnancies during a breeding season and, except at the end of the season, there is no lactation anoestrus. Therefore, in many cases, the lactation and the involution following one pregnancy overlap with the subsequent pregnancy. It is this that complicates the cycle.

(1) *The virgin condition of the mammary glands*

In a virgin animal, at the time of the first oestrus, the mammary glands are represented merely by a thickened area of adipose tissue lying directly under the skin. Scattered in this adipose tissue are accumulations of cells which will give rise to alveoli during the first pregnancy. The ducts do not extend much beyond the nipple region. All are lined with stratified epithelium, and have a thick investment of collagenous tissue. The lumina are small, and their shape irregular.

(2) *Changes occurring in the mammary glands when an animal has its first pregnancy at the end of the breeding season (case* c 1*).*

In this case, the changes taking place in the mammary glands are not complicated by either a previous or a subsequent pregnancy (graph 1).

A. *The development during the pregnancy*

As from the time when the embryos are in the primary amniotic cavity stage of the first pregnancy, there is a steady development of the duct system of the glands. In the rat, proliferation of the ducts is initiated only on the fourth day of pregnancy (Astwood *et al.* 1937), but it starts sooner in the mouse (Cole 1933), where development of the main duct in the nipple region commences after 24 hours of pregnancy. In the latter animal after 2 or 3 days of pregnancy, buds appear along the smaller ducts, and 6 days after copulation there is a definite formation of alveoli (Cole 1933). These buds are comparable to the clusters of epithelial cells which appear around and just ahead of the developing ducts in *Elephantulus* by the time the embryos are in the primary amniotic cavity stage.

In *Elephantulus* alveolar development is very similar to that in the rat, where alveoli first develop on the twelfth day of pregnancy. These alveoli are without a lumen. From this day, until parturition, there is a rapid proliferation of alveoli, as well as the appearance of lumina in the established alveoli. This proliferation of alveoli results in the formation of definite lobules (Astwood *et al.* 1937). However, in *Elephantulus* alveoli first occur when the crown-rump length of the embryo is 8 mm, lumina appear by the time the embryos have attained a length of 10 mm, and these alveoli are lined with a single layer of columnar epithelium. Definite lobules are found once the embryos are 30 mm long.

As this development of glandular elements takes place in *Elephantulus*, the adipose tissue becomes replaced by glandular tissue. There is a similar replacement in the developing mammary glands of the mouse (Cole 1933). However, it is not until lactation commences that the glandular tissue is in excess of the adipose tissue in the glands of this animal, therefore the replacement is somewhat slower than in *Elephantulus*, where this excess is found by the time the embryos are 30 mm long.

Fat droplets first appear in the cells of the alveolar epithelium when the embryos are 30 mm long. Henceforth, until parturition, fat becomes more plentiful, and the droplets larger. Droplets do not appear in the duct epithelium until near full term.

At the same time as fat droplets first appear in the alveolar epithelium, i.e. when the embryos are 30 mm long, the epithelium lining the ducts loses its stratified condition, and henceforth the ducts are lined with a single layer of epithelium, which varies from cuboidal to columnar. In *Mus norvegicus albinus* (Maeder 1922) the transition from stratified epithelium to a single layer of epithelium in the ducts does not occur until the last days of pregnancy.

Secretional activity of an apocrine type commences shortly before the end of the pregnancy, slight activity being apparent when the embryos are 39·8 mm long. At first the secretion present in the alveolar lumina consists of an amorphous, granular substance, but other constitutents are soon added. Fat droplets are the first bodies secreted by the cells of the alveoli, but within a short period cytoplasmic buds are also formed. Thereafter the rate of secretory activity steadily increases. Concurrent with this, the lumina of the alveoli enlarge and are filled with secretion, whilst the epithelium becomes flatter, so that it is of a cuboidal type. Meanwhile the ducts have enlarged and they also contain secretion.

This final condition is shown in figure 4, and is found in the glands by the time the embryos have reached full term. The mammary glands of these animals are fully prepared for lactation, once suckling commences.

B. *Lactation*

After partus, as soon as the mammary glands are stimulated by suckling, a state of full lactation is established, with the rate of secretory activity increasing enormously as compared with the rate at the end of pregnancy.

Unfortunately, there was not sufficient material available to give a comprehensive picture of the course of lactation in this case, when it occurs while the animal is going into anoestrus. The only stages studied were early and late post-partum, 4 and 7 days post-partum. A more detailed picture was presented by the material showing the changes which occur when the animal's first period of lactation overlaps with the second pregnancy. This will be discussed in a later section.

The main features noted during this period of lactation, which occurs during anoestrus, are as follows: The establishment of a state of full lactation is characterized by a marked reduction in the amount of adipose tissue present, particularly between the lobules, which soon merge, so that they are demarcated merely by a thickened septum of collagenous tissue. As a result, the glandular area is comprised almost exclusively of glandular tissue. A similar condition is reported in the albino rat, *Mus norvegicus albinus*, by Maedar (1922).

As well as becoming more compact, the gland of *Elephantulus* also increases in thickness. At full term the thickness was 1·8 mm, but by early post-partum it has increased to 2·8 mm.

With the establishment of lactation, the alveoli differentiate into two definite types, i.e. type 1 alveoli, which are lined with columnar epithelium and have a small lumen, and type 2 alveoli, in which the epithelium is flattened and the lumen large and filled with secretion. These two types of alveoli are distinct by the late post-partum stage, and it appears that, as lactation proceeds, the proportion of type 2 alveoli increases.

The glands taken from animals during this period of lactation show that the chief constituents of the milk are fat droplets and cytoplasmic bodies, both of which are secreted mainly by the alveolar epithelium. Either fat droplets or granular material predominate, but no consecutive order was found for the way in which this varies. The amorphous, granular material present in the secretion is, apparently, formed by the disintegration of the cytoplasmic bodies. In earlier stages, before cytoplasmic bodies are secreted by the alveolar epithelium, the granular material present in the lumina is, most likely, formed by the disintegration of portions of the apical cytoplasm of the epithelial cells.

Once a state of full lactation is established, the ducts of the mammary glands also become distended, and the epithelium thereof exhibits a secretory function, but never as vigorous as in the alveoli. By 4 days post-partum 'finger-like' papillae have developed in the duct walls. These papillae increase the secretory surface of the ducts and constitute centres of secretory activity. It is apparently normal for the ducts to have a secretory function, and this is reported as also occurring in *Mus norvegicus albinus* by Maeder (1922), and in the mouse by Cole (1933). In the mouse the ducts develop 'finger-like' papillae and secrete actively as from the seventh day of lactation which is somewhat later than in *Elephantulus*.

C. *Involution*

In this case, when the first pregnancy occurs at the end of the breeding season, the involution proceeds without the glands being stimulated to further development by a concurrent pregnancy. Unfortunately, there were no data as to exactly how long after weaning these glands were obtained. Glands taken from three animals caught during the month of February show the early stages of involution, whilst later stages of involution are exhibited by glands from animals caught during June, July and August.

The three early stages of involution are marked by a rapid decrease in the thickness of the gland, and an invasion of adipose tissue, which results in the lobules being reduced to small, irregular clusters of alveoli. An invasion of adipose tissue is a feature typical of involuting mammary glands, and occurs in the bat (Jeffers 1940), the mouse (Cole 1933; Lane Williams 1942) and the guinea-pig (Hesselberg & Loeb 1937). In the case of the rat the amount of connective tissue equals that of glandular tissue by the fourth day post-weaning, and thereafter is in excess (Maeder 1922). This excess also occurs in *Elephantulus*.

Another striking characteristic of the early involution is the decrease in the size of the lumina of the alveoli, which ultimately results in the alveoli being reduced to either solid clusters of cells, or there may be an extremely small lumen present. In the rat (Maeder 1922), the alveolar collapse is initiated on the third day after weaning, and by the eighth day the lumina are either very small, or absent. Simultaneously the epithelium lining the alveoli undergoes changes. First it becomes uniformly cuboidal. Thereafter many of the cells show signs of disintegration and, as in the case of the mouse (Lane Williams 1942), the guinea-pig (Hesselberg & Loeb 1937), and in the rat (Jeffers 1935), some of these cells slough off into the lumina. Moreover, there is, during involution, an initial increase in the fat content of the epithelial cells, followed by a decrease, resulting in the complete absence of fat droplets.

Secretory activity apparently ceases very rapidly once the young are weaned. Cytoplasmic bud formation does not occur at all, but in the earliest stage of involution occasional fat droplets are still being discharged from the alveolar epithelium. In this gland, although the majority of the alveoli are filled to half their capacity, the lumina are reduced in size as compared with those during lactation. Therefore, seeing their capacity is so much less than at the end of lactation, secretion must have been removed from the alveoli since the time of weaning. If this were not so the alveoli would, in fact, be even more distended than during lactation, seeing the secretion would be augmented due to the slight secretory activity still taking place in the gland. However, I could find no indications as to the method by which the secretion is removed. Neither was Maeder (1922) able to ascertain how the secretion was removed in *Mus norvegicus albinus*. He suggested that the removal was possibly effected by simple absorption. Maximow & Bloom (1930) agree with this theory, in the case of the human. The same explanation may apply for *Elephantulus*. As a result there is a rapid decrease in the amount of secretion present in the alveoli. This secretion is comprised merely of disintegrating epithelial cells, and a little granular debris formed therefrom.

The various stages in the involution of the ducts do not all occur concurrently with their counterparts in the alveoli. The reduction in the size of the lumina is delayed in the ducts. It ultimately results in the duct lumina becoming small and convoluted, by which stage there is practically no secretion therein. This process is accompanied by an accumulation of fibrous tissue around the ducts, a feature also found in the involuting mammary glands of the mouse (Cole 1933). However, the cessation of secretory activity and changes in the composition of the secretion in the ducts occur concurrently with those in the alveoli. 'Finger-like' papillae are not present in any of the involuting glands.

The final stages of complete involution are shown by the glands from animals caught during the months of June, July and August, and result in a condition slightly more developed than that occurring at the time of the first oestrus (compare figures 14 and 15, plate 1). The lobules are reduced to small strips, lying in a comparatively vast area of adipose tissue, but are larger and contain more dense collagenous tissue than in the virgin gland. Moreover, epithelial cells, both scattered singly and in the form of clusters, which represent the remnants of alveoli, are more plentiful in this collagenous stroma than in the virgin gland. The involuted condition resembles that in the mouse (Cole 1933; Lane Williams 1942) and the bat, *Myotis grisescens*, in which animal it is also found that compact clusters of epithelial cells are more numerous in the involuted glands than at the beginning of the pregnancy (Jeffers 1940).

In the involuted mammary glands of the mouse the ducts are slightly thicker and more branched than in a virgin animal (Lane Williams 1942). In *Elephantulus* the ducts are not strikingly thicker, but there are definitely more branches.

Actually these involuted glands are very similar to those showing the slight development which occurs when the embryos are in the primary amniotic cavity stage of the first pregnancy.

At the end of the period of anoestrus the involuted condition is similar in the glands of all parous animals, regardless of whether the animal has had one or two pregnancies during the previous breeding season. When these animals again fall pregnant at the beginning of the next breeding season (cases *c* 2 and *b* 3), development of the mammary glands commences from the slightly developed condition which is found at the end of the period of involution.

(3) *The cycle in the mammary glands when pregnancy and lactation overlap*

In this section all cases, except case *c* 1, are considered. In these cases the changes in the mammary glands are complicated at some stage or other. Either only the lactation and involution may be complicated due to the onset of another pregnancy (cases *a* 1, *b* 1, *b* 3 and *c* 2), or the effect of the developing embryos may be masked due to the fact that the pregnancy overlaps with the lactation resulting from the previous pregnancy (cases *b* 2 and *a* 3). Finally, both complications may occur (cases *a* 2 and *c* 3). However, the lactation and involution following a third pregnancy present a special case since a fourth pregnancy may follow, but the animal usually dies of old age before the involution is completed.

A. *Cases in which the animal's first pregnancy occurs at the beginning or during the middle of the breeding season (cases* a 1 *and* b 1*)*

During these two pregnancies the course of development of the mammary glands is the same as that during a first pregnancy occurring at the end of the breeding season (case *c* 1). This has already been considered. After parturition there is a post-partum period of approximately 2 days before the next pregnancy sets in, and during this period a state of full lactation is established. These stages in the establishment of lactation resemble the corresponding stages after pregnancy *c* 1. However, the remainder of the period of lactation and all the subsequent involution overlap with the next pregnancy. Therefore the structure of the glands during these periods is dealt with when second pregnancies are considered.

B. *Second and third pregnancies*

B1. *Second and third pregnancies occurring at the beginning of the breeding season (cases* c 2 *and* b 3). These animals have just come out of anoestrus and their glands show the slightly developed condition which is the final result of involution. This development corresponds to that achieved by the time the embryos of the first pregnancy are in the primary amniotic cavity stage. However, once the embryos of these second and third pregnancies have reached the primary amniotic cavity stage, the development of the glands proceeds along the same lines as during a first pregnancy.

The periods of lactation and involution following these second and third pregnancies resemble those occurring after second and third pregnancies which take place during the middle of the breeding season. Therefore they will be discussed in the next section.

B2. *Second and third pregnancies occurring during the middle and at the end of the breeding season* (*cases* a2, b2, a3 and c3). In these cases the changes taking place in the mammary glands during the actual pregnancy are determined by the pregnancy of the individual animal, and not the time of the breeding season. It is only after parturition that the time of the breeding season is important due to the fact that, after pregnancies *a*3 and *b*2, the animals go into anoestrus, whilst after *a*2 and *c*3 there may be another pregnancy.

Since these pregnancies overlap with the periods of lactation and of involution following the previous pregnancies, the glands will show the effects of lactation and involution of a previous pregnancy on the development of the glands during the next pregnancy, and thereafter the development stimulated by the growth of the embryos of the actual pregnancy. Moreover, the size of these embryos gives an indication of the duration of lactation and involution of the glands as related to the previous pregnancy.

(*a*) *Second pregnancy* (*cases* a2 *and* b2). An account of the changes occurring during these stages has already been given. From a study of the glands in which the first period of lactation takes place concurrently with the second pregnancy, some features of interest emerge.

First, the state of full lactation appears to reach a peak when there are one to four cell stages in the embryo chamber, but it is maintained until the embryos of the second pregnancy are about 5 mm long. Throughout this period of lactation, fat droplets are constantly extruded from the epithelium of the alveoli. This results in there being less fat droplets in the cytoplasm of this epithelium than there are in the late stages of the first pregnancy. Jeffers (1940), working on the bat, *Myotis grisescens*, reports a similar reduction at term and during lactation, as compared with the latter half of pregnancy. She found that frequently there was only a large, apical droplet in each cell. This resembles the condition in *Elephantulus*.

Throughout lactation the two types of alveoli, i.e. the large-lumened type 2 alveoli, and the smaller type 1 alveoli, are present. When lactational activity is at its zenith the lumina of both types of alveoli are enlarged, while the epithelium is flattened, in particular that of the type 2 alveoli. Hesselberg & Loeb (1937) report that, in the guinea-pig, the more distended the alveoli, the flatter the epithelial cells thereof, due to pressure exerted by the secreted material. The flattening of the alveolar epithelium in this animal and in *Elephantulus* must be due to the same reason, but as to whether the pressure of the secretion actually flattens the epithelium, or merely distends the alveoli, and this in turn stretches the epithelial cells and so lowers their height, is a debatable but apparently insolvable point.

During the greater part of the period of lactation the type 2 alveoli are in excess of the smaller, type 1 alveoli, which are found to occur mainly down the inner edge of the gland. In the mouse, Lane Williams (1942) reports that, during full lactation, the alveolar epithelium is very flat, but he makes no mention of there being any smaller alveoli with columnar epithelium present in these glands. However, Cole (1933), also working on the mouse, notes that the alveoli on the inner surface of the gland are somewhat smaller; they have higher epithelium and contain less secretion than do alveoli more deeply placed. This agrees with the condition found in *Elephantulus*, as also do the conditions found in the mammary glands of *Mus norvegicus albinus* by Maeder (1922), and in the guinea-pig by Hesselberg & Loeb (1937).

The point of controversy appears to be, not as to whether these smaller alveoli are present or not, but rather as to why the two types of alveoli occur. Maeder (1922) suggests that in *M. norvegicus albinus* these alveoli may constitute a layer of incompletely developed, reserve alveoli. In *Elephantulus* it seems very unlikely that they are incompletely developed, since they form the centres of cytoplasmic bud formation, and surely alveoli fulfilling this important function must be completely developed.

Hesselberg & Loeb (1937) think that these alveoli may be at an earlier stage of the secretory cycle. Jeffers also employs the theory of a secretory cycle to explain the occurrence of two types of alveoli in her work on the mammary glands of the albino rat (1935) and the bat, *Myotis grisescens* (1940), but she expounds it more fully. In her work on the albino rat she reports that there are cells in all stages of the secretory cycle in an actively secreting gland. In alveoli widely distended with milk the cells are cuboidal, and are at the end of one secretory cycle or the beginning of another. When a new cycle is established, a drop of fat forms in the region of the cytosome bordering on the lumen. The cells again become columnar while materials continue to accumulate therein, and when the alveoli collapse due to the removal of milk. In turn the products are discharged from these columnar cells, and their alveoli fill with secretion so that the lumina become distended, and the epithelial cells flatten again. This explanation would hold if the type 1 alveoli occurred in any position in the gland, since all alveoli must pass through the cycle. However, in *Elephantulus*, as previously mentioned, the type 1 alveoli are nearly always confined to a peripheral zone along the inner border of the gland. Moreover, the cells of these alveoli do not appear to be at the end of one secretory cycle or beginning of another because they are secreting cytoplasmic buds very actively. It seems more likely that, in *Elephantulus*, the alveoli of this zone become specialized for the formation of cytoplasmic buds, although fat droplets are also extruded. This activity is almost lost in the large-lumened alveoli, where the chief secretory product is fat droplets; the bulk of the cytoplasm secreted here is in the form of crescents enclosing these droplets.

From the material studied for this work, it appears that alveoli may either open directly into branches of the duct system, or connect with other alveoli. Therefore, in the case of type 1 alveoli, besides those which drain directly into ducts, there are others which open into type 2 alveoli (figure 10). Moreover, these type 2 alveoli may either drain into a duct, or lead into other alveoli of their type (figure 10). Of course it is practically impossible in some instances to distinguish between large alveoli and end-branches of the duct system. Thus it appears that secretion formed in an alveolus may either pass straightaway into a duct, or first pass through other alveoli before reaching the duct system. In the latter case cytoplasmic buds, as well as fat droplets, may be secreted in the type 1 alveoli, pass into the type 2 alveoli and accumulate there. In the meantime the secretion is enriched by the addition of more fat droplets and cytoplasm, mainly in the form of crescents, secreted by the epithelium of the type 2 alveoli. Possibly the greater part of the water content of the milk is also added in these type 2 alveoli. The fact that the cytoplasm in the buds and crescents has a more granular, disintegrating appearance in the milk contained in the type 2 alveoli than in the type 1 alveoli, suggests that the buds in the type 1 alveoli are more newly formed than those in the type 2 alveoli. This also supports the assumption that secretion formed in the type 1 alveoli may accumulate in type 2 alveoli. Maximow &

Bloom (1930) state that in the human the cellular fragments in the milk autolyze between the time when they are formed and when the milk is secreted from the glands.

The secretion, accumulated in the type 2 alveoli, passes into the ducts, which also become very distended.

As previously mentioned, the state of full lactation is maintained unaltered until the embryos are about 5 mm long. At this stage a distinct change occurs in the mammary glands. This change is most likely due to the cessation of suckling by the offspring. It has already been stated that the gestation period in *Elephantulus* is about 8 weeks (van der Horst 1946). Since it is estimated that at mid-term the embryos are roughly 5 mm long (van der Horst 1950), this change in the mammary glands, when the embryos are about 5 mm long, occurs after 4 weeks of pregnancy. Moreover, the young of *Elephantulus* are sexually mature within 5, or at the most 6 weeks after birth (van der Horst 1946), so it seems more than likely that suckling would definitely cease by the time the animals are 4 weeks old.

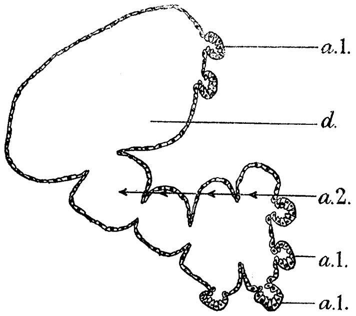


FIGURE 10. A diagram to illustrate how alveoli may either open directly into branches of the duct system, or connect with other alveoli. The diagram shows two type 1 alveoli draining into a duct, while others lead into type 2 alveoli. Moreover, these type 2 alveoli either drain into other alveoli of their type, or have a direct connexion with the ducts. This diagram is a composite drawing since these connexions would not all occur at the same level. *a* 1, type 1 alveolus; *a* 2, type 2 alveolus; *d*. duct.

Another point favouring the idea that the young are weaned by the time that the embryos of the next pregnancy are about 5 mm long is that the changes occurring in the glands at this stage resemble those occurring both during the involution following pregnancy *c* 1, and also in the glands of other animals at the time of weaning. The slight general contraction of the whole gland, and therefore a decrease in thickness, reported by Cole (1933) as occurring in the mouse 3 days after weaning, also occurs in *Elephantulus* when the embryos are about 5 mm long. The decrease in secretional activity reported in these glands occurs in the involuting glands after pregnancy *c* 1, as well as in the glands of the rat, 3 days after the cessation of suckling (Maeder 1922). Similarly, the sloughing off of cells of the alveolar epithelium, which is a feature apparent at this stage, appears to be a normal phenomenon of involution, and occurs after pregnancy *c* 1 as well as in the mouse (Lane Williams 1942), in the guinea-pig (Hesselberg & Loeb 1937) and in the rat as from the first day after weaning (Jeffers 1935).

It is difficult to estimate exactly how long after the cessation of suckling these glands from an animal with embryos 5 mm long were obtained, but it cannot be more than 1 or 2 days, since the majority of the alveoli are still somewhat distended with secretion.

A similar state occurs in the rat 2 days after weaning (Jeffers 1935), but in the mouse the alveoli have collapsed by this time (Cole 1933).

Involution proceeds until the embryos of the second pregnancy measure about 33 mm, when the most involuted condition found during this pregnancy occurs. The course of this involution has already been described. One of the main features noted is that there is an invasion of adipose tissue which, in the final stage, results in at least a third of the gland being comprised of this tissue. In the rat this invasion is more accentuated, and Maeder (1922) reports that in the involuting mammary glands of that animal the amount of connective tissue equals that of glandular tissue by the fourth day post-weaning, and thereafter is in excess.

During involution the size of the lumina of the alveoli is reduced, and there is an equalization of the epithelial heights of the two main types of alveoli, so that, eventually, the alveoli are all of the same type, with the lumen either extremely small or absent. Moreover, there is a steady increase in the fat content of the alveolar epithelium. Maeder (1922), working on the rat, states that the increase in size of the fat droplets in the epithelium is one of the first signs of involution. In *Elephantulus* the increase in the fat content is initially achieved by an enlargement of the fat droplets, but after the embryos have attained a length of 17 mm the droplets become smaller, and it is the number thereof that is increased. As a result, shortly before the end of this period of involution, the condition of cytoplasm of the alveolar epithelium resembles that found at this stage in the rat (Jeffers 1935) and the bat (Jeffers 1940), since it has become riddled with small fat droplets. However, in *Elephantulus*, the number of fat droplets is again reduced just before the involution is completed.

Meanwhile, the boundaries of the cells lining the alveoli and ducts become very indistinct, and there are signs of cellular disintegration after the embryos reach a length of 17 mm. In the mouse there are no cell boundaries visible from weaning onwards (Cole 1933), but in the rat, as in *Elephantulus*, cellular degeneration is delayed somewhat. In the rat it only occurs as from the fourth day of involution; after the sixth day it is difficult to distinguish cell boundaries (Jeffers 1935).

The initial decrease in the rate of secretory activity at the time of weaning, although not apparent in these preparations, must have been very marked. If this were not so, the alveoli would have soon become very greatly distended, since suckling no longer occurred. However, these preparations do show that cytoplasmic bud formation has ceased completely by the time the embryos are 17 mm long. In the mouse the cessation of secretory activity is more rapid, and none occurs after the first day post-weaning (Lane Williams 1942), but in the rat the condition resembles that in *Elephantulus* more closely, being more gradual, and there are still a few signs of secretory activity on the fourth day (Maeder 1922).

The amount of secretion present in the type 2 alveoli only exhibits a distinct decrease once the embryos have attained a length of 20 mm. Thereafter it becomes very scanty, and consists mainly of granular material. This granular material seems comparable to the type of secretion occurring in the mammary glands of the mouse on the twelfth day of involution (Cole 1933). In the mouse the period of glandular engorgement is very brief, and diminishes after 1 day (Lane Williams 1942), but in the rat there are still a few distended alveoli 5 days after weaning (Jeffers 1935). The latter resembles the condition in *Elephantulus*, where there is a gradual decrease in the amount of retained secretion, more closely than does the former.

However, the secretion in the type 1 alveoli decreases more rapidly. Initially both cytoplasmic buds and fat droplets are present, but by the stage when cytoplasmic bud formation has ceased, i.e. when the embryos have attained a length of 17 mm, these alveoli are practically devoid of secretion. By the time the embryos are 29 mm long, different types of alveoli can no longer be distinguished, and from this stage, until the end of involution, any secretion present in the alveoli is of the granular type described above.

Throughout involution the size of the ducts decreases, with the final result that the duct lumen is either slit-like, or convoluted. In the meantime the thickness of the collagenous sheath surrounding the ducts increases, finally becoming two to four times as wide as the epithelial layer of the ducts. In the mouse the reduction in the size of the ducts occurs 6 days after weaning, and this is accompanied by an accumulation of fibrous tissue around these ducts (Cole 1933).

The final state of involution, which is found in the glands from an animal with embryos 32·6 mm long (figure 15, plate 1), closely resembles the state of development occurring in the glands from animals with embryos of this size in the first pregnancy. Therefore, the extent to which involution proceeds is governed by the size of the developing embryos; the glands do not involute further than the state of development expected by the time the embryos have attained that particular size.

The mammary glands of *Elephantulus* have started developing again in preparation for lactation by the time the embryos of the second pregnancy are 36 mm long. From this stage until partus the course of development of the glands is the same as that found at the corresponding stages of the first pregnancy.

(*b*) *Lactation and involution following a second pregnancy*. When the second pregnancy occurs at the end of the breeding season (case *b* 2) the periods of lactation and involution following this pregnancy occur during anoestrus, and resemble those following pregnancy *c* 1. If, however, the second pregnancy has occurred either at the beginning of the breeding season (case *c* 2), or during the middle of the season (case *a* 2), a third pregnancy is initiated within approximately 2 days. In these cases the stages in the establishment of full lactation during the 2 days post-partum period are the same as those following a first pregnancy. The remainder of the period of lactation, and all of the subsequent involution, occur during the third pregnancy, and are therefore considered in the next section.

(*c*) *Third pregnancy* (*cases* a 3 *and* c 3). The young from the second pregnancy are being suckled during the first part of this pregnancy. The structure of these lactating glands is essentially similar to that of the corresponding stages of a second pregnancy, when the young from the first pregnancy are being suckled. It is not until the young are weaned, and involution sets in, that any significant differences are apparent between the glands of the second and third pregnancies.

Involution is initiated at the same stage, i.e. when the embryos are approximately 5 mm long, during both the second and third pregnancies. At this stage the glands from the two pregnancies are similar. However, the most advanced involution occurs sooner after the second period of lactation than after the first; in the former case it is when the embryos of the third pregnancy are 13·7 mm long, while in the latter it occurs only when the embryos of the second pregnancy have attained a length of 32·6 mm. In both cases involution follows the same general course except that, during the second period of involution, certain changes are accelerated, while others are retarded as compared with the first period of involution.

The main features which show an acceleration are the decrease in size of the lumina of the alveoli, the slowing down and cessation of secretory processes and, lastly, the reduction in the amount of secretion retained in the alveoli and ducts. The course followed by these changes is essentially similar to that in the second pregnancy; it is merely that they are more rapid.

There are only two processes which are apparently retarded. These both concern the fat content of the gland. First, the increase in the amount of fat present in the alveolar epithelium is not striking until involution is completed. Secondly, the invasion of adipose tissue is only apparent in the final stages of involution.

As already stated, the most involuted condition occurring during the third pregnancy is found when the embryos measure 13·7 mm. The whole structure of the glands from animals at this stage presents a more involuted condition than is achieved at the end of the first period of involution (compare figures 15 and 16, plate 1). This more pronounced involution can be attributed to the fact that apparently the extent to which involution proceeds is dependent on the stage reached by the developing embryos. After the second period of lactation complete involution occurs when the embryos of the third pregnancy are 13·7 mm long, whereas after the first period of lactation it does not occur until the embryos of the second pregnancy are 32·6 mm long. The glands would naturally be less developed when the embryos are only 13·7 mm long than when they are 32·6 mm long.

It was not possible to compare this involuted mammary gland after the second period of lactation with a gland from an animal with embryos of the same size in the first pregnancy, since no material of this stage was available. However, the involuted gland presents a condition fairly similar to the stage of development reached when the embryos of the first pregnancy are 10 mm long, i.e. when there has been no concurrent lactation nor involution. The development would not progress much between this stage of the first pregnancy and that when the embryos attain a length of 13·7 mm. This similarity also supports the presumption that the size of the developing embryos governs the extent of involution.

By the time the embryos of the third pregnancy are 16 mm long the glands are developing once more. Development proceeds along the normal lines until partus. When the embryos are 33 mm long the gland is essentially similar to that showing the final stages of involution during the second pregnancy, i.e. the stage with 32·6 mm embryos. Therefore, again in the case of the second pregnancy, this is further evidence in favour of the view that, when involution following one pregnancy overlaps with the next pregnancy, the size of the developing embryos limits the extent to which the involution proceeds.

(*d*) *Lactation and involution following the third pregnancy*. At the end of the third pregnancy the ovary may still retain a few follicles, which are sufficient for the animal to survive either another period of anoestrus (case *a* 3), or the early stages of a fourth pregnancy (cases *b* 3 and *c* 3). In the former (case *a* 3), the periods of lactation and of involution are the same as those occurring during anoestrus after any other pregnancy at the end of the breeding season.

However, when a fourth pregnancy follows, it appears that the animal usually dies of old age after the early stages of this pregnancy. Therefore, although lactation occurs, the animal dies before involution is established. The few glands available indicate that the period of lactation overlapping with the fourth pregnancy is the same as those overlapping with either a second or a third pregnancy.

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Description of Plate 1

Figure 11. Photomicrograph of a section of a gland from a virgin animal at the time of the first oestrus. Stained with Mallory's triple stain, and photographed through a Wratten no. 11 green filter. Note the early stages of the branching of the main duct, and the accumulations of cells in the connective tissue around the duct. (Magn. × 100.)

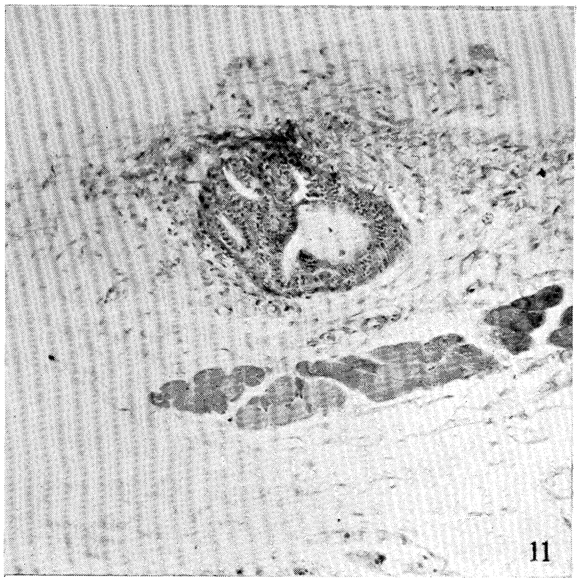
Figure 12. Photomicrograph to show the structure of a gland in full lactation. Stained with Hansen's haematoxylin and eosin, and photographed through a Wratten no. 11 green filter. Note the two types of alveoli, the smaller type 1 alveoli along the edge of the gland at the left lower corner, and the larger type 2 alveoli lying within the body of the gland. On the right-hand side of the photograph there is a portion of a large duct. (Magn. × 100.)

Figure 13. Photomicrograph of a portion of another gland in a state of full lactation. Stained with Hansen's haematoxylin and eosin, and photographed through a Wratten no. 11 green filter. Again note the two types of alveoli. In this gland the lumina of the type 1 alveoli are somewhat larger than those in figure 12. (Magn. × 100.)

Figure 14. Photomicrograph of a portion of a gland showing the final stage of involution during anoestrus. Stained with Hansen's haematoxylin and eosin, and photographed through a Wratten no. 11 green filter. In the centre of the photograph there is an artery, and to the left thereof a duct, the lumen of which is very reduced. Note that the glandular tissue is reduced to small islands, scattered in a comparatively vast area of adipose tissue. These islands consist of dense collagenous tissue in which numerous epithelial cells are scattered. Portions of two of these islands are situated at the upper margin of the photograph. Other islands are shown lying across the middle of the field. (Magn. × 100.)

Figure 15. Photomicrograph of a portion of a gland from an animal in its second pregnancy when the embryos are 32·6 mm long. Stained with Hansen's haematoxylin and eosin, and photographed through a Wratten no. 11 green filter. This shows the most advanced state of involution occurring after the first period of lactation when this and the second pregnancy overlap. Note the invasion of adipose tissue resulting in the shape of the lobules being irregular. Many of the alveoli are without a lumen. On the right, in the lower corner, there is a duct, the lumen of which is rather reduced and contains very little secretion. (Magn. × 100.)

Figure 16. Photomicrograph of a portion of a gland from an animal in its third pregnancy, when the embryos are 13·7 mm long. Stained with Hansen's haematoxylin and eosin, and photographed through a Wratten no. 11 green filter. This shows the most advanced state of involution occurring after the second period of lactation when it overlaps with the third pregnancy. Again note the invasion of adipose tissue, which here reduces the lobules to mere clusters of alveoli. In approximately the centre of the photograph there is a duct, the lumen of which is reduced and convoluted. The duct is surrounded by a wide area of collagenous tissue. The irregular clusters of reduced alveoli, the lumina of which are either small or absent, lie to the left and right of the duct. There is a moderately wide sheath of collagenous tissue surrounding each alveolus, and in it there are a number of epithelial cells scattered around the organized epithelial layer of the alveolus. Clusters of epithelial cells also occur in the collagenous tissue, and these are the remnants of retrogressed alveoli. (Magn. × 100.)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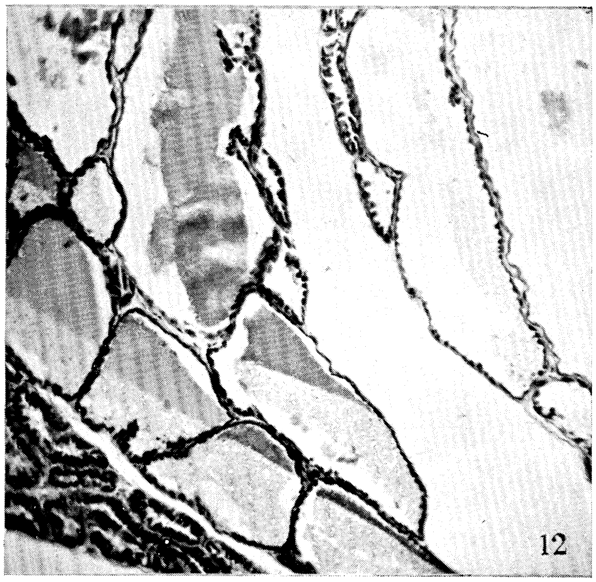
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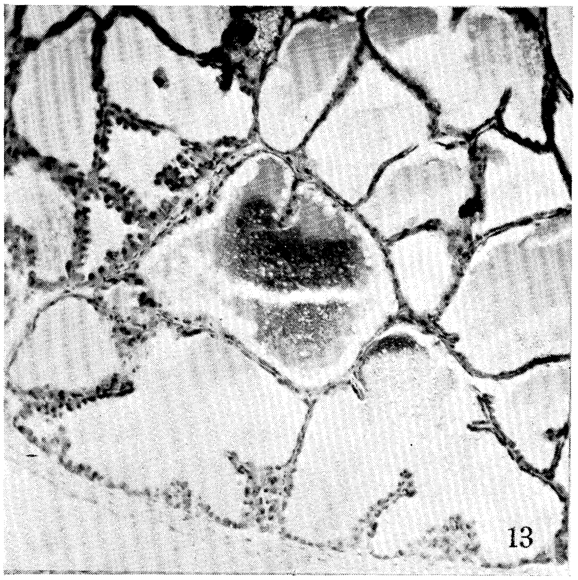
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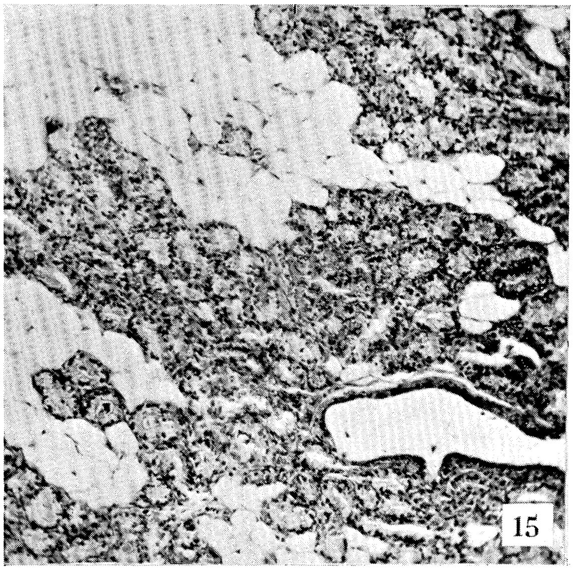
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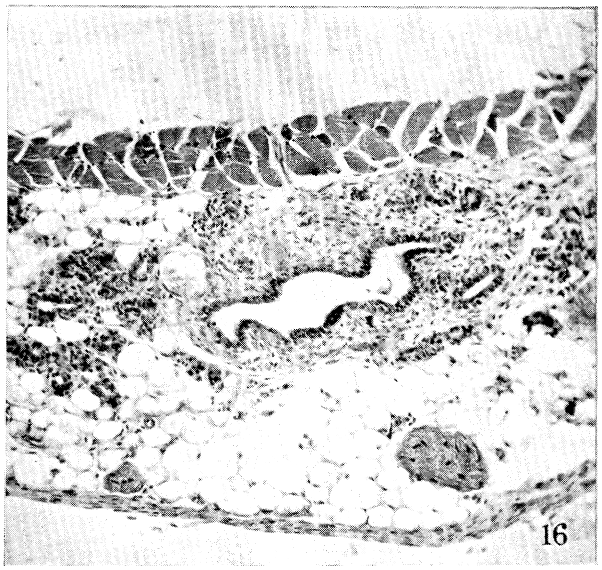
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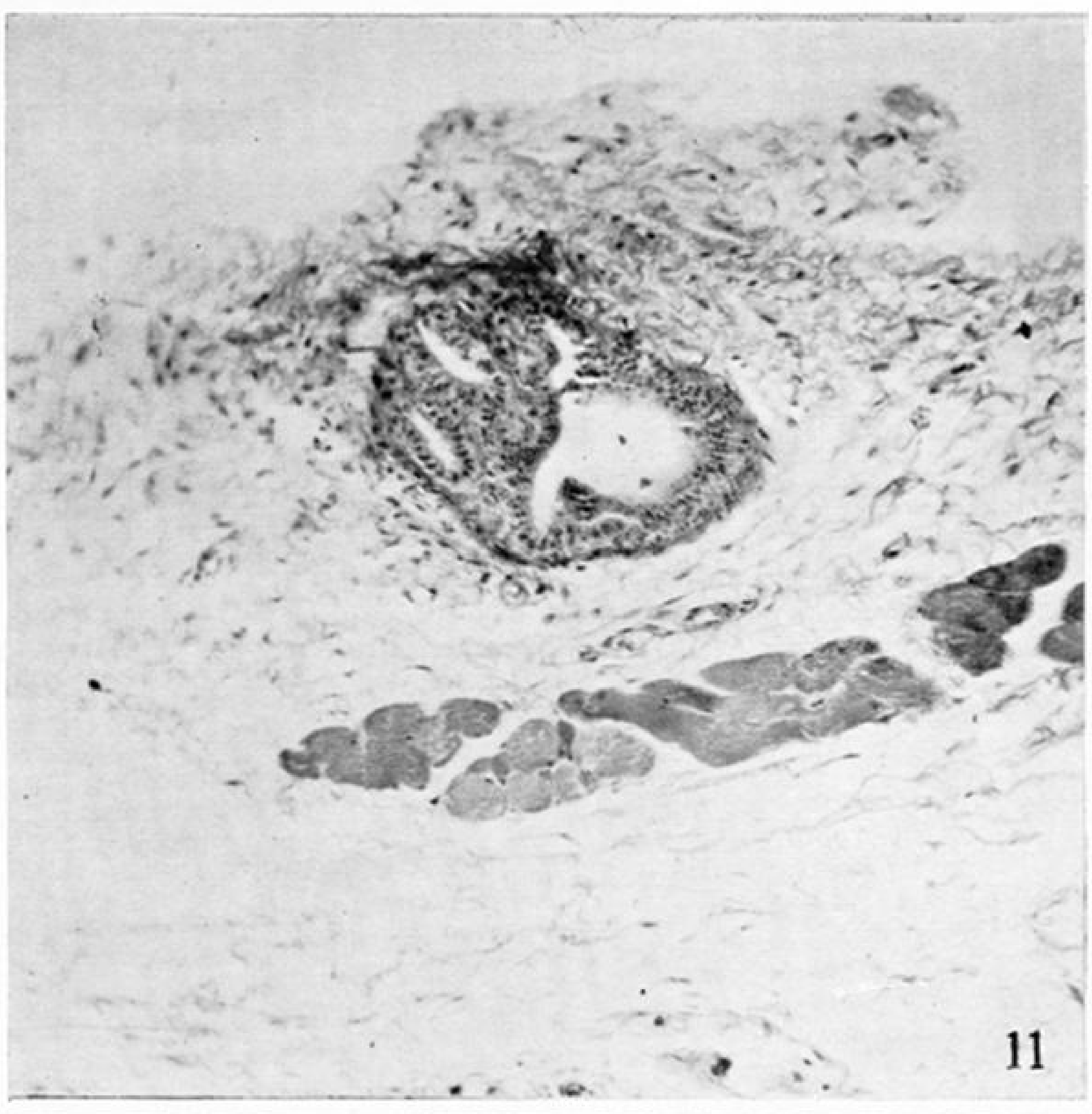
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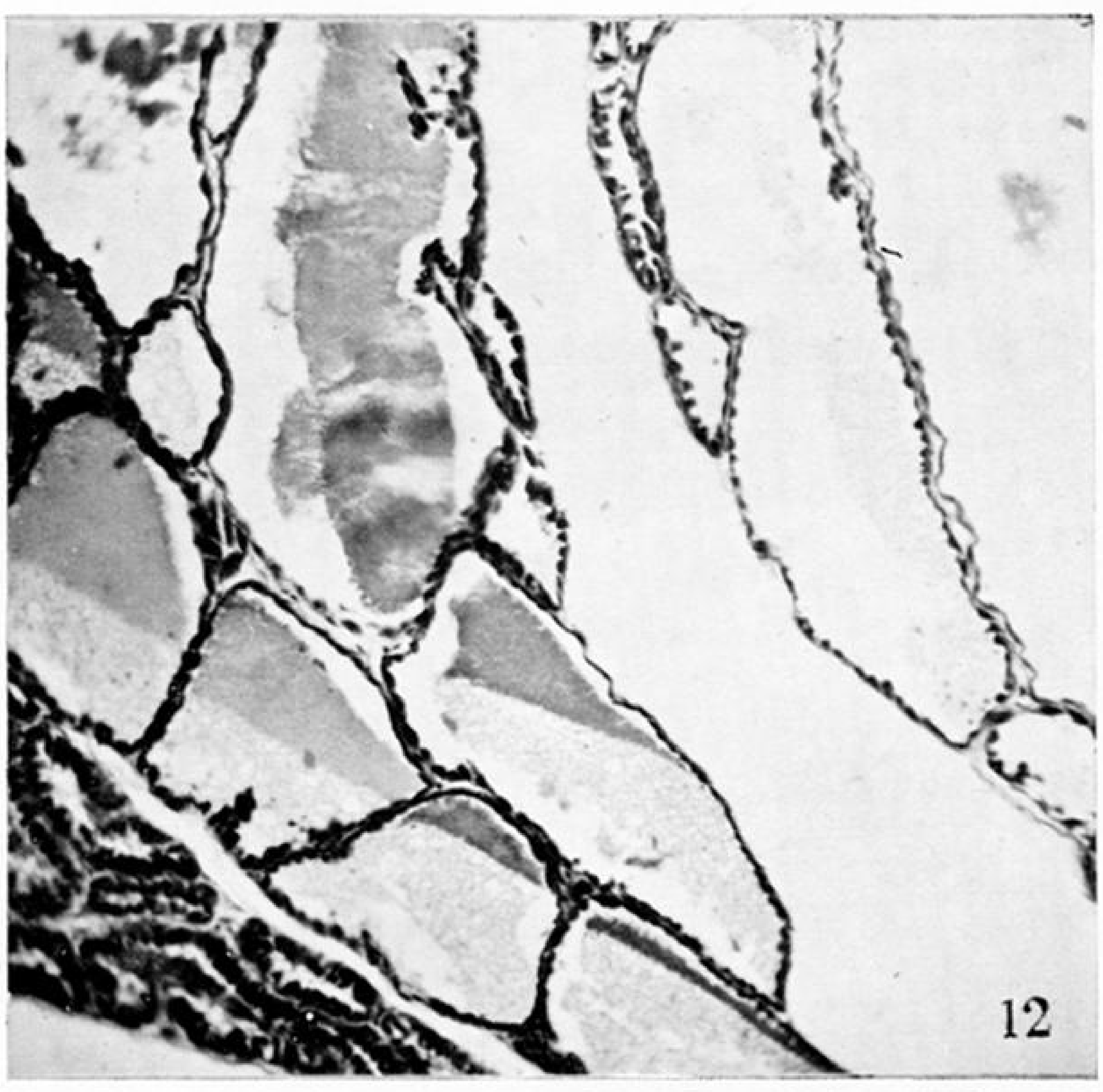
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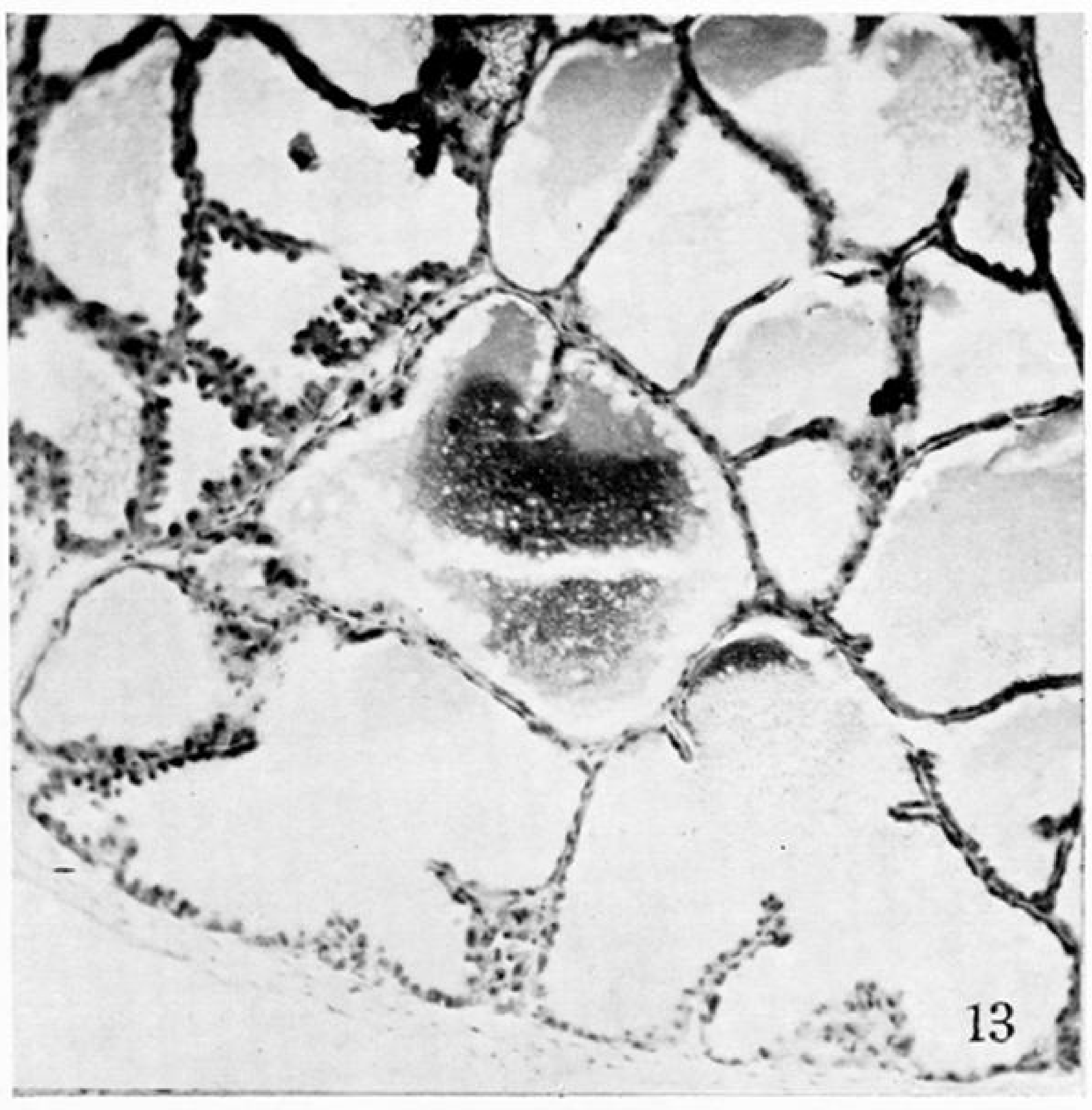
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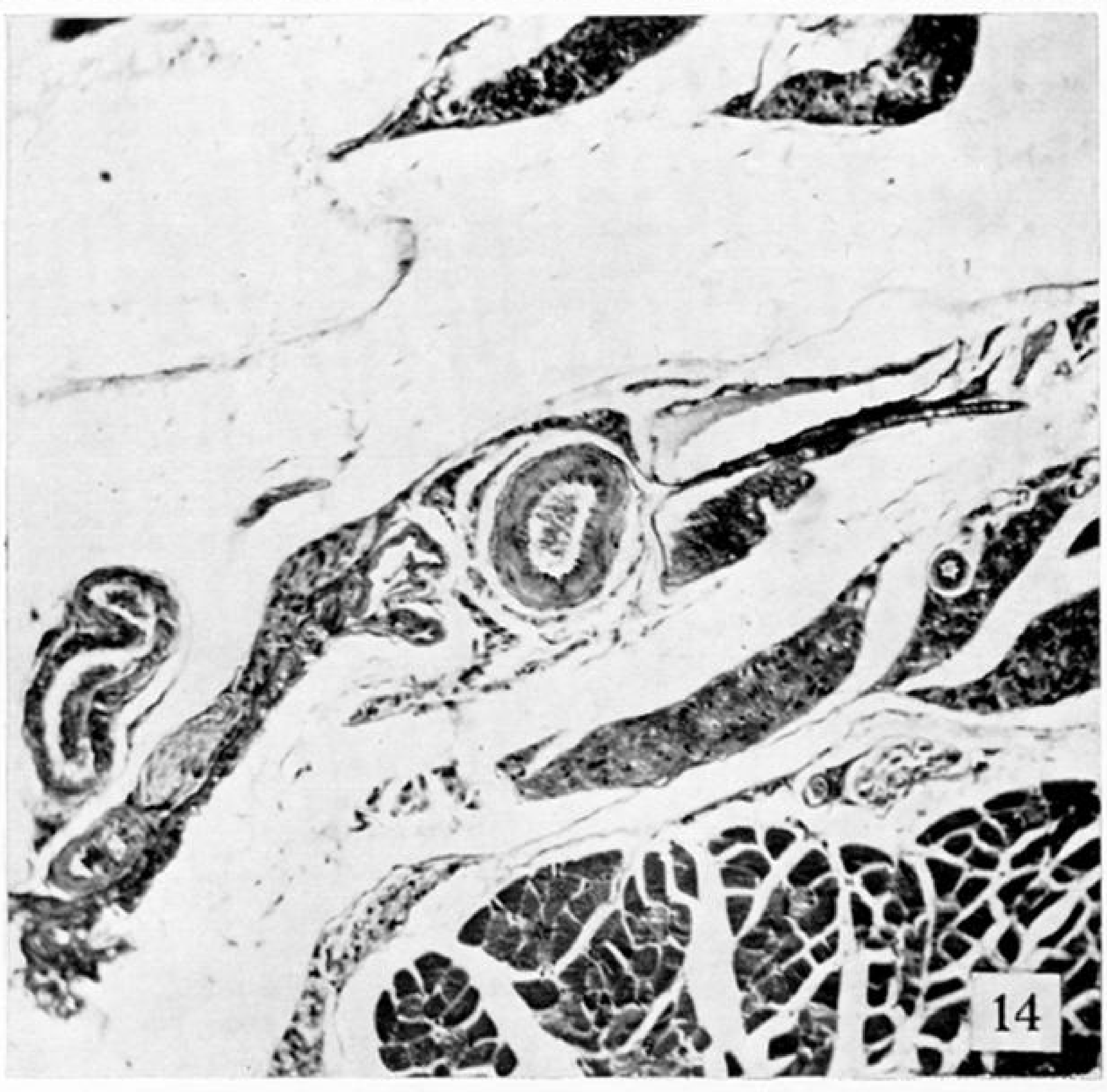
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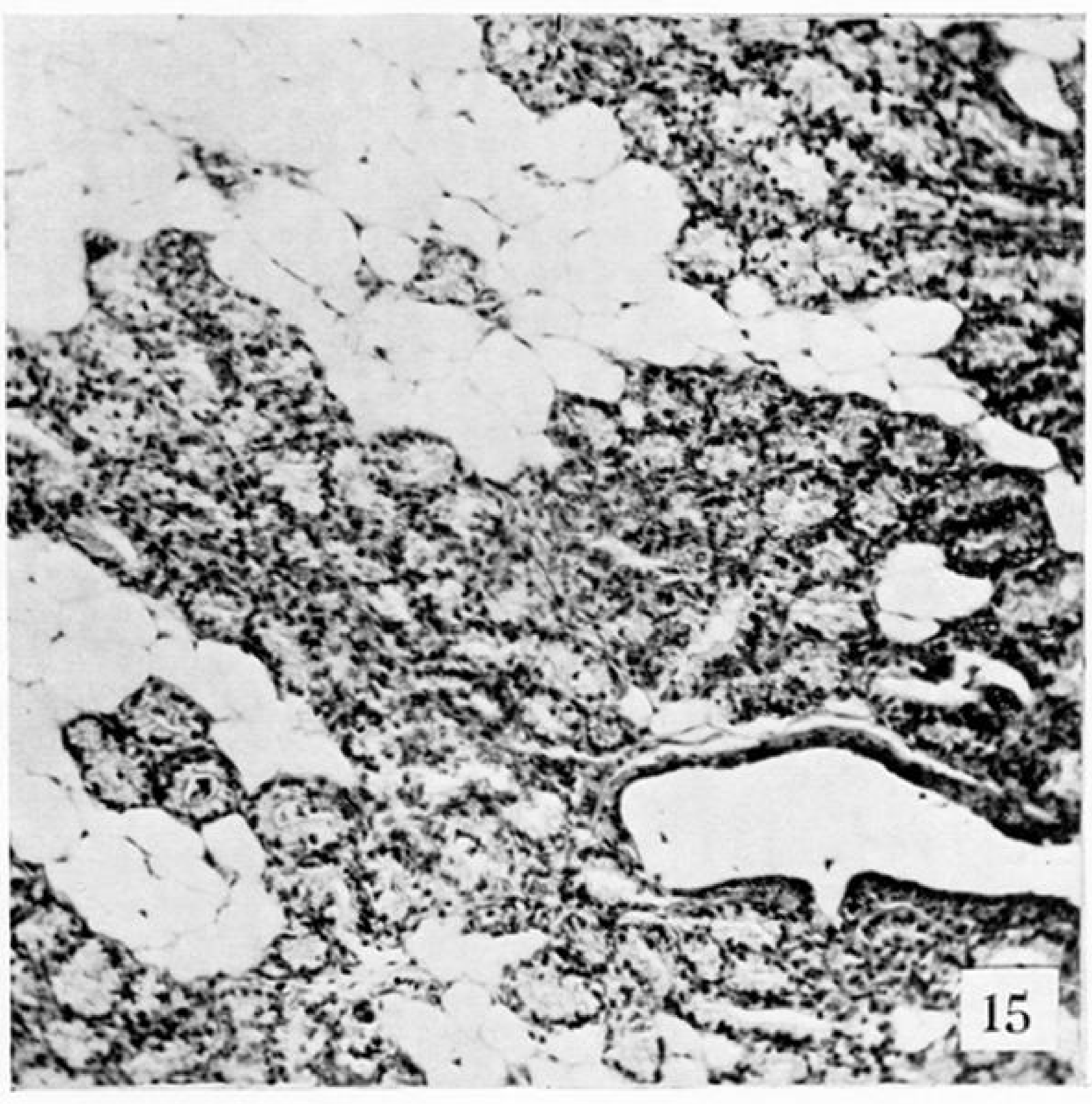
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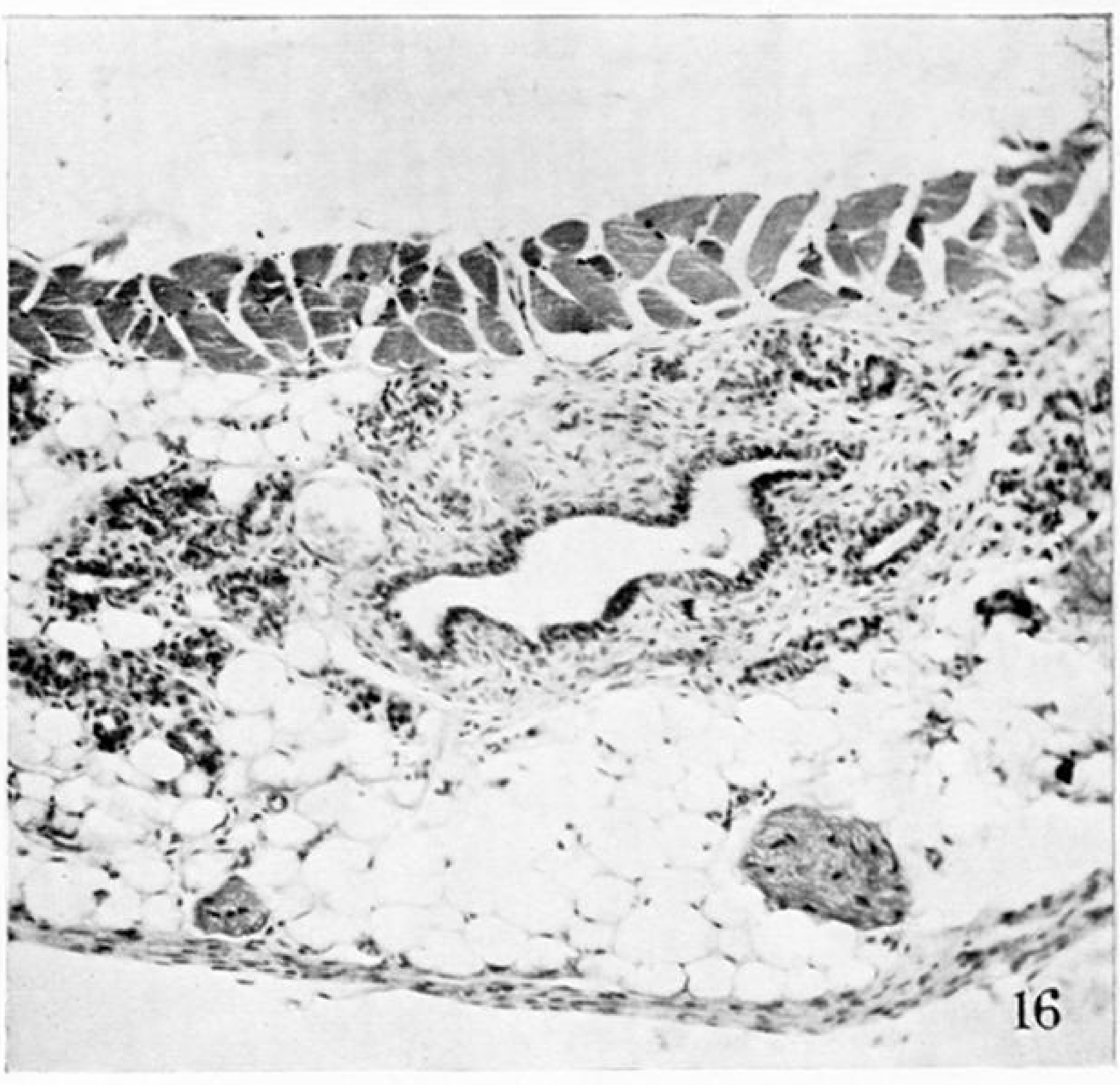
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Description of Plate 1

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