THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee
of the Graduate School, have read the accompanying
thesis submitted by Frank Lester Roberts.
for the degree of   Master of Arts.
They approve it as a thesis meeting the require-
ments of the Graduate School of the University of
Minnesota, and recommend that it be accepted in
partial fulfillment of the requirements for the
degree of   Master of Arts.

C. M. Jackson
Chairman

J.......................

F. C. Mann

May 29   1919

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given   Frank Lester Roberts final oral examination for the degree of Master of Arts   . We recommend that the degree of Master of   Arts   be conferred upon the candidate.

Minneapolis, Minnesota

May 29 1919   *C. M. Jackson*
                         Chairman

J. B. Sedgwick.
H. C. Mann
E. T. Bell.
Richard Scammon.
A. T. Rasmussen

Changes in the Mammary Gland of the Albino Rat
(Mus Norvegicus Albinus) during the Second
Half of Pregnancy.

A thesis submitted to the
Faculty of the Graduate School of the
University of Minnesota
by
Frank Lester Roberts
In partial fulfillment of the requirements
for the degree of
Master of Arts.

1919

Changes in the Mammary Gland of the Albino Rat (Mus norvegicus albinus) during the Second Half of Pregnancy.

Table of Contents.

I. Introduction

The primary purpose of the present study is to determine the changes which occur in the mammary gland of the albino rat during pregnancy, and thereby to establish for this animal a norm which would serve as a basis for experimental work. The subject is old, and much has been written concerning it. But a survey of the literature will show that there has been very little detailed work on the gland during pregnancy on any one animal form, with the exception of Schil's work on the rabbit. Hence it was thought desirable to investigate the subject in the rat, supplementing the researches of Myers, who has worked out the earlier stages of the development of the mammary gland in this animal.

II. Literature

In reviewing the early history of research on mammary gland changes in milk formation most of the earlier works are so rare that I was unable to consult them in the original. Also some of the later works, especially doctors' dissertations, were not available, and hence are cited from reviews. That the author, in most cases, has been correctly quoted, is assured by the agreement of various reviews.

From a review of the literature, there appear at least six milestones in the history of mammary gland research in regard to its changes during gestation. These are, as Bab ('04) pointed out: (1) the mechanical theory of milk formation by Hippocrates; (2) the discovery, by Leeuwenhoek (1680), of milk spherules; (3) Donné's discovery, in 1837, of colostrum corpuscles; (4) Virchow's ('58) theory of the necrobiotic formation of milk; (5) Heidenhain's ('68) decapitation theory; and (6) Czerny's ('90) theory of the leucocytic origin of colostrum corpuscles.

We can trace the gradual evolution of the question from the theorizing of the ancient philosophers, the unscientific attitude of the middle ages, and then a seeming quiescent stage in research until the invention of the microscope. After a certain time, however, the limit of possibilities in milk research was reached until modern microscopical methods were introduced and from the time of Reinhardt we can note a rapid advance in our knowledge of milk secretion from the viewpoint of anatomy. In the following review of the literature the species studied was human, unless otherwise stated.

Reinhardt (1847) was the first writer to attempt seriously to trace the colostrum corpuscles to their origin in the gland. He concluded as a result of his studies that colostrum corpuscles arise from the epithelial cells which undergo fatty changes. Reinhardt describes colostrum corpuscles as being large, finely granular, possessing as a rule no nucleus, but having a well defined cell membrane. He noticed active molecular movements of these granules, stating, "Öfters bemerkt man auch ein lebhafte Molecularbewegung feiner Körnchen innerhalb der angedehnten Zelle". He was able, further, to trace all the steps of the transformation of an epi-thelial cell into a colostrum corpuscle.

In 1849, v. Beuren also stated that colostrum corpuscles are formed by the fatty metamorphosis of epithelial cells. In the following year Kölliker and Will likewise held that colostrum corpuscles are fatty degenerated epithelial cells. Kölliker, however, derived the epithelial cells undergoing this degeneration from the solid alveoli. These alveoli later hollow out and the cells thus detached become colostrum corpuscles.

In 1855, Scanzoni also regarded fatty metamorphosed epithelial cells as giving rise to colostrum corpuscles.

In 1858, Virchow formulated his famous theory that the mammary gland acts like sebaceous glands in that its cells degenerate to form milk. He regarded colostrum corpuscles as fatty, broken off, but not completely metamorphosed epithelial cells. Virchow thought that the membrana propria of the gland served as the matrix for the development of new cells, and that the acini of the mammary gland have more layers of epithelial cells than the sebaceous glands. Not until modern microscopical technique was developed was anyone able to overthrow Virchow's work. This theory, as Michaelis ('98) pointed out, is interesting in view of the fact that it persisted for a long time in so many text books of histology and physiology, even after it had been disproved.

In 1866, Stricker demonstrated that colostrum corpuscles show active ameboid movements, and stated that these cells come from fatty metamorphosed epithelial cells, or broken off parts of such.

Beigel (1868) succeeded in staining the nucleus of colostrum corpuscles.

Fürstenburg (1868) described the membrana propria of the acini of the mammary gland of the cow as a homogeneous membrane.

Kehrer (1871) stated that milk globules develop by necrobiosis of the epithelial cells, and colostrum corpuscles represent fatty degenerated epithelial cells. He described the gland epithelium as being single layered in the rabbit, and described a proliferation of these cells during secretory activity.

Langer (1871) concluded that colostrum corpuscles are fatty epithelial cells from the duct epithelium and none of them came from the alveoli, since he could find no corpuscles in the alveolar

lumina. He described fat granules in the nucleus of the epithelial cells. The gland epithelium is composed of a single layer of polyhedral cells, and the cells of the membrana propria stand in close relation with the interalveolar connective tissue. Elastic tissue surrounds the excretory ducts.

De Sinety (1875) stated that colostrum corpuscles come from fatty metamorphosed epithelial cells.

Buchholz (1877) described two kinds of colostrum corpuscles, those with, and those without a membrane or nucleus, both of which are genetically related and are derived from fatty epithelial cells.

Kollessnikow (1877) described the alveolar epithelium as being double layered. The cells of the inner layer are larger than the outer ones and contain most of the fat. The membrana propria is composed of flat, star-shaped cells which are firmly bound together.

Schmid (1877) described the necrobiosis of the epithelial cells which are, according to him, replaced by new cells.

Winkler (1877), working on guinea pigs, gave lymphocytes as the origin of milk spherules. He described the membrana propria as a translucent layer, which is united by processes to the interalveolar connective tissue.

Rauber (1879) was the first author to expound fully the theory that colostrum corpuscles come as a result of fatty metamorphosis of leucocytes. He described many leucocytes in the interstitial tissue, between the epithelial cells, and in the lumina of the alveoli of the active gland. This was an important contribution to the studies on milk formation and was destined to stimulate many other investigators along the same line.

Jakowski (1880) described the smooth muscle in the nipple as forming a constrictor. The excretory ducts possess a double layer of epithelium, an inner layer of high cylindrical cells and an outer layer of small round cells. The membrana propria is formed by a compact layer of flat anastomosing cells.

Partsch (1880) described the epithelium in the glands of the dog and rabbit as being single-layered. He also mentions the large number of mast cells in the gland stroma during pregnancy.

Saefftigen (1881) stated that colostrum corpuscles are fatty metamorphosed lymphocytes. He described mitotic figures in the epithelial cells of the active gland of the guinea pig.

Barfurth (1882) opposed Kölliker's theory of colostrum formation from a hollowing out of the solid alveoli. He also called attention to the fact that the nucleus is surrounded by a rim of non-vacuolated cytoplasm. Many mast cells are to be found in the interstitial tissue of the mammary gland during gestation.

Talma (1882) could observe no mitoses in the actively secreting gland.

Heidenhain (1883) gave a new theory of milk formation, -- the theory of "decapitation". That is, the inner portion of the epithelial cells of the gland is broken off and goes to form milk. The degenerating nuclei which are observed in the lumina of alveoli and ducts are destined to give rise to the nuclein content of the milk. The epithelium of the gland is single layered and the membrana propria consists of a network of fine star-shaped cells. With regard to Virchow's theory that milk is formed by a degeneration of the epithelial cells--Heidenhain showed that the cow would have to replace her udder completely almost three times a day in order to account for the organic constituents of the milk. This

same fact was demonstrated in the same year by Mülheim Schmidt.

In 1886 Franz Nissen, working with dogs, rabbits and cats, agreed with Heidenhain that the nuclein content of the milk comes from a degeneration of the nuclei of the cast-off epithelial cells. In the nuclei of the intact epithelial cells the chromatin is arranged about the periphery. Some of the nuclei show signs of indirect cell division, but Nissen could not find a single mitosis. His figures show the acini with a single layer of epithelium.

In 1887 Bizzozero and Vassale, studying the glands of guinea pigs, dogs, and rabbits, described the mammary secretion as a product of the secretory activity of the epithelial cells. According to these authors the height of the epithelial cells is dependent upon the amount of secretion in the alveoli and upon the stretching of the alveolar wall. Numerous mitoses were observed during pregnancy which ceased with the beginning of lactation. There is no degeneration of nuclei to form nucleo-proteins for the milk.

Billroth (1887) called attention to the large number of leucocytes in the connective tissue stroma of the gland and states he was unable to find any membrana propria.

Coen (1888) stated that colostrum corpuscles are fatty epithelial cells, and that mitoses are plentiful during pregnancy in order that the desquamated epithelial cells may be replaced. Acini have a double layer of epithelium in many places and between the acini and in the interlobular connective tissue large numbers of mast cells are to be seen.

Kadkin (1890) using the mammary glands of rats, guinea pigs, mice and rabbits, described colostrum corpuscles as being derived from fatty metamorphosed epithelial cells. He observed a few

mitoses during pregnancy but states that the gland proliferates during gestation mostly by amitosis.

Czerny, in 1890, took another great step in the study of colostrum. He injected human milk into the lymphatic system of frogs and found that the leucocytes took up the fat globules and in so doing increased their size, often six to eight times. Subcutaneous injections of milk into rabbits gave the same result and the fat-laden leucocytes appeared identical with colostrum corpuscles. Czerny formulated his theory as follows:

"Die Colostrumkörper sind Leukocyten, welche in die Brustdrüsenraume einwandern, sobald in diesen Milch gebildet, aber nicht durch die Ausführungsganse entleert wird, welche dann daselbst die unverbrauchten Milchkügelchen aufnehmen, zertheilen und behufs Weiterer Rückbildung in die Lymphbahn aus der Drüse abführen."

Benda (1893) also ascribed to leucocytes an important rôle in colostrum formation and said that desquamated epithelial cells and fragmented nuclei are artefacts, which may be avoided by proper technique. The membrana propria is homogeneous and between it and the epithelial cells of the gland are flattened cells which are analagous to the myoepithelial cells of the sweat glands.

Duclert (1893) stated that mitoses are the more numerous the closer the time of delivery approaches.

Steinhaus (189̂), working on guinea pigs, formulated a new theory of colostrum formation, namely, that mast cells undergo fatty metamorphosis and are transformed into colostrum corpuscles. In the pregnant gland he found many mitotic figures resulting in double nucleated cells. Peculiar granules are found in the cytoplasm of the epithelial cells colored by the Altmann method.

These are probably mitochondria, although Steinhaus, of course, did not call them that.

La Croix (1894) described myoepithelial cells in the mammary gland.

Szabo (1896) reviewed some of the more important works in this field and as a result of his own work (on guinea pigs) decided that the epithelial cells are single-layered, often multi-nucleated, and are not destroyed by the process of secretion. The nuclei of these cells during the secretory activity of the gland have a characteristic arrangement of the chromatin, which is concentrated about the periphery of the nucleus, with a well defined nucleolus in about the center.

Von Ebner (1896) in Kölliker's Handbuch reviewed the literature and decided on the basis of the morphology of the nucleus that colostrum corpuscles represent fat-laden leucocytes. The epithelium of the ducts and acini is single layered. The membrana propria is a structureless membrane in which basket cells are found. These cells/ Von Ebner considered analogous to the myo-epithelial cells of the sweat glands.

Michaelis (1898) supplied a useful review of the literature on the active mammary gland and from his own studies on the guinea pig concluded: (1) The secreting epithelium of the gland is single layered; (2) the change in the form of the alveolar epithelium is not wholly dependent upon the amount of secretion in the lumen of the acini, but may during lactation become high cylindrical cells as a result of their own secretory activity; (3) the nuclei which disintegrate in the lumen of the alveoli to form nucleo-proteins for the milk are replaced by a process of direct cell division; (4) leucocytes wander into the alveolar lumina, take up fat, and

are transformed into colostrum corpuscles; and (5) the fat content
of the milk is a result of the secretory activity of the gland
cells and is not the result of the degeneration of these cells.

Unger (1898) concludes, "Ich halte also Czerny's Theorie der
Entstehung der Colostrum Körperchen für die richtige.....". He
modifies Czerny's view, however, in that he gives mast cells the
foremost rôle in the formation of colostrum corpuscles. He
describes these cells as being found in the interalveolar spaces
and sending up processes between the epithelial cells, around the
blood vessels, and about the ducts, between the fat cells of the
retro-mammary fat tissue, in the perialveolar lymph and blood
spaces, and in the axillary and retro-mammary lymph nodes. Some of
these cells Unger describes as undergoing a fatty metamorphosis.
In addition to human material, Unger used mammary glands of guinea
pigs.

Kollowsow (1898), by special staining methods, demonstrated
intercellular bridges of the epithelium of the mammary gland of a
pregnant cat. The duct epithelium is double layered and Kollossow
suggested that the basal layer of cells might be transformed, by
the end of pregnancy, into typical smooth muscle.

Bizzozero and Ottolenghi (1899) supply a useful review of the
literature of the mammary gland in pregnancy, and in describing
the glands of pregnant guinea pigs, call attention to the large
number of leucocytes in the stroma, and the great percentage of
mast cells among these leucocytes.

Sticker (1899) described the epithelium of the acini of the
active gland of the cow as being double-layered. Between the
acini, capillary loops are found which often lack even a connective
tissue support.

Palazzi (1900) described colostrum corpuscles as being derived from epithelial cells, although he mentions the fact that in milk one often sees fat-laden leucocytes.

Cohn (1900) described colostrum corpuscles as having their origin from fat-laden leucocytes. Some of these corpuscles are smaller and contain less fat than the others, and such forms are to be regarded as younger cells. Most of the colostrum corpuscles come from leucocytes with neutrophilic granulation; none of them come from mast cells. Cohn assumes that there must be a positive chemotalis exerted by the gland or its secretion in order to account for the immigration of the leucocytes to this region.

Bierrich (1900) deals with the amount of elastic tissue in the breast under normal and pathological conditions. His data indicate that the older the woman the more elastic tissue there is in the gland. But since Bierrich says nothing regarding the number of pregnancies in each case, his results are uncertain, unless we assume that in each case it was the first pregnancy. He described the membrana propria as consisting of several layers of closely packed cells.

In 1901 Ottolenghi called attention to the large number of mast cells in the stroma of the mammary gland of pregnant white mice and described large fat-laden leucocytes in colostrum.

Bab's thesis appeared in 1904, in which he described his work on colostrum formation. This work is really a repetition of Czerny's experiments on a very elaborate scale. His conclusions were: (1) By intra-peritoneal injection of milk, corpuscles are formed which are morphologically identical with colostrum corpuscles; (2) the phagocytosis not only concerns itself with milk-fat but also with the albuminous part of the milk; (3) intra-

peritoneal milk resorption takes place in the salamander in much the same way as in warm-blooded animals. (4) In every detail, the physiological process of colostrum formation is analogous to the pathological process of inflammation. (5) Cohn's hypothesis that colostrum corpuscles develop from small leucocytes with neutrophilic granulations is untenable. (6) From colostrum to milk there is a gradual transition, since leucocytes and colostrum corpuscles can be found constantly in milk. (7) Colostrum is an excellent regulatory apparatus for the fat-content of the first milk and serves also as a laxative and nutritious fluid for the newborn.

Bertkau (1907) described myoepithelial cells in the mammary gland of the cow. With regard to the secretory activity of the gland, Bertkau claims that "decapitation" of cells, as described by several authors, is an artefact, and no necrobiotic processes, complete or partial, take part in milk formation. The changes and variations in the height of the epithelial cells of the secreting alveolus are due to folds in the membrana propria brought about by the contraction of the so-called myoepithelial cells. These folds are removed when the alveoli are filled with secretion.

In 1911, Berka gave a detailed account of the histology of the human mammary gland during pregnancy. In the first half of pregnancy the "gland fields" have an infiltrated appearance as a result of the large number of cells, epithelial, connective tissue, and leucocytic in type. The typical lobules are formed by the beginning of the second half of pregnancy. The acini have a single layer of epithelial cells, while the ducts are double-layered throughout. The membrana propria is composed of a fibrous non-nucleated network. In the stroma there are numerous leucocytes, and prominent among these are the large number of mast cells.

These, however, have nothing to do with colostrum corpuscle formation, and in none of them was he able to demonstrate any fat. He gives a detailed account of the elastic tissue content of the breast. Berka was unable on the basis of his material to decide definitely which plays the more prominent rôle in elastic tissue formation, age or the number of pregnancies. Both seem to influence the production of this tissue.

Ancel and Bouin (1911) published one of their many articles on the gland and especially studied the relation of the corpus luteum to the development of the breast during pregnancy. They divide the pregnant cycle of the mammary gland of the rabbit into two phases,--one the growth phase and the other the glandular activity phase. The first phase occupies the first half of gestation and the second one begins with the beginning of the second half of pregnancy. The figures published in connection with this paper show the ducts with a double layer of epithelium and the acini with a single layer.

Schil, in 1912, also described these two phases of the development of the mammary gland in the gravid rabbit and believes that the growth of the gland has reached its maximum at the beginning of the second phase, as he was unable to find any mitotic figures during the second phase.

O'Donoghue (1911) described the mammary apparatus of Dasyurus. With the onset of pregnancy the milk ducts become more and more branched. The alveoli are first solid and later become hollowed out and finally have a single layer of epithelium surrounding their lumina. Regarding Kollossow's suggestion that the myoepithelial cells might be transformed into typical smooth

muscle cells by the end of pregnancy, O'Donoghue says that such a condition is not probable, since if it were true we would have muscle derived from ectoderm. He regards the membrana propria as being not an independent structure but a part of the general gland stroma, which is closely applied to the acini by a process of stretching.

In 1915, Wahl described the blood supply to the mammary gland of the pregnant albino rat and gave a brief description of the method of proliferation of the ducts and acini. In the early stages of pregnancy, lobular ducts begin to appear on the ends and sides of the main ducts. These lobular ducts may be simple or compound. The simple ducts arise as solid bulbular projections which elongate, become hollowed out and their sides become studded with knob-like processes which later expand and become true alveoli. The compound ducts, in addition to the foregoing processes, give off secondary branches which in turn become studded with alveoli.

Since 1915, so far as I can learn, there have been no articles on the histology of the breast during pregnancy. In the last decade, however, an enormous amount of experimental work has been done on the source of the stimulus which causes the growth of the mammary gland. Prominent among these researches are those by Ancel and Bouin, Lane-Claypon and Starling, Schil, O'Donoghue, and Frank and Unger.

III. Material and methods.

The material for the present study was presented to me by Dr. J. A. Myers to whom, as well as to Professor Hal Downey, the writer is also greatly indebted for aid and advice during the

course of the investigation. The material comprises the glands of ten pregnant albino rats (Mus norvegicus albinus), all primiparae from the colony in the Institute of Anatomy. The material represents stages from the thirteenth day of pregnancy to within a few hours of delivery and includes the following six stages: thirteen days; sixteen days plus twelve hours; eighteen days plus twelve hours; nineteen days; twenty days; and twenty days plus nine hours. Material for the earlier stages of pregnancy was not available.

The technique of removal and preparation of the glands has been described by Myers ('16) which briefly is as follows: The animals were killed with ether and the skin, together with all the mammary glands, was immediately removed, pinned out on a sheet of cork and placed in Zenker's fluid. The hairy surface of the skin of course was next to the cork sheet.

Cleared preparations were made in the same manner as described by Myers ('16) which is essentially the method Lane-Claypon and Starling used in work on the rabbit. These preparations were mounted in xylol-gum-damar in shallow glass cells.

In preparing material for microscopic sections, portions of the fixed gland were removed carefully and imbedded in hard paraffin. But in sectioning the nipples it was found almost impossible to cut the tough skin when it was imbedded in ordinary paraffin. Therefore Johnston's asphalt method (Guyer '17), as modified, I believe by Dr. J. T. Patterson, was employed. Crude rubber was cut into very fine bits with a pair of sharp curved scissors. About three teaspoonfuls of this finely divided rubber was put into a pint of paraffin, M.P. 65°C., and the mixture was left in the oven for three days. During this time the mixture was

frequently stirred, and by the end of three days most of the
rubber had passed into solution. After three days, however, the
mixture was removed from the oven and cooled. A part was melted
only when needed for imbedding, since it was soon ascertained that
if the mixture is kept melted for much more than three days, the
rubber tends to precipitate. This treatment toughens the paraffin
to such a degree that the skin imbedded in it can easily be cut.

Sections of the mammary gland and nipple were cut at 3, 4, 5,
7 and 10 micra in thickness. Some sections were stained with
Mallory's connective tissue stain; some with Weigert's iron
haematoxylin counter-stained with Van Gieson's stain; and some with
a 1 per cent aqueous solution of cresyl violet. Another series
were stained with Unna's orcein for elastic tissue. A few (fresh)
frozen sections were stained with osmic acid and Sudan III, in
order to show the distribution of fat.
        The cresyl
        ₐviolet stain deserves particular mention because of the
beautiful results obtained and because of some of the difficulties
encountered in using it. Paraffin sections were passed in the
usual way from xylol to water, and from water were placed in the
stain for twenty minutes. From the stain the sections, after
rinsing well in water, were plunged directly into 95 per cent
alcohol for a few seconds; then for the same length of time into
absolute alcohol; then into a mixture of absolute and xylol a few
seconds; and finally into xylol for three minutes. They were
finally mounted in toluol-gum-damar.

The reason for the short treatment with the alcohol and xylol
is because it was observed that, for some reason the alcohols and
xylol quickly decolorize the sections to such a degree that there
is no longer any differentiation of the tissue elements.

Cresyl violet, as is well known, gives a most excellent differentiation to the mast cells, sharply marks off the glandular from the connective tissue, and gives very good cytological details.

In studying the increase in the size of the nuclei, cells, and alveoli, a Leitz ocular micrometer was used with a Spencer's mechanical stage. The nuclei measured were those which showed most clearly the nucleolus. The alveoli measured were those in which the lumen was most clearly outlined and nearly circular. The entire alveolus was measured from the base of one cell to the base of the opposite cell. The lumina were measured in their transverse diameters, and the epithelial cells were measured along the diameter perpendicular to the circumference of the alveolus. There was no attempt at extremely accurate measurements as the only thing desired was to determine roughly the change in the size of the alveoli and cells from the thirteenth to the twentieth day.

A few measurements were taken by calipers on the nipples of the pregnant animals and served merely to confirm what could easily be seen with the naked eye, namely, that there is a marked increase in the size of the nipple during pregnancy.

IV. Gross changes in the mammary gland during pregnancy.

The only gross change that can be observed externally in the gland of the living animal during pregnancy is the increase in the size of the nipples. If a pregnant rat is suspended by the head or tail the nipples are seen to be very prominent. Part of this prominence is due of course to the stretching of the abdominal skin, but by far the most of it is due to an actual increase in the size of the nipples. This increase in size is apparent to the naked eye and was easily verified by a few rough measurements with the calipers on virgin and pregnant animals.

The increase in the length of the nipple during pregnancy is associated with the complete degeneration of the 'epithelial hood' which surrounds, at least in part, the virgin nipple. Myers ('19) has described a partial degeneration of this hood at the time of puberty, but in virgin rats even four months old this hood has not entirely disappeared and is only partly degenerated on one side. During pregnancy, however, the degeneration is completed. At the end of pregnancy there is not a remnant of this hood left, excepting some necrotic debris in the groove which has taken its place. (See fig. 1.)

The nipple, accordingly, in pregnant and lactating rats may easily be drawn out to nearly double its apparent length. In fact it is drawn out during the act of suckling, and the purpose of the degeneration of the epithelial hood is doubtless to allow for this increase in the length of the nipple for the accommodation of the young.

Upon removing the skin from the freshly killed animal, the mammary glands are seen as whitish masses raised slightly above the surface of the rest of the skin. The glands are streaked with numerous blood vessels, which indicate the extremely rich blood supply which the glands have during gestation. The arrangement of the individual glands is best made out with the aid of the cleared preparations.

In the cleared specimens we can see that there has been a tremendous increase in the amount of the glandular tissue, which, roughly, forms two horseshoe-shaped masses, anterior and posterior, with their concavities facing each other. This appearance is brought about as follows. There is an overlapping of the glandular

tissue off the abdominal and inguinal glands of the same side.
The second inguinals of either side overlap across the mid-ventral
line of the body and thus the caudal C-shaped mass of glandular
tissue is formed. Likewise there is an overlapping of the three
thoracic glands of the same side, and a transverse overlapping of
the glandular tissue of the first thoracic glands of opposite
sides. There is no overlapping of the last thoracic and abdominal
glands--and no transverse overlapping anywhere except in the two
regions noted above.

Myers ('16) has called attention to this arrangement of the
glandular tissue in the virgin animal and this overlapping is
merely emphasized by the tremendous proliferation of the glands
during pregnancy.

A closer study of the cleared preparations show that there is
one main duct leading to the nipple pore and all the rest of the
ducts branch off from this one, as shown by Myers ('16). From the
sub-branches of the secondary ducts the buds are seen which in the
thirteen-day stage are nearly spherical while they are larger and
roughly ellipsoidal in the twenty-day stage.

These bud-like outgrowths on section are seen to consist each
of a group of alveoli. Thus each bud represents a lobule, while
a secondary duct with its lobules constitutes a lobe. However, it
should be noted that the excretory duct of each lobe opens into the
main duct before the main duct passes through the substance of the
nipple.

Although (as will be shown later) practically no mitoses or
amitoses appear in the gland during this period, the cleared pre-
paration in the gland parenchyma of the 20 day stage showed more

lobules than that of the 13 day stage. It is very probable, however, that this represents an individual variation rather than a multiplication of elements in the second part of pregnancy. Some individual variation in the size and arrangement of the mammary glands of the albino rat is always to be expected. This was clearly shown by Myers ('16), who was working on rats from the same colony from which the material for this study was taken.

Further evidence that this apparent increase in the number of lobules is due to individual variation is that there is no visible difference in the number of lobules in the glands of the 13 day stage and those of the 16 day and the 18 day stages. The cleared preparation of the 19 day stage was lost and hence was not available for a comparison, but it is very improbable that a multiplication of the elements would not begin before the 19th day of pregnancy. If there were an increase in the number of lobules of the mammary gland in the latter part of pregnancy we would expect to find some evidence of the increase in the time elapsing from the 13th day to the 19th day stage.

It might be objected that this lack of difference in the stages studied represents an individual variation while the increased number of lobules represent the true conditions. In the absence of evidence of cell division, however, I believe it safe to conclude from the material thus far studied that there is probably no increase in the number of glandular elements in the second period of pregnancy, from the 13th day onward.

V. Changes in microscopic structure.

1. The nipple.--Aside from the increase in the size of the nipple and the disappearance of the epithelial hood, the main difference in the structure of the nipples of pregnant and virgin

animals is in the amount of smooth muscle about the main duct.
This smooth muscle, described by Myers ('19) in virgin animals, is
well developed by the end of pregnancy. Since it is attached
externally to the processes of the epithelium which project into
the corium of the nipple and internally is continuous with the
smooth muscle layer directly under the skin, it is probable that
the purpose of this muscle is to aid the elastic tissue in re-
tracting the nipple after it has been drawn out in the act of
suckling (fig. 1). It also may aid in compressing the main duct
of the nipple and thus facilitate the flow of milk; but to my mind
this is rather the minor rôle of this muscle. The emptying of the
ducts appears to be rather a passive process, and it seems un-
necessary to assume that there must be some muscular elements (as,
for example, the myoepithelial cells of La Croix and others) to
aid in expelling the secretion.

The lumen of the main duct of the nipple is enlarged con-
siderably during pregnancy. This enlargement is purely mechanical
and is due to the increase in the amount of secretion in the duct
as pregnancy advances. There is apparently no change in the
epithelium of the duct from that in virgin animals, which was
accurately described by Myers ('19).

2. General structure. A low power view of a section of the
thoracic mammary gland of a 13 day pregnant rat gives a picture
which, excluding the panniculus carnosus, represents in general the
picture of any one of the mammary glands at any stage of pregnancy.
The picture differs from that of a section of a virgin gland in the
presence of lobules, more ducts, more blood vessels, and more
cellular elements in the stroma.

A relatively broad band of striated muscle (the panniculus carnosus) is seen running through the center of the section. On either side of this muscle is arranged the glandular parenchyma in the form of lobules and ducts scattered through the fatty tissue. Only occasionally do we see a duct opening directly into a lobule, an appearance which is brought about by the arrangement of ducts and lobules. It is to be remembered that the lobules bud off irregularly from the duct and hence in many places the duct will be cut and not the lobule; in other places the lobule will be apparently isolated, and only when the section happens to cut the narrow stalk connecting the lobule and duct will we see the true arrangement.

Blood vessels and nerves are scattered throughout the section. The large nerves lie in close proximity to the muscle, which they supply. The ducts and lobules are surrounded by sheaths of fibrous connective tissue and the rest of the stroma is mostly adipose. In fact the whole picture, excluding the muscle, gives an impression of small islands of glandular tissue in a lake of fat. (See fig.2.)

This distribution of the adipose tissue is interesting from the point of view of the development of the mammary gland. Myers ('19) has shown that in young albino rats that fat is soon laid down in certain definite regions and into these regional pads of fat the mammary gland pushes its way. Hence we see the explanation for the large amount of adipose tissue in sections of the pregnant gland.

In discussing the finer structure of the gland the following structures will be described in turn: (1) Changes in the stroma; (2) Changes in the ducts; and (3) Changes in the acini.

3.   Changes in the stroma.--Any stage from the 13th to the 20th day might be described with reference to the stroma and the resulting picture with a few minor changes would represent the stroma of any of the other stages. That is, the stroma is the more constant element in the gland, while the parenchyma is under-going radical changes.

Each duct is surrounded by a dense sheath of connective tissue, which is prolonged about the lobules which are drained by this duct. (Fig. 3.) From the lobular sheath prolongations extend inwards and surround the individual alveoli. Where the sheath leaves the duct to pass about the lobule, it suddenly becomes very much thinner, an arrangement which is easily under-stood if we but refer to Wahl's ('15) description of the mode of proliferation of the ducts and lobules.

Here an explanation is given for the appearance of the stroma of the gland after all the lobules and acini are formed. The first bud which pushes out carries with it a covering of fibrous connec-tive tissue derived from the sheath of the parent duct. This bud enlarging, keeps pushing ahead of it the connective tissue sheath, which as a result becomes very much thinned. Of course this thinning out would not result if the growth of the stroma of the gland kept pace with that of the parenchyma; but Duclert ('93) and Steinhaus ('9$) have clearly shown that the stroma lags far behind the parenchyma as regards growth. None of the specimens studied showed any structure resembling a membrana propria.

Turning from the covering of the ducts and lobules to the covering of the individual acini, one sees that in the 13 day stage it is relatively loose (fig. 4), being composed of elastic

and collaginous fibers and traversed by capillaries. By the end of pregnancy, however, the acini have enlarged to such an extent that this sheath has been stretched. As a result, it appears more dense and is closely applied to the base of the alveolar epithelium, giving an appearance of a membrana propria.

That this appearance of a membrana propria is a result of the stretching of the interlobular connective tissue and that the membrana propria, as such, does not exist in the mammary gland of the pregnant albino rat, is shown by a study of the stages used for this work. Figure 4 shows that the sheath is loose and only when the acini are distended with secretion do we get the appearance of a basement membrane (fig. 5). Since the sheaths of the ducts are imbedded in soft adipose tissue, no distention of the ducts stretches the sheath since the adipose tissue yields to the pressure. Hence there is no appearance of any structure resembling a membrana propria around the ducts.

Besides the prolongations about the lobules, the sheath about the ducts send out projections which become continuous with the sheaths around the blood vessels of this region and everywhere are continuous with the fibrous framework of the adipose tissue. (Fig. 2.) Where a duct lies next to a muscle, as in the thoracic glands, the sheath about the duct is continuous with the perimysium of the muscle. In the region of the nipple, the ducts merely pass through the subcutaneous tissue and lose the well-defined sheaths which they possess deeper in the gland.

A close study of the sheaths about the ducts, lobules, and acini reveals many elastic fibers in these sheaths. There have been several authors who have attempted to point out a connection

between pregnancy and the amount of elastic tissue in the stroma of the mammary gland. As shown in the review of the literature, little has been accomplished towards a solution of the problem. In my material there is an unfortunate arrangement of the age of the specimens. With two exceptions the further advanced the pregnancy the older was the rat. As a result all I can do is to describe the elastic tissue as I found it and to point out some factors suggested by the findings.

In sections stained with Unna's orcein, the elastic tissue fibers are seen to be running lengthwise in the connective tissue sheaths about the ducts, and are prolonged in the form of a few fine fibers about each lobule. From this lobular sheath fine fibrils are sent in which, in the latter stages of pregnancy, surround each alveolus.

In the 13 day stage, in most cases it is extremely hard to see any elastic tissue about the individual alveoli, but by the end of 18 days and 12 hours it is easily seen that each alveolus is completely surrounded by a fine network of elastic tissue.

In the earlier stages the elastic fibers are fine and delicate, while in the later stages most of them are exceedingly coarse. The sections show that in the specimens used for this study there is apparently a great increase in the amount of elastic tissue about the ducts from the 13th to the 20th day. This increase is a progressive one; that is,—the 15 day stage has more than the 13, the 18 day stage more than the 15 day stage, and so on.

On the face of it, this would indicate that pregnancy causes a proliferation of elastic tissue in the mammary gland, but as previously pointed out, the age of these specimens, with two exceptions, is arranged parallel with the advance of pregnancy.

For reference I will arrange the representative stages in order
as the amount of elastic tissue increases and indicate the age of
each specimen:

1. Rat No. 102,   5-1/2 months old,   13 days pregnant.
2. Rat No. 106,   6   months old,   15 days pregnant.
3. Rat No. 105,   10   months old,   16 days,12 hrs.pregnant.
4. Rat No. 101,   3-1/2 months old,   18 days,12 hrs.pregnant.
5. Rat No. 104,   10   months old,   19 days pregnant.
6. Rat No. 103,   9   months old,   20 days,9 hrs. pregnant.

Between 1 and 2 it was sometimes difficult to say which had the
more elastic tissue, and this was also the case with 5 and 6. But
with 1 and 4 there was no question at all. Rat 4 had much more
elastic tissue than did rat 1, although the former was two months
younger than the latter. (See figs. 6 and 7.)

With regard to the interalveolar elastic tissue, therefore,
one pertinent fact ~~that~~ stands out. The alveoli are new formations
which appear after the beginning of pregnancy, and hence age can
play no direct part in their formation. But during pregnancy, the
further advanced the pregnancy, the more prominent do the inter-
alveolar elastic tissue fibers become. The picture thus presented
suggests very strongly that pregnancy stimulates the proliferation
of elastic tissue in the mammary gland of the albino rat.

The adipose tissue is very plentiful in the stroma of the
gland in all stages studied, and its distribution and significance
have already been referred to under the general structure of the
gland.

The stroma of the mammary gland is very rich in cellular
elements, many of which are of the wandering type. A great number
of these wandering cells are mast cells, which are scattered

throughout the stroma, in the connective tissue sheaths about the ducts, in the walls of blood vessels, in the meshes of the adipose tissue, and between the alveoli. It should be noted here that mast cells are not only common in the mammary gland of the albino rat during pregnancy but, according to Professor Downey, are present in large numbers in this animal during its whole life. They are found most plentifully in the subcutaneous tissues, but are also found free in the peritoneal fluid and in the lymph nodes.

Many other wandering cells of leucocytic or lymphocytic type were found in the stroma in all the stages studied. In many cases where these cells were found between the alveoli it was difficult to decide whether they were inside or outside the capillaries.

Scattered throughout the stroma, but tending for the most part to be located in close proximity to the lobules are many arterioles which send capillary branches in to supply the lobules. These intralobular, or more accurately, interalveolar capillaries are very thin walled and in most places are represented only by endo-thelial cells. In the 13 day stage, these capillaries show no distention and the nuclei of the endothelial cells are large and vesicular, showing no evidence of being subjected to any pressure. In the 16 day (plus 12 hours) stage, however, these capillaries are beginning to dilate, and the endothelial nuclei are flatter as a result of the increased pressure from the expanding alveolus. In the 18 day (plus 12 hours) and in the 19 day stages, the capillaries are greatly dilated. At 20 days (plus 9 hours)(a few hours before delivery), they are enormously distended with red blood corpuscles, and the endothelial nuclei are very much flattened.

In regions where striated muscle is seen, large nerve trunks also appear lying in close proximity to the muscle. They are

probably destined for the most part to the supply of these
muscles. No attempt was made in this investigation to study the
nerve supply to the gland substance.

4. Changes in the ducts.--The epithelium lining the ducts in
the mammary gland of the white rat is double-layered throughout.
The inner cells are cylindrical or cuboidal, regularly arranged,
with their long axis perpendicular to the axis of the duct.

The cells making up the deep or peripheral layer of the duct
epithelium are smaller and more irregularly arranged. They are
irregularly flattened cuboidal and are arranged with their short
axis parallel with the long axis of the inner layer of cells, i.e.,
they tend to encircle the duct. (Fig. 8) In many places these
cells appear to send processes up for a short distance between the
cells making up the inner layer.

In the 13 day stage, the cytoplasm of the inner layer of duct
cells is finely granular, almost homogeneous, and shows no sign of
any secretory activity. This condition holds true until the 16
day (plus 12 hours) stage. In this stage a few fine vacuoles can
be seen in the cytoplasm of the inner layer of cells. These
vacuoles are not numerous but tend to show that secretory activity
on the part of these cells has begun.

From 16 days (and 12 hours) up to within a few hours of
delivery the vacuolization of the cytoplasm of the inner layer of
epithelial cells becomes progressively more and more pronounced and
at the end of pregnancy these cells appear to be actively secreting
milk. (Fig. 9)

The cytoplasm of the peripheral layer of duct cells is finely
granular and almost homogeneous, and even in the last stages of
pregnancy these cells show no sign of secretory activity.

In the 13 and the 15 day stages, the nuclei of the inner layer of cells are in many places elliptical and in others round. They occupy about the middle of the cell. They are for the most part so crowded that as a consequence there seems to be very little cytoplasm at the sides of the nuclei and the line of demarcation between the individual cells is extremely poorly defined. The chromatin is scattered in the form of granules, more or less uniformly throughout the nucleus, but is not plentiful enough to make the structure look dark. The nuclei are rather vesicular in appearance, but not so much so as the nuclei of the acini, which will be described later. The explanation for the fact that in some places the nuclei appear elliptical and in other places round may be that most of them are actually ellipsoidal, but appear round only when cut transversely to their long axis. After secretory activity begins in the cells of the inner layer, the nuclei of these cells appear to be more rounded and there is more cytoplasm between the individual nuclei.

The nuclei of the deep layer of cells are round, relatively small, and are placed in each case near the center of the cell.

It is these basal cells that have probably been mistaken by many authors (Benda, Bertkau, La Croix and others) for myo-epithelial cells. Their shape and the fact that they send up small processes between the cells above them suggest the myo-epithelial cells of the sweat glands. Their staining reactions and general appearances, however, in the ducts of the mammary gland of the albino rat, indicate that they are merely a basal layer of irregularly shaped and arranged epithelial cells. Some of the more common differential stains (Mallory's connective tissue stain, Van Gieson's stain, and cresyl violet) were used on these

preparations, and all of them show conclusively that these cells are epithelial in structure.

Whether or not they possess contractile properties I am unable to state, but as mentioned under the discussion on the smooth muscle in the nipple, there seems to be no reason for assuming that these cells must have contractile properties in order to aid in emptying the milk ducts.

5. Changes in the acini. -- The changes in the acini will be discussed in the following order: (1) Changes in the size of the alveoli, cells, and lumina; (2) cytoplasmic changes including secretion; and (3) nuclear changes.

The alveoli, in all stages, are lined with a single layer of columnar or cuboidal cells, which are arranged about a central lumen. The lumina of the alveoli increase in size during the latter half of pregnancy, from an average of 5.8 micra in the 13 day stage to 17.5 micra in the 20 day (plus 9 hours) stage. This increase progresses as follows:--13 days, 5.8 micra; 15 days, 7.2 micra; 16 days and 12 hours, 8.0 micra; 18 days and 12 hours, 19.1 micra; 19 days, 13.7 micra; 20 days and 9 hours, 17.5 micra.

While these changes were going on in the lumen of the alveoli, the average diameter of the alveoli was increasing as follows:-- in the 13 day stage, 30.8 micra; 15 day stage, 31.0 micra; 16 days and 12 hours, 32.0 micra; 18 days and 12 hours, 35.9 micra; 19 days, 36.8 micra; 20 days and 9 hours, 40.6 micra.

In the same stages, the average height of the epithelial cells was as follows:--13 days, 11.1 micra; 15 days, 10.7 micra; 16 days and 12 hours, 9.3 micra; 18 days and 12 hours, 8.5 micra; 19 days, 11.7 micra; 20 days and 9 hours, 12.1 micra.

The foregoing figures represent an average of 20 measurements for each stage. They are only approximate, of course, as the relatively few observations cannot be expected to give more than a rough index of the changes in the successive stages. However, the figures indicate that there has been a marked increase in the size of the lumina of the alveoli, and that there has been an increase in the total diameter of the alveolus, while the alveolar cells have undergone but little change in height.

The explanation for the slight progressive decrease in the height of the epithelial cells in the 15, 16 and 18 day stages is probably twofold. In the first place, the cells perhaps have not reached their full growth in width and hence are of necessity flattened out when the alveolus is becoming rapidly distended. In the second place the lumina of the acini in these stages are distended to their greatest capacity and the sheaths about them are stretched, it appears, to the limit. At the same time the lumina of the ducts are not so distended; in the next stage, however, the ducts are more distended and the amount of secretion in the lumina of the acini seems to have decreased, a result probably brought about by the emptying of the secretion out of the acini into the ducts, thus relieving the pressure on the alveolar epithelial cells. This might permit the increase in height noted at 19 and 20 days.

It might be asked here how the acinus could increase in size, the lumina increase in diameter, and at the same time the height of the epithelial cells remain practically constant, without an increase in the number of these cells? This leads us to the question of mitoses in the gland which will be taken up later. But a count of thirty acini in each stage showed that the average number

of cells about the lumina of the acini is eight,--the extremes in a single acinus being six and ten.

The explanation of the increase in the size of the acini with no increase in the number or decrease in the height of the epi- thelial cells, is that the cells enlarge, lose their high columnar form, widen out, and become low columnar or cuboidal. Thus the acinus can increase in size without an increase in the number of cells lining it, and with no marked change in absolute height.

The increase in the diameter of the entire alveolus is accounted for by the increase in the size of the lumen of the alveolus. The total increase from the 13th to the 20th day has been 10 micra, and in the same time the lumen has increased 11.7 micra. This discrepancy would probably disappear if a larger number of measurements on more specimens was taken.

Secretory changes.--The cytoplasm of the alveolar epi- thelium of the 13 day stage has a foamy appearance, which is due to the presence of a great many fine vacuoles. These vacuoles are for the most part small, round, discrete, and arranged irregularly throughout the cell. As before mentioned, they are so great in number and so close together that they give the cytoplasm of the cell the appearance of a fine lacework. In many places some of these vacuoles are seen to be confluent.

By the 15th day the vacuolization of the cytoplasm is much more pronounced than in the 13 day stage. This appearance is due no doubt to the enlargement of the single vacuoles and the coalescence of many others. As yet there is no sign of any secre- tion in the lumen of the alveoli or ducts (see fig. 10).

The cytoplasm of the acinar cells in the 16 day 12 hours stage shows merely an exaggeration of the condition in the previous stage.

That is, the vacuoles in the cytoplasm are much larger.)

In many places one observes what appears to be a "decapitation" of the cells, that is, a detachment of the inner portion adjacent to the lumen. Indeed, as mentioned in the review of the literature, some authors have held that the secretion of the mammary gland owes its origin, for the most part, to such a process.

A careful study of these cells shows, as a rule, one of two things. We find either a portion of the section which has been poorly cut, or a location in which a secretion droplet is so large that it has ruptured the cell membrane and carried before it the inner end of the cell, or (rarely) even the nucleus.

In regard to the first possibility it should be emphasized that unless one is extremely careful in all the steps of his technique, the specimen will show various distortions and artefacts. In sections cut with a knife that is slightly dulled one will often get sections in which the whole epithelial lining of a duct is being displaced en masse, leaving the naked connective tissue behind, or there will be alveoli fragmented and blood vessels ruptured. This, of course, would be an extreme case, but much less distortion might readily produce appearances of "decapitation" in the lining epithelial cells. This fact was pointed out by O'Donoghue ('11) in the course of his work on the marsupials. In this connection it may be noted that if the tissue happens to be a piece of the gland imbedded while still attached to the skin, and it is cut so that the knife passes through the skin before it passes through the gland, there will be many artefacts, which will not be present if the knife passes first through the softer glandular part of the tissue.

As regards the second possibility, many of the cells contain secretion droplets larger than the nucleus of the corresponding cell. It would seem impossible for such large droplets to get out of the cell without doing at least some damage to the cell membrane. It must be borne in mind that here we are dealing with a condition different from that which obtains in certain mucous cells in which extremely large secretion droplets are formed. In the latter, the nucleus and cytoplasm are pushed to the base of the cell and the cell membrane--if any--has a chance to regain its integrity before the cytoplasm can be extruded. In the mammary gland cell, however, some of the cytoplasm may be pushed ahead of the secretion droplet and forced out with it. Occasionally, as before mentioned, even the nucleus is carried ahead of the droplet.

Still another fact leads me to believe that this so-called "decapitation" process is accidental and not an essential part of milk formation, and that is the relative scarcity of such appearances. Indeed the more perfect the section, the fewer of these "decapitated" cells appear.

In other places in the 16 day stage one sees a seemingly ragged edge to some of the alveolar epithelial cells next to the lumen, an appearance which is probably due to an actual secretion being extruded from the cell. (   )

In this 16 day stage, there is, in the lumina of the ducts and acini, quite an amount of secretion which is homogeneous, lightly staining, and contains many vacuoles, resulting perhaps from the dissolving out of the fat contained in it.

The cytoplasm of the acinar epithelial cells is very much more vacuolated in the 18 day (plus 12 hours) stage than in any of the stages previously described. There are many more of the vacuoles

which are larger than the nucleus of the containing cell than there were in the preceding stage. There are also many more examples of secretion granules or droplets being extruded from the alveolar cells. (Fig.11)

In the lumina of the acini, besides the secretion already described, there is some granular debris which by its staining reaction with cresyl violet appears to be of nuclear origin. This debris perhaps comes from the nuclei which have been pushed out by the extremely large secretion droplets in the manner described in the foregoing stage. Does this nuclear debris give rise to the nucleo-proteins of the milk? It undoubtedly contributes, at least in these stages studied, but as to whether it forms an essential constituent of the secretion or not I am unable to state.

By the 19th day the height of the secretory activity of the alveolar cells seems to have been reached, at least no further vacuolization of the cytoplasm is observed. From this stage up to within a few hours of delivery no changes in the cytoplasmic struc-ture of the cells can be observed. There is abundant secretion present in the lumina of the ducts and acini. (Fig.12)

The nuclei of the acinar cells show no observable change during the period from the 13th day to within a few hours of birth. These nuclei are about 6.07 micra in diameter (an average of twenty measurements in each stage). Since they do not increase in size as the epithelial cell increases, they appear relatively smaller in the latter stages of pregnancy.

The chromatin of these nuclei has a very definite arrangement. The chromatin masses are arranged about the periphery of the nucleus and a well defined nucleolus in about the center. (Fig. 12) This arrangement of the chromatin corresponds to that

described by Szabo ('96) in the epithelial cells of the active
mammary gland of guinea pigs.

Only two unquestionable mitoses were observed in the stages
studied. Both of these were in the 13 day stage. (Fig. 12   )
Furthermore, no definite evidence of amitosis could be observed.
The absence of cell division, together with the fact that there is
no increase in the number of cells about the alveoli in the stages
studied, indicates that the gland has already reached its maximum
growth (so far as number of cells is concerned) by the 13th day of
pregnancy.

6. Colostrum formation.--In studying the epithelium of the
ducts and acini, nuclei which resembled lymphocytic or leucocytic
nuclei were observed in many places. These nuclei were apparently
passing through the wall of the ducts and acini. Unfortunately
none of the material used in this study had been fixed in Helly's
fluid and hence no satisfactory Giemsa or Dominici stain could be
used, which would have shown more clearly whether or not these
nuclei belong to the blood elements.

There are, however, evidences indicating that these nuclei
belong to cells of the wandering type. First, one must bear in
mind that there is not a great deal of irregularity in the
position of the epithelial nuclei of the ducts or acini, that is,
they are about on the same level. These suspected nuclei, however,
are usually seen either above or below the level of the epithelial
nuclei. They are much smaller and much more densely staining than
the nuclei of the epithelial cells, and their chromatin masses,
though dense, have fairly definite outlines. (Fig. 8)
Occasionally they are surrounded by a clear space and in one case

at least presented a definite, pale green, narrow rim of surround-
ing cytoplasm, in a section stained with Cresyl Violet.

Since these sections were stained with iron haematoxylin or
cresyl violet, it is possible that these nuclei might be pycnotic
epithelial nuclei. If they were, however, one would not expect to
see the chromatin masses so clearly outlined and the whole nucleus
should appear more uniformly shrunken and black in color. Their
position, the appearance of their chromatin, and their relation to
the epithelial cells, all point strongly to the conclusion that
these nuclei belong to wandering cells.

There are other more general phenomena which support this
conclusion. Leucocytic infiltration of the gland has been observed
by various investigators in several other species, in the first
half of pregnancy. The stroma is rich in cellular elements in the
second half of pregnancy and (as noted in the literature) in
several other forms leucocytes and lymphocytes have been found a
constant constituent of colostrum.

So far as this study goes, all I am able to state is that my
material does not prove that these nuclei are of the wandering cell
type, but the appearance they present strongly indicates this con-
clusion. As previously mentioned, sections with specific blood
stains must be carefully studied before a final conclusion is
justified.

This leads to a consideration of the origin of colostrum
corpuscles in the rat. In the lumina of the ducts (less often of
the acini) in the latter stages of pregnancy fatty cells are
observed which are lying free in the mass of secretion. These cells
begin to appear in the 16 day 12 hour stage and increase in number
from then until the end of pregnancy. Previous to the 16 day

12 hour stage a few desquamated epithelial cells were observed in the lumina of the ducts, but since they were not fatty they do not represent true colostrum corpuscles. The cells in the latter stages are in most cases as large as the epithelial cells of the gland; they have many fatty vacuoles, and the nucleus for the most part is large, round, and vesicular, appearing identical with the nuclei of the epithelial cells.

In other cases, these cells are smaller, the cytoplasm is not quite so vacuolated, and the nucleus resembles that of a leucocyte or lymphocyte. A third class of these cells, which occur more rarely, resembles macrophages, in that they have a crescent shaped nucleus and abundant, lightly-staining cytoplasm, filled with a great many vacuoles. (For the various types of colostrum corpuscles see fig. 9)

What is the origin of these cells? I have already discussed the probability that the cells resembling lymphocytes and leuco-cytes might actually be such, which have wandered through the walls of the ducts and acini. Such cells, either in the lumina of these structures or before they pass through their walls, may have become laden with fat, although no fatty leucocytes were observed anywhere in the stroma.

As to the origin of those occasional cells which resemble macrophages, it is impossible to state definitely. The different shape of the nucleus might be accidental or due to some degenerative process. However, Professor Downey (who kindly examined some of my preparations) called my attention to the presence of some large, apparently phagocytic cells in the interalveolar spaces. A careful study with special stains might possibly show that these large phagocytes occasionally pierce the walls of the ducts or acini.

The nuclei of the large interalveolar cells appeared rounded, however, rather than crescent shaped, as found in the intra-alveolar cells. But the nucleus is often variable in this type of cell.(Fig.13)

Most of the colostrum corpuscles, however, are undoubtedly desquamated cells derived from the epithelium of the ducts and acini. I have been able to trace them in all stages from the beginning of their desquamation to the time that they have become rounded, fully formed colostrum corpuscles. Some appear breaking off from the epithelial wall but still partly attached. Others are lying close to the wall of the duct, but still maintaining a more or less cubical shape. Finally they appear as rounded cells, this shape being assumed when the cells become free in a liquid medium. (For these stages, see fig. 14)

Occasionally two adjacent cells may be detached at the same time, lying free in the lumen of the duct though still remaining attached to each other. (Fig. 9)

Further evidence that these cells are epithelial in origin is shown by the fact that when they are observed in the ducts of the 16 day 12 hour stage they are not as vacuolated as they are later. In other words the cytoplasm of the earlier colostrum corpuscles is in the same condition as that of the epithelial cells lining the ducts. The nuclei, with the exception of the crescent shaped, and the smaller (probably leucocytic) nuclei, appear in most cases identical with the epithelial nuclei.

The scarcity of these cells in the lumina of the acini, indicates that by far the greater number of them come from the duct epithelium.

It should be emphasized that this desquamation of epithelial cells is not a pathological condition, nor is it an artefact due to faulty technique. I have excluded all cases where there is an extensive desquamation of cells, or where the walls of the ducts, acini, or blood vessels indicate a previous injury or abnormality. Only those cases were chosen for study in which the gland appeared perfectly normal in structure.

Before leaving the question of the origin of colostrum corpuscles, we may consider the theory of Steinhaus ('93) and Unger ('98), according to which mast cells are thought to be transformed into colostrum corpuscles. Attention has already been called to the large number of mast cells in the stroma of the mammary gland of the albino rat during pregnancy. That This is a fairly constant occurrence in other forms, as well as in the rat, is indicated by the reports of Unger ('98), Steinhaus ('9$\overset{2}{5}$), Michaelis ('98) and Berka ('11).

Steinhaus ('9$\overset{2}{5}$) states, after describing the number and appearance of the mast cells in the mammary gland of the pregnant guinea pig, "S$\overset{\varrho}{\text{o}}$ viel es scheint, sind die Colostrum$\overset{k}{\text{kö}}$rperchen einfach Mastzellen mit verfetteten eosinophilen Körnern". A close study of his figures, however, fails to show a single mast cell undergoing fatty metamorphosis, or passing through the walls of the ducts or acini, or in the lumina of these structures. Furthermore the granules of mast cells are obviously not eosinophilic.

Unger ('98) is more definite in his statements regarding the relation of mast cells to colostrum. He found these cells in the glands of humans and guinea pigs, in large numbers around the blood vessels and ducts, between the fat cells in the retro-mammary fat tissue, between the alveoli and sending up processes between the

epithelial cells, in the perialveolar lymph and blood spaces, and in the axillary and retro-mammary lymph nodes. He claims to have demonstrated fat granules in the bodies of these cells, but other authors, particularly Bertkau ('07) and Berka ('11) have since questioned whether these cells were actually mast cells.

Unger concludes by approving Czerny's theory of the leucocytic origin of colostrum corpuscles, but goes farther in ascribing to the mast cells the major rôle in this process.

Berka ('11), however, in discussing this theory, states that the mere presence of these mast cells in the gland during pregnancy does not prove that they aid in forming colostrum. Furthermore he was unable to demonstrate any fat in a single one of the mast cells.

In the case of the rat, it is an easy matter to settle this question, for here we have mast cells in great numbers. Their distribution has already been described under stroma. (Fig.3)

If the mast cells take any part in the formation of colostrum, evidence should appear in the following respects. (1) If the mast cells undergo fatty metamorphosis, this process should appear even in ordinary stained sections. (2) Some of these cells should be seen passing through the walls of the ducts or acini. (3) Some of them should be seen free in the lumina of these structures.

In a careful study of hundreds of sections, I have not seen a single mast cell undergoing fatty metamorphosis and in no case was a mast cells observed in the walls of the ducts or acini or within the lumina. It is therefore certain that in the rat, x at least, mast cells take no direct part in colostrum formation.

The picture presented, then, is one in which there is an increasing number of cellular elements in the secretion of the mammary gland as pregnancy advances. These cells present various

stages of fatty vacuolization and represent true colostrum corpuscles. The following conclusions therefore appear justified on the basis of the evidence which has been presented.

1. Colostrum corpuscles in the rat are independent nucleated cells. This confirms the view of practically all modern investigators with the exception perhaps of Benda ('93) who regards free cells in the lumina of the ducts and acini of the mammary gland as artefacts.

2. Colostrum corpuscles originate for the most part from desquamated epithelial cells. This agrees with the conclusions of Reinhardt ('47), v. Beuren ('49), Will ('49), Scanzoni ('55), Stricker ('66), Kehrer ('71), Langer ('71), de Sinety ('75), Buchholz ('77), Coen ('88), Kadkin ('90), and Palazzi ('00), but disagrees with Rauber ('79), Saefftigen ('81), Czerny ('90), Benda ('93), Steinhaus ('93), v. Ebner ('96), Unger ('98), Cohn ('00) and Bab ('04).

3. Some of the colostrum corpuscles may arise from immigrated lymphocytes or leucocytes. Palazzi ('00), so far as I know, is the only author who calls specific attention to the double origin of colostrum corpuscles.

4. Mast cells play no direct part in the formation of colostrum corpuscles. This is in agreement with the conclusions of Cohn ('00) and Berka ('11), and opposed to the claims of Steinhaus ('93) and Unger ('98).

VI. Two principal phases.

The picture presented by the mammary gland of the albino rat in the latter part of pregnancy is that of a gland which has already reached its maximum growth, so far as a multiplication of

elements is concerned. From the thirteenth day on, any enlarge-
ment is due chiefly to a mechanical distention of the hollow
structures of the organ, together with hyperemia, although there
is also hypertrophy of the glandular cells.

The gland is undergoing striking histological changes, which
are directed towards the elaboration of secretion. As a result,
the parenchyma is the part of the gland that is changing, while
the stroma (aside from the increased vascularity) remains
practically unchanged, excepting secondary changes due to pressure
associated with the growth and expansion of the parenchyma.

The secretory activity of the gland is evidenced by a hyper-
trophy of the glandular elements. The acini increase in size,
their lumina enlarge, and their epithelial cells become much
larger. The cytoplasm of the epithelial cells becomes progressive-
ly vacuolated, first in those of the acini, and later in those
lining the ducts. The cells remain clearly outlined and do not
lose their definite form, and the nuclei do not change in position
or appearance. These facts indicate that the cytoplasmic
vacuolization is the result of the secretory activity of the cell,
and is not a sign that the cell is undergoing fatty metamorphosis
or necrobiosis.

Two stages can therefore be recognised in the changes of the
mammary gland of the albino rat during pregnancy. These two
phases, as described by Ancel and Bouin ('11) and Schil ('12), in
the rabbit, are (1) a phase of growth activity, and (2) a phase of
secretory activity. The first (which occurs in stages earlier
than those covered in the present paper) is marked by the addition
of new elements--an increase in the number of ducts and lobules,

and an infiltration of lymphocytes and leucocytes. The second
phase begins in the latter half of pregnancy (the period studied
in the present investigation). Here the epithelial cells begin
to elaborate secretion and from now on any growth is due chiefly
to cellular hypertrophy and enlargement by accumulation of
secretion and by hyperemia. At the same time the leucocytic
infiltration is becoming progressively less prominent. The forma-
tion of colostrum also falls within this period.

VII. Summary.

The conclusions from a study of the mammary gland in the
albino rat from the 13th to the 20th day (or end of pregnancy)
may be summarized briefly as follows.

1. The nipple increases in size during pregnancy, but has
apparently reached its maximum growth by the 13th day. The epi-
thelial hood about the nipple entirely disappears during
pregnancy, resulting in an elongation of the nipple, and at the
same time the smooth muscle about the main duct has become well
developed.

2. The stroma, aside from progressive increase in the blood
vessels, and an increase in the elastic tissue (especially around
the ducts) shows no marked changes. There is no membrana propria
around the acini, aside from a thin connective tissue sheath
formed by their distention.

3. There is a tremendous proliferation of the mammary
parenchyma during pregnancy. Hyperplasia is evidently confined
bhiefly to the first half of pregnancy, as mitoses are rare from
the 13th day and amitoses apparently absent. The ducts and acini
continue to increase in size, however, partly through hypertrophy

of the epithelial cells, and partly by accumulation of secretion within the lumina.

4. Progressive cytoplasmic vacuolization, indicating secretory activity, is apparent in the acinar epithelium from the 13th day, and some days later in the cells lining the ducts. The maximum degree of vacuolization is reached by the 19th day. "Decapitation", or detachment of the inner portion of the acinar cells during secretion, is a rare occurrence and does not represent the usual mode of secretion in the stages observed. The secretory cells usually remain intact.

5. Colostrum corpuscles are derived chiefly from desquamated epithelial cells of the ducts and acini. Some may be derived from immigrated lymphocytes or leucocytes. Mast cells are abundant in the stroma, but take no direct part in the formation of colostrum corpuscles.

VIII. Bibliography.

Ancel and Bouin, 1912 Sur l'évolution de la glande mammaire pendant la gestation; determinisme de la phase glandulaire gravidique. C.R. Soc. Biol., T. LXXII, p. 129.

Bab, Hans 1904 Die Colostrumbildung. Diss. Berlin.

Barfurth, D. 1882 Zur Entwickelung der Milchdrüse. Diss. Bonn. (Cited by v. Ebner and Bizzozero and Ottolenghi.)

Beigel, H. 1868 Über die mikroskopische Zusammensetzung der Milch des Weibes. Virchow's Arch., Bd. 42, p. 442.

Benda, C. 1893-4 Das Verhältnis der Milchdrüse zu den Hautdrüsen. Dermatol. Zeitschr., Bd. I, p. 94-110. (Cited by Bizzozero and Ottolenghi.)

Berka, F. 1911 Die Brustdrüse verschiedener Alterstufen und während der Schwangerschaft. Frankfurter Zeitschrift für Pathologie. Bd. 8, p. 203-256.

Bertkau, F. 1907 Ein Beitrag zur Anatomie und Physiologie der Milchdrüse. Anat. Anz., Bd. 30, p. 161-180.

Bierich, R. 1900 Untersuchungen über das elastische Gewebe der Brustdrüse im normalen Zustande und bei Geschwülsten. Diss. Königsberg.

Billroth, Th. 1887 Diseases of the Female Mammary Glands. Cyclopedia of Obstetrics and Gynecology. Vol. IX. Wm. Wood and Company, New York.

Bizzozero and Ottolenghi, 1899 Histologie der Milchdrüse. Ergeb. f. Anat. u. Entw., Bd. 9, p. 253.

Bizzozero e Vassale, 1887 Sulla produzione e sulla rigenerazione fisiologica degli elementi ghiandolari. Arch. f. le scienze mediche. Vol. XI. (Also) Über die Erzeugung und die physiologische Regeneration der Drüsen zellen bei den

Säugetieren. Virchow's Arch., Bd. 110, p. 155.

Buchholz, W. 1877 Das Verhalten der Kolostrumkörper bei unter-
lassener Säugung. Diss. Göttingen. (Cited by Bizzozero and
Ottolenghi.)

v. Beuren, R. L. 1849 Die Entwickelung der Formbestandteile der
Milch. Nederl. Lancet, Juli. (Cited by Michaelis, Bab, and
Bizzozero and Ottolenghi.)

Coen, E. 1888 Beiträge zur normalen und pathologischen
Histologie der Milchdrüse. Ziegler's Beiträge z. path. Anat.
u. Physiol., Bd. II, p. 83-100.

Cohn, Mich. 1900 Über Frauenmilch. Münchener Med. Wochenschrift,
p. 753. (Cited by Bab and Bizzozero and Ottolenghi.)

Czerny, A. 1890 Über die Brustdrüsensekretion beim Neugeborenen
und über das Verhältnis der Sogenannten Kolostrumkörperchen
zur Milchsecretion. Sep. Abdr. aus d. "Festschr. zu
E. Hennochs 70. Geburtstag". 34 p. (Cited by Michaelis,
Bab, Steinhaus and Unger.)

Donné, Al. 1837 Du lait et en particulier de celui des nourrices,
considéré sous le rapport de ses bonnes et de ses mauraises
qualités nutrives et de ses altérations. Paris. (Cited by
all the workers on colostrum.)

Duclert, L. 1893 Étude histologique de la sécretion du lait.
Montpellier, These. 79 p. (Cited by O'Donoghue, and
Bizzozero and Ottolenghi.)

v. Ebner, V. 1896 Kölliker's Gewebelehre. 6 Auflage, Bd. III,
p. 593.

Frank and Unger, 1911 An Experimental Study of the Causes which
produce the Growth of the Mammary Gland. Arch. of Int. Med.,
Vol. 7, p. 812⁴

Fürstenberg, M. H. F., 1868 Die Milchdrüsen der Kuh. Leipzig.
215 p. (Cited by Bab.)

Heidenhain, R. 1880 Die Milchabsonderung. Hermann's Handbuch d.
Physiol. Leipzig.p.374-406. (Cited by v. Ebner, Bab, Unger,
Schmidt.)

Jakowski, S. M. 1880 Über die Milchdrüse des Menschen und der
Thiere. (Russian). Abhandlungen u. Sitzungsberichte der
Naturwiss.-mathem. Classe der Akad. der Wissensch. zu
Krakau. Bd. 7, (Reviewed in Jahresbericht über Anat. u.
Physiol., Bd. IX, 1880, p. 272.)

Kadkin, 1890 Beiträge zur Anatomie der Milchdrüsen während ihrer
Thätigkeit. Diss. Petersburg. (Original in Russian, reviewed
in Jahresbericht über Anat. u. Physiol., Bd. 20, 1891, p.481.)

Kehrer, F. A. 1871 Zur Morphologie des Milchkaseins. Arch. f.
Gyn., 28 p.

Kölliker, Th. 1850 Beiträge zur Kenntniss der Milchdrüse.
Verhandl. der Phys.-Med. Gesellschaft zu Würzburg, N.F. Bd. 14,
18 p., 3 Taf. (Cited by Bab.)

Kolessnikow, N. 1877 Die Histologie der Milchdrüse der Kuh und
die pathologisch-anatomischen Veränderungen derselben bei der
Perlsucht. Virchow's Arch., Bd. 70, p. 531-546.

Kollossow, A. 1898 Eine Untersuchungsmethode des Epithelgewebes,
besonders der Drüsenepithelien und die erhaltenen Resultate.
Arch. f. mikr. Anat. u. Entw., Bd. 52, p. 1-44?2

Lacroix, E. 1894 De l'existence de cellules en paniers, dans
l'acinus et les conduits excreteurs de la glande mammaire.
C.R. de l'Acad. des Sciences de Paris. T. 119, p. 748-751.
(Cited by Bizzozero and Ottolenghi.)

Langer, C. 1871 Die Milchdrüse. Stricker's Handbuch der Lehre
von den Geweben. I. Bd., p. 627-634.

Lane-Claypon and Starling, 1906 An experimental enquiry into the
factors which determine the growth and activity of the
mammary glands. Proc. of the Royal Soc., Series B, Vol. 77,
p. 505-

Leeuwenhoek, 1680 Arcana Natura Detecta.

Michaelis, L. 1898 Beiträge zur Kenntnis der Milchsekretion.
Arch. f. Mikr. Anat. u. Entw., Bd. 51, p. 711-747.

Myers, J. A. 1916 Studies on the mammary gland. I. The growth
and distribution of the milk-ducts and the development of the
nipple in the albino rat from birth to ten weeks of age. Am.
Jour. Anat., Vol. 19. (Abstract also published in the Anat.
Rec., 1916, Vol. 10, p. 230.)

_____ 1917 Studies on the mammary gland. II. The fetal develop-
ment of the mammary gland in the female albino rat. Am. Jour.
Anat., Vol. 22. (Abstract also published in the Anat. Rec.,
1917, Vol. 11, p. 390.)

_____ 1919a Studies on the mammary gland. IV. The histology of
the mammary gland in the male and female albino rat from birth
to ten weeks of age. Am. Jour. Anat., July, 1919. (Abstract
also published in the Anat. Rec., Vol. 14, p. 46.)

_____ 1919b Studies on the mammary gland. V. The effects of
inanition on the developing mammary glands in male and female
albino rats. Amer. Jour. of Diseases of Children, May, 1919.

Myers, J.A. and Myers, Frank J. 1919 Studies on the mammary
gland. VII. The distribution of the subcutaneous fat and
its relation to the developing mammary glands in male and
female albino rats from birth to ten weeks of age. Abstract
in Anat. Rec., May.

Nissen, F. 1886 Über das Verhalten der Kerne in den Milchdrüsen-
zellen bei der Absonderung. Arch. f. Mikr. Anat. u. Entw.,
Bd. 26, p. 337-342.

Ottolenghi, S. 1889 I corpuscoli del colostro ed i globuli lattei
in rapporto alla medicina legale. Giorn. della R. Accad. di
Med. Torino. Nr. 8, p. 1-7. (Cited by Bizzozero and
Ottolenghi.)

O'Donoghue, C. H. 1912 Growth Changes in the Mammary Apparatus
of Dasyurus. Quarterly Journal of Micro. Science. Vol. 57.

Partsch, C. 1880 Über den feineren Bau der Milchdrüse. Diss.
Breslau. 30 p. (Cited by Bizzozero and Ottolenghi.)

Palazzi, G. 1894 Sopra alcune differenze microscopiche fra la
secrezione mammaria durante la gravidanza e quelle finito
l'allattamento. Ann. di Obstetricia e Ginec., Agosto,
p. 1-31. (Cited by Bab.)

Rauber, A. 1879 Bemerkungen über den feineren Bau der Milchdrüsen.
Schmidt's Jahrb., Bd. 182, p. 7-8.

Reinhardt, B. Über die Entstehung der Körnchenzellen. Virchow's
Arch., Bd. 1, p. 52-64.

Saefftigen, 1881 Bull. de l'Academie des Sciences de
St.Pétersbourg, T. 28, p. 70. (Cited by Bab and Szabo.)

Scanzoni, 1855 Ueber die milch-secretion und die entzündlichen

  Anschwellungen der Brustdrüsen bei Neugeborenen. Verhandl.

  der Physic.-Med. Gessellschaft in Würzburg, Bd. II, p. 302.

Schil, L. 1912 Recherches sur la glande mammaire, sur les phases

  qu'elle présente au cours de son évolution et leur.

  determinisme. Thèse, Lyon.

Schmid, H. 1877 Zur Lehre von der Milchsekretion. Diss.

  Würzburg. (Cited by Bizzozero and Ottolenghi.)

Schmidt, Mül. 1883 (Cited by Bab without reference being given.)

de Sinety, M. 1875 Recherches sur la mamelle des enfants

  nouveaux-nés. Arch. de phys. norm. et pathol., T. 2,

  p. 291-302. (Cited by Bizzozero and Ottolenghi.)

Steinhaus, J. 1892 Die Morphologie der Milchabsonderung. Arch.

  f. Anat. u. Phys. Suppl. Bd. Physiol. Abt.

Sticker, A. 1899 Zur Histologie der Milchdrüse. Arch. f. mikr.

  Anat. u. Entw., Bd. 54, p. 1-23.

Stricker, S. 1866 Über kontraktile Körper in der Milch der

  Wöchnerin. Sitzungsber. d. Akad. d. Wiss., Wien, Bd. 53,

  II. Abt., p. 184.

Szabó, J. 1896 Die Milchdrüse im Ruhezustande und während ihrer

  Thätigheit. Arch. f. Anat. u. Physiol., Anat. Abt.,

  Jahrg. 1896, p. 352-359.

Talma, S. 1882 Beitrag zur Histogenese der Weiblichen Brustdrüse.

  Arch. f. mikr. Anat., Bd. 20, p. 145-

Unger, E. 1898 Das Kolostrum. Virchow's Arch., Bd. 151.

Virchow, R. 1858 Cellularpathologie. I. Aufl.

Wahl, H. M. 1915 Blood Vessels of the Mammary Gland. Am. Jour.

  of Anat., Vol. 18, p. 515-

Will, J. G. F. 1850 Über die Milchabsonderung. Akad. Festschr.
    Erlangen. (Cited by Bab.)

Winkler, F. N. Beitrag zur Histologie und Nervenverteilung in
    der Mamma. Arch. f. Gyn., Bd. 11, p. 294-303.

IX.   Explanation of Figures.

Fig. 1.   Drawing of nipple.   Rat 16 days, 12 hours pregnant. Shows that epithelial hood has entirely disappeared leaving a groove, E.H., in its place.   This groove contains some granular debris, D.   This also shows the well developed smooth muscle, S.M., about the main duct, M.D., of the nipple.   The smooth muscle does not extend up to the nipple pore, N.P.   A hair follicle, H.F., and a sebaceous gland, S.G., are shown on the left.   Zenker's fixation, Weigert's iron haematoxylin counterstained with Van Gieson's stain.   7 micra.   X 100 (approx)

Compare this section with fig. 14 in Myers' ('19b) paper, which shows only a slight degeneration of the hood.

Fig. 2.   This is a lower power drawing of a section of one of the abdominal mammary glands of a rat 18 days, 12 hours pregnant. Drawing does not show panniculus carnosus muscle.   BV, blood vessel; AT, adipose tissue; D, duct; DS, duct sheath; L, lobule; IA, interalveolar connective tissue; A, alveolus.   Zenker's fixation.   Mallory's connective tissue stain.   5 micra.   X 120

Fig. 3.   Drawing through duct and neighboring acini.   Rat 15 days pregnant.   This section shows the presence of mast cells, as well as the rapid thinning of the fibrous sheath of the duct, as this sheath passes about the lobule at Lb.   BL, basal layer of duct epithelium; L, lumen of duct; M, mast cell; M', mast cell between two alveoli; IL, inner layer of duct epithelium.   At this stage shows no vacuolization.   LA, lumen of alveolus; AC, vacuolated alveolar cytoplasm; CTC, cellular elements of connective tissue.   Zenker's fixation.   Cresyl violet stain.   4 micra.   X 300

Fig. 4. Alveolus from gland of rat 13 days pregnant. Connective tissue sheath, CT, is relatively loose. Endothelial cell nuclei, E, show no pressure effects. Mitotic figure, M, shown in one of the cells. Zenker's fixation. Weigert's iron-haematoxylin, counterstained with Van Gieson's stain. 4 micra.

Fig. 5. Acini from gland of rat, 18 days, 12 hours pregnant. IACT, interalveolar connective tissue is seen to be stretched about acinus giving appearance of a membrana propria. A mass of secretion, S, is seen in the lumen of the central acinus. Secretion granules, S, are seen being extruded from one of the acinar cells. A distended capillary, C, and a mast cell, MC, are also shown. Zenker's fixation. Cresyl violet stain. 4 micra.

Fig. 6. Sketch showing elastic fibers, E, about a duct, D. A well defined elastic sheath, LS, is seen about the lobule. The alveoli, AL, show almost no elastic tissue about them. An arteriole is seen at A. Section from gland of a rat 5-1/2 months old, 13 days pregnant. Zenker's fixation. Unna's orcein stain. 4 micra.

Fig. 7. Compare this section with the one shown in fig. 6. Here there are a great many more elastic fibers, E, about the duct, D. The lobular sheath, LS, is well defined and the acinar elastic sheaths, AS, have appeared. An alveolus is labeled at A. AT is adipose tissue. Section from rat 3-1/2 months old; 18 days, 12 hours pregnant. Zenker's fixation. Unna's orcein stain. 4 micra.

Fig. 8.   Section through wall of duct of gland of rat 13 days pregnant.   The basal layer of cells, BL, are shown with a process, P, sent up between the cells of the inner layer, IL.   This section also shows a lymphocyte (?),L, in the wall of the duct and surrounded by a clear space, CS.   Another suspected nuclei is shown at L'.   Zenker's fixation.   Cresyl violet stain.   5 micra.

Fig. 9.   Portion of wall of duct and colostrum corpuscles in lumen.   From gland of rat 19 days pregnant.   IL, inner layer of cells showing secretory activity of cytoplasm; BC, basal cell. No vacuoles are present in any of the basal cells.   DS, duct sheath; C, colostrum corpuscle with nucleus identical with those of the inner layer of epithelial cells of the ducts; C', colostrum corpuscle with double or fragmented nucleus; M, colostrum corpuscle which resembles fatty macrophage.   Note crescent shaped nucleus and abundant cytoplasm.   EC, desquamated epithelial cells still adherent to each other--seemingly end to end.   Zenker's fixation.   Cresyl violet stain.   6 micra.   X 750

Fig. 10.   Acinus from gland of rat 13 days pregnant.   Shows presence of many fine vacuoles in cytoplasm of the cells.   Section is tangential so some of the cells appear to have more than one nucleus.   A lymphocytic-like nucleus is shown at L, surrounded by Zenker's fixation.   Cresyl violet stain.   6 micra.   X 750

Fig. 11.   Acinus from gland of rat 18 days, 12 hours pregnant. Cytoplasm much more vacuolated than in fig. 10.   S, secretion mass; ND, nuclear (?) debris; Se, secretion being extruded from cell. Zenker's fixation.   Cresyl violet stain.   6 micra.   X 750

Fig. 12. Acinus from gland of rat 19 days pregnant. Secretion mass with fat vacuoles, F, seen in lumen. This secretion shows characteristic arrangement of chromatin in nucleus, N. A well defined nucleolus, Ns, is shown. Zenker's fixation. Cresyl violet stain. 6 micra. X 750

Fig. 13. Portion of wall of duct of gland of rat 19 days pregnant. A large cell, M, is seen in the wall of the duct. This cell has staining reaction different from that of the epithelial cells. This cell might possibly be a macrophage. C represents a greatly distended capillary. Zenker's fixation. Mallory's connective tissue stain. 7 micra. X 300.

Fig. 14. A, Section through wall of large duct of gland from rat 18 days, 12 hours pregnant. Epithelial cell, EC, being desquamated. Basal cells shown at BC.

B, Wall of smaller duct from same section. Epithelial cell, C, shown free in lumen; it has not yet lost its cylindrical shape. BC, basal cell.

Zenker's fixation. Mallory's connective tissue stain. 6 micra. X 750
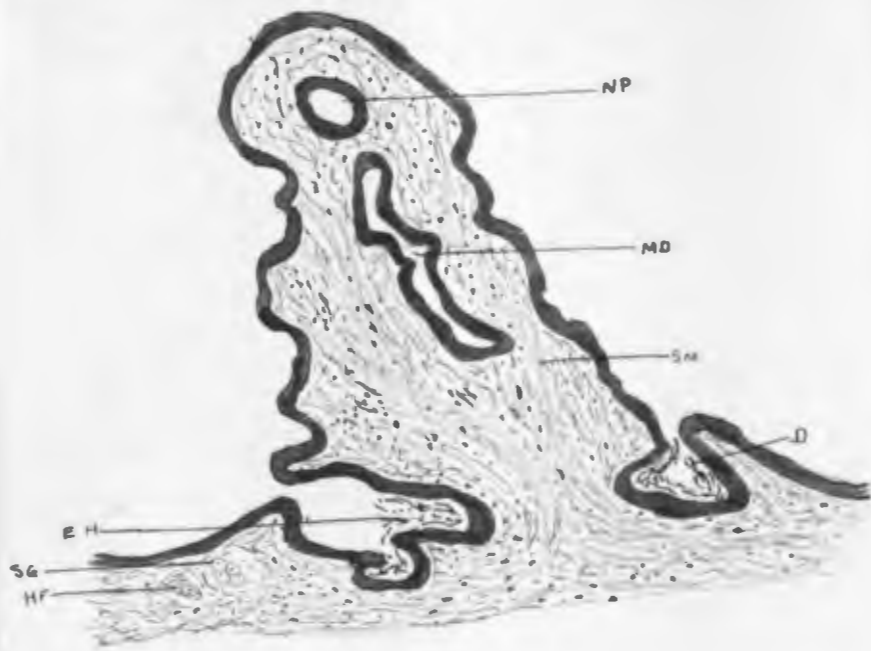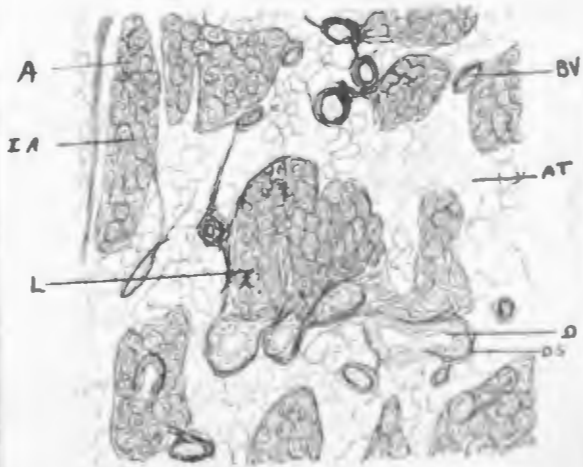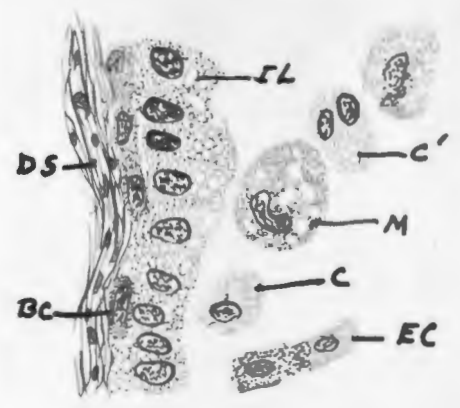
Fig. 1.

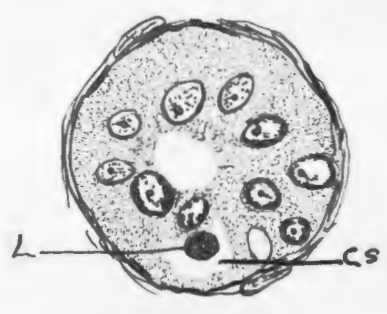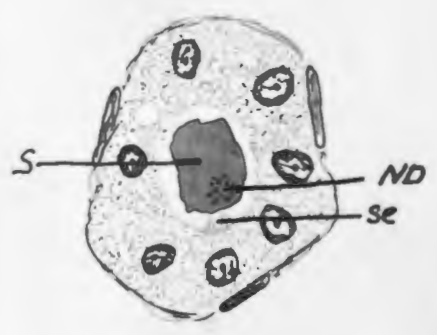Fig. 2.   x

Fig. 3
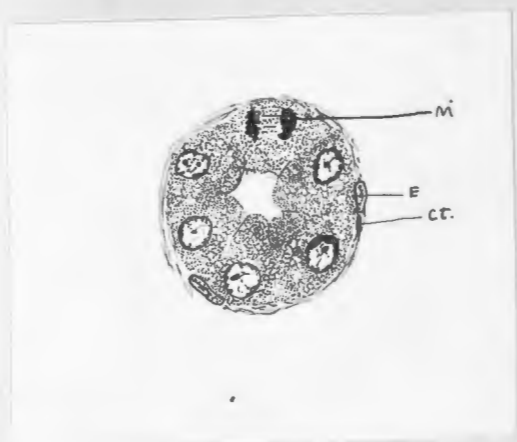
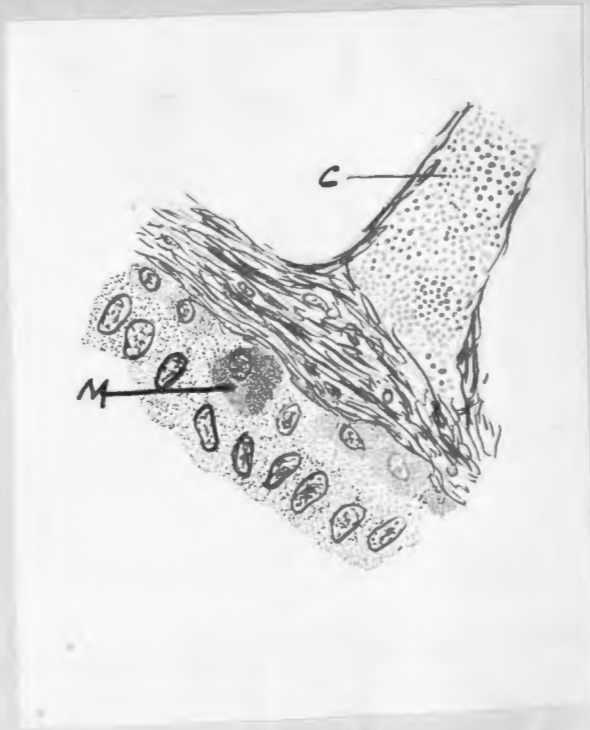Fig. 4.
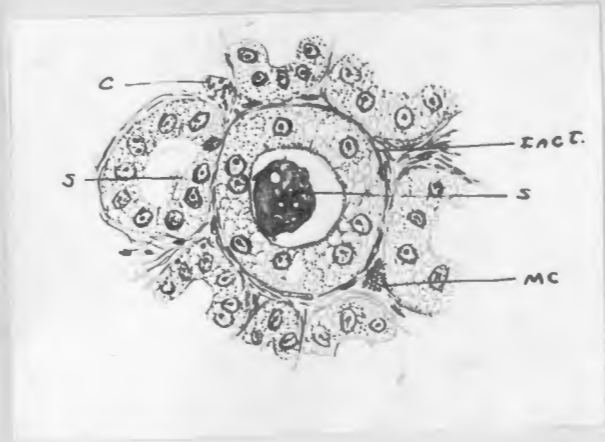
Fig. 5.

Fig. 6.
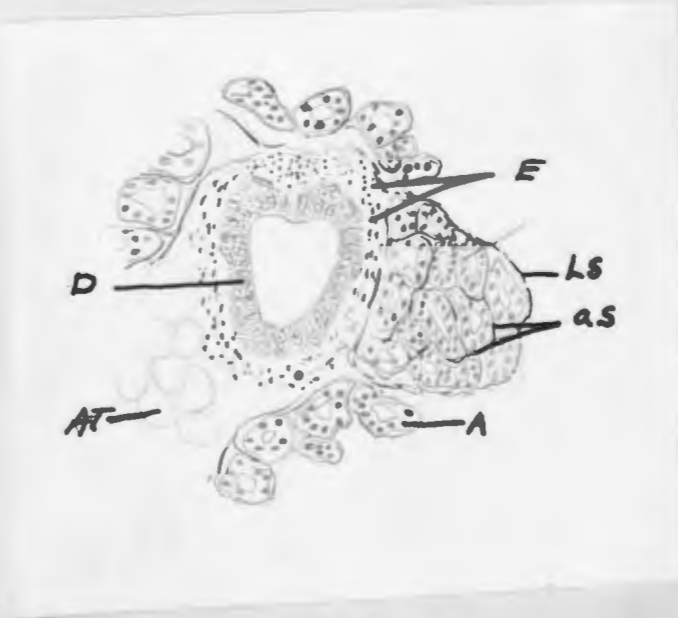
Fig. 7.
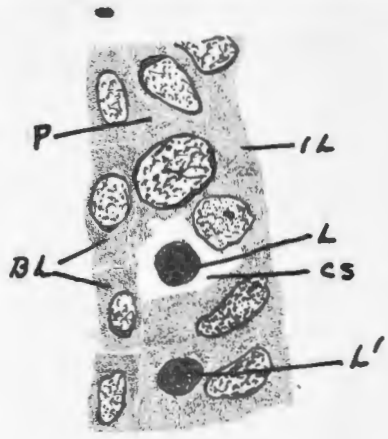
Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

-A-
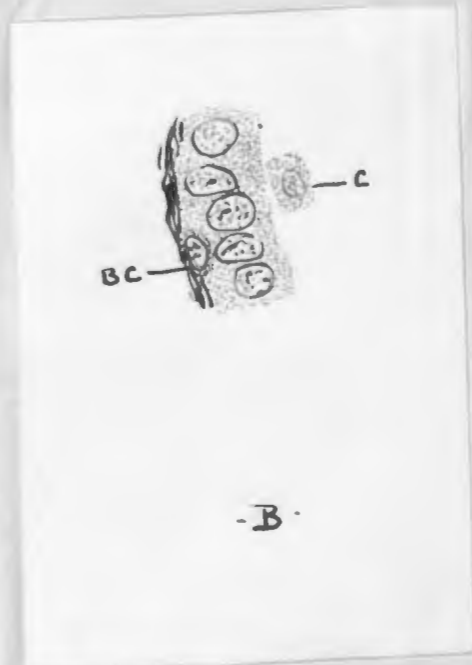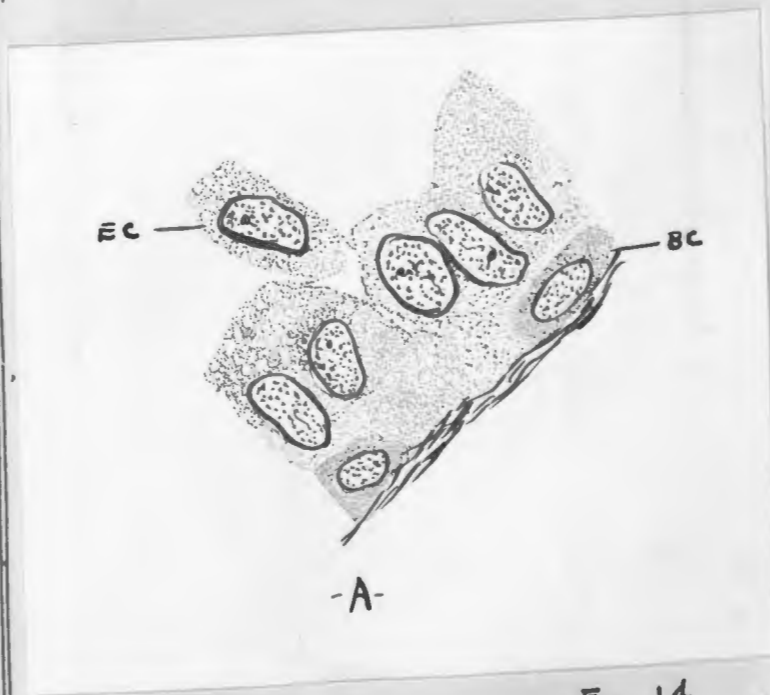

-B-

Fig. 14.