UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
RESEARCH BULLETIN 145

The Effect of the Estrus Producing Hormone on the Growth of the Mammary Gland

(Publication authorized May 20, 1930.)

COLUMBIA, MISSOURI
JULY, 1930

UNIVERSITY OF MISSOURI — COLLEGE OF AGRICULTURE

Agricultural Experiment Station

STATION STAFF, JULY, 1930

WALTER WILLIAMS, LL.D., Acting President

F. B. MUMFORD, M.S., D. Agr., Director — S. B. SHIRKY, A. M., Asst. to Director

MISS ELLA PAHMEIER, Secretary

AGRICULTURAL CHEMISTRY
A. G. Hogan, Ph.D.
L. D. Haigh, Ph.D.
W. S. Ritchie, Ph.D.
A. R. Hall, B.S. in Agr.
E. W. Cowan, A. M.
Robert Boucher, Jr., A.M.
L. V. Taylor, A.B.
Robert Pilcher, M.S.
Luther W. Richardson, A.M.
U. S. Ashworth, A.B.

AGRICULTURAL ECONOMICS
O. R. Johnson, A.M.
Ben H. Frame, A.M.
F. L. Thomsen, Ph.D.
Preston Richards, B.S. in Agr.
C. H. Hammar, Ph.D.

AGRICULTURAL ENGINEERING
J. C. Wooley, M.S.
Mack M. Jones, M.S.
R. R. Parks, B.S. in Agr. Eng.
D. D. Smith, B.S. in A.E.

ANIMAL HUSBANDRY
E. A. Trowbridge, B.S. in Agr.
L. A. Weaver, B.S. in Agr.
A. G. Hogan, Ph.D.
F. B. Mumford, M. S., D. Agr.
D. W. Chittenden, A.M.
H. C. Moffett, A.M.
J. E. Comfort, A.M.
F. F. McKenzie, Ph.D.

BOTANY AND PHYSIOLOGY
H. W. Rickett, Ph. D.
I. T. Scott, Ph.D.

DAIRY HUSBANDRY
A. C. Ragsdale, M. S.
Wm. H. E. Reid, A.M.
Samuel Brody, Ph. D.
C. W. Turner, Ph.D.
Harry Herman, B.S. in Agr.
Warren Gifford, A.M.
E. R. Garrison, A. M.
M. E. Powell, A.M.

ENTOMOLOGY
Leonard Haseman, Ph.D.
T. E. Birkett, B. S. in Ed.

FIELD CROPS
W. C. Etheridge. Ph.D.
C. A. Helm, A. M.
L. J. Stadler, Ph.D.
R. T. Kirkpatrick, A. M.
B. M. King, A.M.
Miss Clara Fuhr, M.S.*
W. R. Tascher, Ph. D.
S. F. Goodsell, A.M.

HOME ECONOMICS
Mabel Campbell, A. M.
Jessie A. Cline, A. M.
Margaret C. Hessler, Ph.D.
Adella Eppel Ginter, M.S.
Sylvia Cover, A.M.

HORTICULTURE
T. J. Talbert, A.M.
H. G. Swartwout, A.M.
J. T. Quinn, A.M.
A. E. Murneek, Ph.D.

POULTRY HUSBANDRY
E. M. Funk, A.M.
Earl W. Henderson, A.B.

RURAL SOCIOLOGY
E. L. Morgan, A.M.
Henry J. Burt, A.M.
Miss Ada C. Niedermeyer, A.M.
Walter Burr, A.M.
Howard E. Jensen, Ph.D.
George A. Gemmell, A.M.

SOILS
M. F. Miller, M.S.A.
H. H. Krusekopf, A.M.
W. A. Albrecht, Ph. D.†
Hans Jenny, Ph.D.
George Z. Doolas, M.S.
Lloyd Turk, A.M.
Harold F. Rhoades, B.S.A.
James F. Lutz, B.S.A.

VETERINARY SCIENCE
J. W. Connaway, D.V.M., M.D.
O. S. Crisler, D.V.M.
A. J. Durant, A.M., D.V.M.
Andrew Uren, D.V.M.
Ellmore F. Sanders, D.V.M.

OTHER OFFICERS
R. B. Price, B.L., Treasurer
Leslie Cowan, B.S., Sec., of Univ.
A. A. Jeffrey, A.B., Agricultural Editor
J. F. Barham, Photographer
Leon Waughtel, Ass't Photographer
Jane Frodsham, Librarian

*In service of U. S. Department of Agriculture. †On leave of absence.

TABLE OF CONTENTS

The Effect of the Estrus Producing Hormone on the Growth of the Mammary Gland

C. W. TURNER AND A. H. FRANK*

Abstract.—As a result of previous work at this Station indicating that the rate of excretion of the estrus producing hormone in the urine of pregnant cattle increases during the course of gestation, a study was made of the effect of the hormone on the growth of the mammary gland in the rabbit. In the normal rabbit after continued estrus, the mammary glands show extreme extension of the duct systems resembling the naked branches of a tree. If pregnancy or even pseudo-pregnancy now ensues, the ducts develop lobules containing large numbers of alveoli, resembling the budding of leaves from the smaller branches. It was found that the daily injection of 20 rat units of the estrus producing hormone recovered from pregnant cow's urine for 30 days in male castrate rabbits and in female rabbits castrated previous to puberty caused the growth of the duct system of the glands equal to that produced during continued estrus in the normal female. The results obtained seem to warrant the conclusion, that the estrus producing hormone will cause the growth of the ducts equal to that produced during estrus, that the rate of development is not hastened by increasing the dosage, that there was present in none of the oils used a hormone or hormones which would produce the type of growth characteristic of pregnancy.

In connection with a study of the physiological cause of the growth of the mammary glands and the initiation of milk secretion, it has been demonstrated at this Station that during pregnancy cattle excrete increasing amounts of the estrus producing hormone. As other workers have shown it to be present in the blood; it is assumed, until evidence is available, that the increasing rate of excretion in the urine indicates an increasing concentration of the hormone in the blood during the course of pregnancy.

The fact that the estrus producing hormone increases during pregnancy makes very plausible the theory that the development of the mammary gland during pregnancy is regulated in part at least by the increasing concentration of the hormone in the blood.

The object of the present paper is to report the results of a study of the effect of the estrus producing hormone on the development of the mammary gland of the rabbit. As the rabbit has been found to be especially valuable in studies on the development of the mammary gland, it seemed desirable to make a careful review of the literature describing the anatomy and physiological development of the gland as a background for this and future studies.

*The data presented in this paper formed part of a thesis presented by A. H. Frank in partial fulfillment of the requirements for the Degree of Master of Arts in the Graduate School of the University of Missouri, 1930.

Studies of the Anatomical Development of the Mammary Gland in the Rabbit

Before it is possible to study the effect of the estrus producing hormone on the growth of the mammary glands of the rabbit it is necessary for purposes of comparison, to have a complete picture of the development of the mammary gland of both the male and female during all stages of their development.

The growth of the rabbit gland has been divided into the following stages of development. I. *Development during embryonic and fetal life.* This stage includes the development from the appearance of the anlagen of the mammary line to the initiation of the canalization of the primary sprouts. II. *Development from birth to puberty.* During this stage canalization of the primary sprouts is completed but there is very little growth of the mammary gland. Near the end of this stage there is a sex differentiation in the size of the gland due to an increase in growth of the female gland, while the male gland remains rudimentary. III. *Development during puberty.* A rapid growth of the ducts is produced under normal continuous estrus, but there may be an abnormal condition called pseudo-pregnancy, resulting where ovulation occurs, followed by persistent corpora lutea. During the persistence of the corpora lutea the ducts increase to full length with a complete growth of lobules. IV. *Development during pregnancy.* A growth equal to that of pseudo-pregnancy is produced during the first 14 days, while there is an increase in the size of the lobules followed by secretion in the last half of pregnancy. V. *Development during lactation followed by regression.* The gland remains essentially the same anatomically during lactation.

At weaning time there is a gradual decline in milk secretion followed by a reabsorption of the secreted milk. Many have observed the gradual absorption of alveolar tissue during regression.

DEVELOPMENT DURING EMBRYONIC AND FETAL LIFE

The Mammary Line.—Schultze (1892) found the mammary line in rabbits to mark the beginning of the mammary apparatus. It was observed in the form of a fine line running from the fore limb bud to the inguinal region.

In a study of the blood vessels of the mammary gland Wahl (1915) found that the mammary line rises comparatively early but appears to exert no influence on the formation of the ventral cutaneous vessels. The latter are formed in response to the needs for more direct vascular connections in the ventral region of the abdominal wall as the umbilical veins change their courses and the distance from the dorsal axis increases.

Mammary Bud Development.—In an extensive study of the mammary gland development in the rabbit Rein (1881-82) did not observe the mammary line stage but described the beginning anlage as the hillock stage, which is the beginning of the mammary bud formation. The progressive development of the mammary bud was divided into four stages by Rein whose description is summarized below: (1) the hillock stage, (2) the lens shaped stage, (3) the plug shaped stage, and (4) the flask shaped stage.

Hillock Stage.—The beginning of this stage is marked by small spherical thickenings which develop at intervals along the mammary line. These areas become thicker enclosing the structures which form the mammary buds.

Several of these formations were found on either side of the median line, generally four placed symmetrically, similar to the position of the teats in the adult, except that they are farther from the median line.

The region of the mammary apparatus consists of several cell layers appearing as slight thickenings of the ectoderm. The anlage of the epidermis consists of two layers, the epitrichium, a very thin layer of flattened cells (not observed by Rein), and the Malpighian, a short cuboidal or cylindrical stratum. The Malpighian layer is underlain by a fine membrana propria or basement membrane, which separates it from the underlying embryonic mesenchyme layer. The mesenchyme develops into the derma or corium of the skin. It consists of spindle shaped cells with large masses of intercellular substance. A few capillary blood vessels were observed in this layer.

The mammary bud is formed as a result of the proliferation of the Malpighian layer into an intermediate layer several cells in thickness. Rein counted 25 cells which were arranged in four rows at the highest point in some of the earliest anlagen.

The age of the embryos were estimated at 11 to 12 days after conception. They measured about 1.5 centimeters in length. (Figures 1, 2 and 3).

Lewis (1905) made an extensive study of the development of the lymphatic system in rabbit embryos. He states that in 1.45 centimeter embryos the deep subcutaneous outgrowth from the jugular sac has become greatly dilated in its distal portion. A large lymphatic space is found, near the beginning of the external mammary vein, wedged between two converging venous branches. This is not connected with the veins. A few slender detached lymphatics follow the external mammary vein.

The Lens Shaped Stage.—The areas of the hillock stage gradually sink into the underlying mesenchyme changing into a lens shape.

Fig. 1.—Sagittal section of the hillock stage in the development of the mammary bud. Embryo 1.5 centimeters long. Rein (1882).

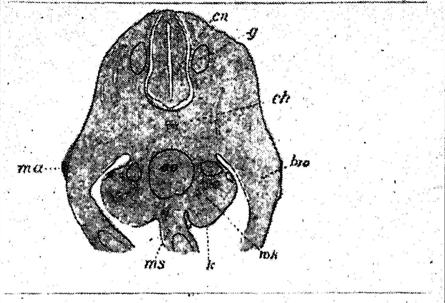

Fig. 2.—Cross section (m. a., mammary gland anlage). Embryo 1.5 centimeters long. Rein (1882.)

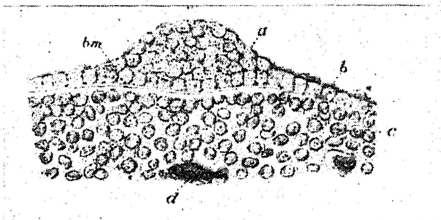

Fig. 3.—Sagittal section showing some development of the mammary hillock. Embryo 1.6 centimeters long. Rein (1882).

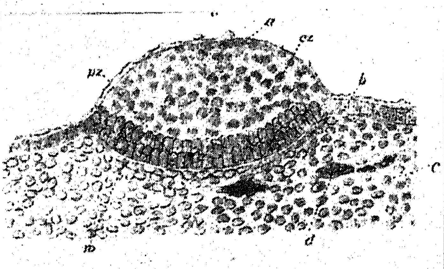

Fig. 4.—Sagittal section of the lens-shaped stage of the development of the mammary gland. Embryo 1.7 centimeters long. Rein (1882).

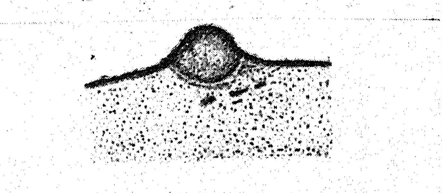

Fig. 5.—Cross section of lens-shaped stage. Embryo 1.7 centimeters long. Rein (1882).

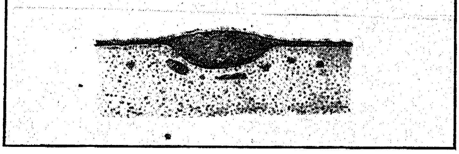

Fig. 6.—Lens-shaped stage with flattened surface advanced deep into the underlying mesenchyme. Embryo 1.8 centimeters long. Rein (1882).

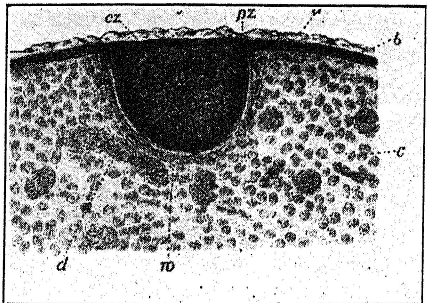

Fig. 7.—Plug-shaped stage. Embryo 1.8 centimeters long. Rein (1882).

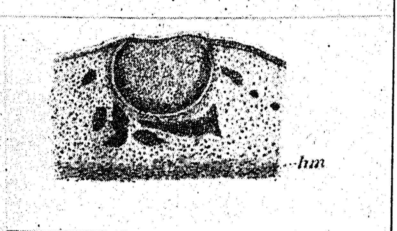

Fig. 8.—Section showing the transition from the plug-shaped to the flask-shaped stage. Embryo 2,0-2,1 centimeters long. Rein (1882).

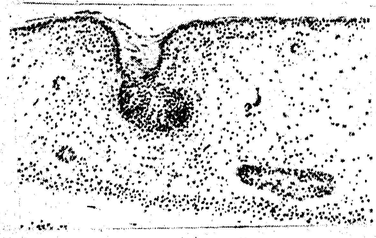

Fig. 9.—Cross-section through the skin of a rabbit embryo showing the mammary bud and sections of several blood vessels. Embryo 2.3 centimeters long. Wahl (1915).

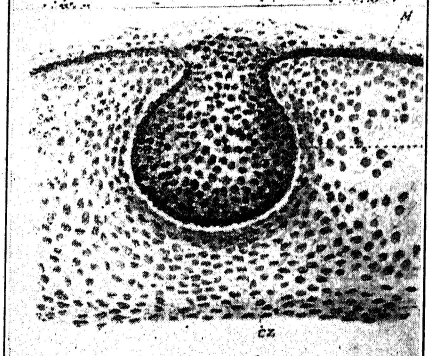

Fig. 10.—Flask-shaped stage. Embryo 2.9 centimeters long. Rein (1882).

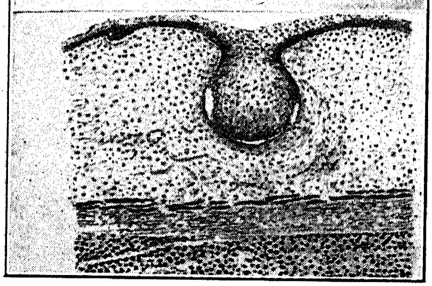

Fig. 11.—Further development of flask-shaped stage showing beginning of indentation of skin. Embryo 3.5 centimeters long. Rein (1882)

The growth continues downward until the upper surface is level with the surrounding epidermis. (Figures 4, 5, and 6).

Rein states that this stage follows very rapidly the former or hillock stage. Embryos presenting both stages may be found in the same uterus.

The Plug Shaped Stage.—As the mammary bud sinks deeper into the mesenchyme, the upper surface becomes flattened while the lower surface becomes more convex taking on the shape of a plug. At this stage the bud increases in height with the upper surface lying on a level with the epidermis. (Figures 7 and 8). Histologically, the structure remains the same.

As the anlage advances a slight indentation begins to form at the center of the upper surface. Cornification of the cells in the center of the bud begins. Macroscopically the anlage appears as a conical elevation in the center of which there appears a small cavity This cavity may be in the center or to one side.

The Flask-shaped Stage.—Sinking deeper into the mesenchyme, the mammary bud assumes a characteristic flask shape. (Figures 10, 11, 13 and 14). At this time the bud is fully formed.

A differentiation of the cells of the mammary bud begins at this stage. The cells lining the wall of the mammary bud consisting of the basal Malpighian layer with several layers of epithelial cells above are similar to the earlier stages, staining easily with carmine, while the central cells are becoming irregular in form and resist staining similar to the cornified cells of the epidermis.

The mesenchyme cells are becoming more fibrous with an increased amount of intercellular substance. Small islands of subcutaneous adipose tissue appears in the older individuals of this stage. At this period the formation of the hair and sebaceous glands begin.

The changes observed in the development of the mammary bud begins in 1.5 centimeter embryos and continues up to about the 4.5 centimeter stage.

The first noticeable influence of the mammary gland region on the cutaneous blood vessels was observed by Wahl (1915) to appear at the period when the individual gland anlagen (the hillock stage) begin to differentiate in the mammary line. At this time in the deep layer of the skin the branches of the thoraco-epigastric vein spread out widely and anastomose. Superficial to these larger branches there is a network of smaller capillaries. This network becomes especially well developed about the lenticular thickenings (the mammary bud Figure 9) of the epidermis. (Figure 12).

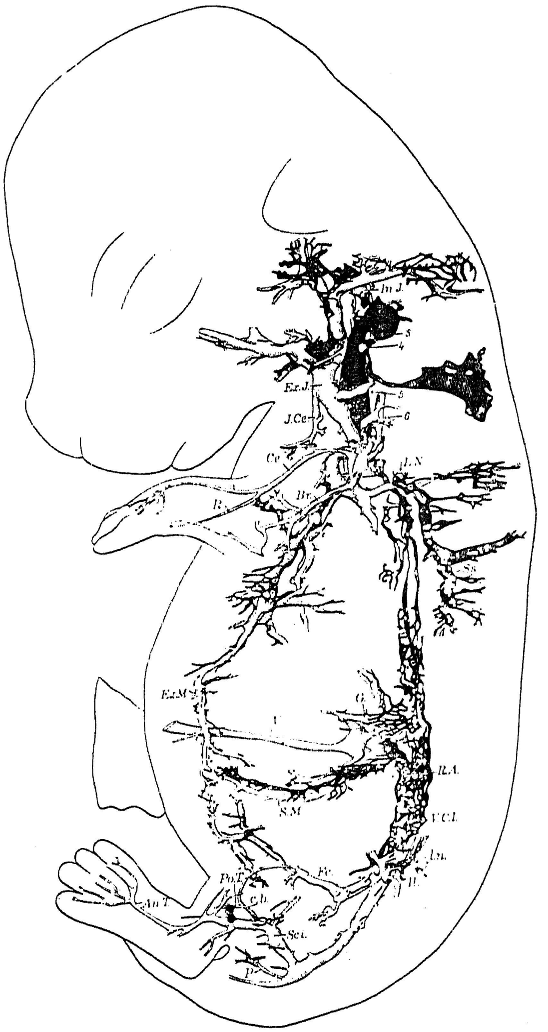

Fig. 12.—The development of the blood and lymph vessels in a 2.9 centimeter rabbit embryo. Lewis (1905).

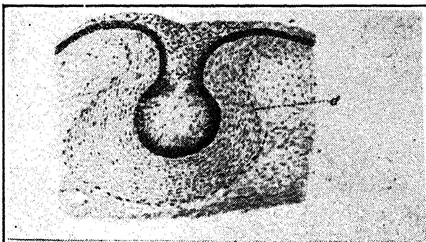

Fig. 13.—Development in the flask-shaped stage showing the lengthened neck of the mammary bud. Embryo 4.5 centimeters long. Rein (1882).

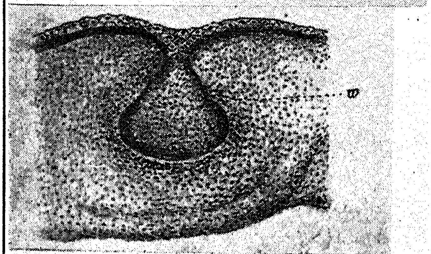

Fig. 14.—Mammary bud showing flask-shaped stage in another 4.5 centimeter embryo. Rein (1882).

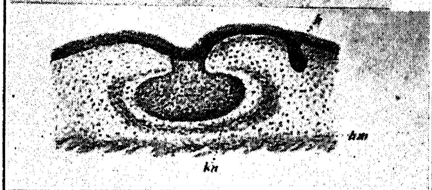

Fig. 15.—Section showing transition from the mammary bud stage to the stage of sprout formation. Embryo 5 centimeters long. Rein (1882).

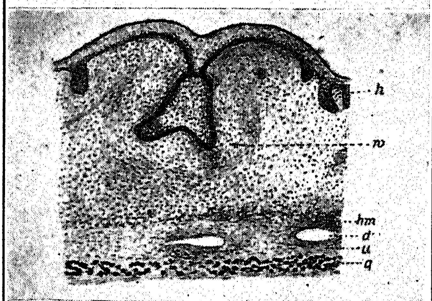

Fig. 16.—Two sprouts are beginning to extend from the bud. Embryo 7-8 centimeters long. Rein (1882).

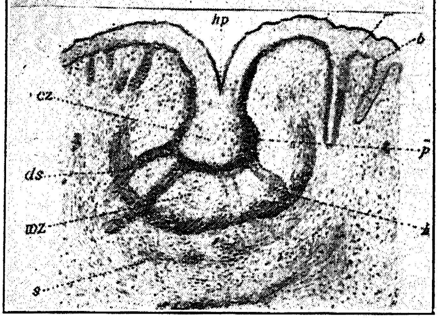

Fig. 17.—Further growth of the primary sprouts, and cornification of the central part of the bud. Embryo 7.5-8 centimeters long. Rein (1882).

Primary Sprout Development.—The mammary bud gradually loses its oval shape, becoming triangular in cross-section. (Figure 15). This change in form is caused by the development of the primary sprouts. (Figures 16 and 17). The sprouts are composed of the basal epithelial cells of the mammary bud from which they grow into the underlying mesenchyme tissue.

The total number of primary sprouts was not reported. Four was the largest number observed in a single cross-section.

During this stage a new cellular formation appears. A newly formed mass representing the anlagen of the connective tissue of the future gland begins to develop between the skin and muscle layer. While these changes are taking place the cells of the mammary bud remain practically the same except for a further cornification of the central cells.

During the formation of the primary sprouts the embryos increase in size from 4.5 centimeters to 8 centimeters in length.

While this development is taking place there is also a development of the vascular system. Thus Wahl observed a special capillary plexus developing in the connective tissue as the primary sprouts project down into it.

In the oldest rabbit embryos Lewis (1905) found that the lymphatic system had invaded the skin extensively. The subcutaneous vessels of the shoulder are attached by rich networks. These veins are the external mammary and another which is ventral to the scapula and posterior to the shoulder joint. (Figure 12).

DEVELOPMENT FROM BIRTH TO PUBERTY

Canalization of the Primary Sprouts.—Rein (1881-82) observed that the primary sprouts gradually increase in circumference and canalize, forming lumen. In one day old rabbits the lower parts of the sprouts have formed rather wide lumen while the upper parts leading to the bud remained solid (Figure 19). Incomplete canalization was still observed at 5 days of age; however, it was complete at 15 days of age. (Figure 20). From these primary sprouts secondary sprouts develop. (Figure 18).

The canalized sprouts appear as tubes extending into the underlying mesenchyme tissue. They are lined with a single layer of low cuboidal or cylindrical epithelium. The primary sprouts may divide into two secondary sprouts before advancing far toward the base of the teat and thus in a basal section of the teat there would appear a larger number of teat cisterns than there were primary sprouts. (Figure 22).

The Vascular Supply and Gland Growth.—Wahl (1915) observed veins and arteries surrounding the anlage of the gland in the new born

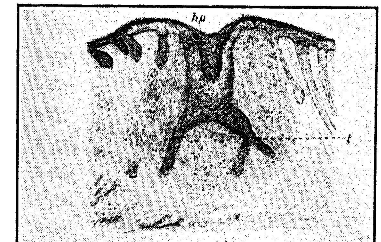

Fig. 18.—The primary sprout has divided into secondary sprouts Embryo 9 centimeters long. Rein (1882).

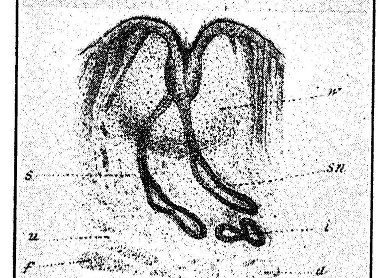

Fig. 19.—Further increase of the teat elevation, and canalization in primary sprouts. Female rabbit one day old. Rein (1882).

Fig. 20.—Further development of teat. Complete canalization. Female rabbit 15 days old. Rein (1882).

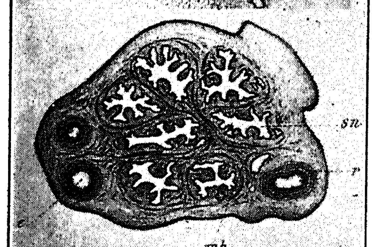

Fig. 21.—Horizontal section through teat, in the upper third part, at time of puberty, showing 7 teat cisterns and 3 streak canals. Rein (1882).

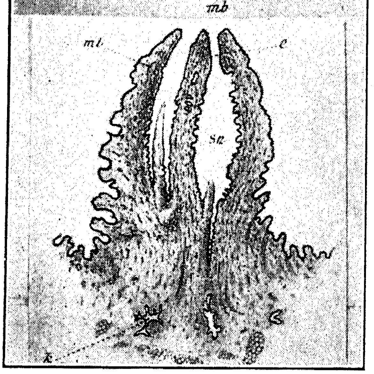

Fig. 22.—Vertical section of female rabbit teat at puberty. Rein (1882).

rabbits. (Figure 23). The vascular system is essentially the same in location at this period as in the adult. The mammary ducts extend only a short distance from the base of the teat. (Figure 24).

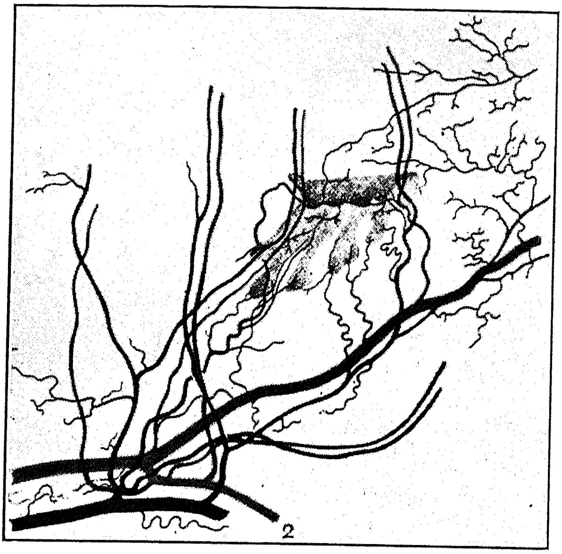

Fig. 23.—Mammary gland of a new born rabbit, seen from below; the epidermis and nipple have been removed. The blood vessels are injected; the veins are shown lighter colored than the arteries. The smaller ducts of the gland have been bent back over the nipple area in mounting the specimen and are shown darker than the rest of the gland. Length of longest duct, 2 mm. Magnified 8 diameters. Wahl (1916).

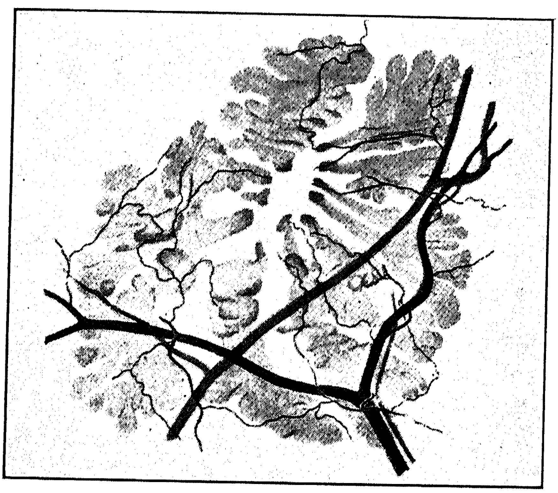

Fig. 24.—Mammary ducts of rabbit before reaching puberty. The ducts are cut off near the base of the nipple; only a few of the larger blood vessels are shown; length of longest duct 5 mm. Diameter of gland about 1 centimeter. Magnified 8 diameters. Wahl (1905).

At two months of age the vascular supply of the mammary gland has increased considerably. Special vessels supply the teats and ducts lying close to the teats. Other vessels from the cutaneous and muscular plexuses supply the outer extremities of the mammary ducts and tissue with well developed capillaries.

Brouha (1905) made an extensive study of the mammary gland in the rabbit. In 4, 15, and 25 day old rabbits the gland consisted of eight mammary ducts extending only a short distance from the base of the teat. He observed seven streak canals. One divided before reaching the base of the teat, thus giving eight mammary ducts.

During the period from birth to puberty Ancel and Bouin (1911) found that the mammary gland retains its infantile characteristics. It consists of 8 to 10 ducts which hardly reach beyond the base of the teat, covering about one centimeter in diameter. The male and female glands remain the same size until puberty or slightly before when there is a rapid increase in length and branching of the ducts in the female, while the male gland remains in the infantile stage.

Time of Reaching Puberty.—The beginning of puberty is very irregular and cannot be set at any definite age. Hammond (1925) shows that the time of acceptance of coitus in the virgin doe is governed mainly by two factors, the plane of nutrition and time of the year the doe is dropped.

Does dropped from October to December will copulate as early as four months of age, while those dropped in March and April require seven to eight months. If we consider the time of reaching puberty as the time of first coitus, then puberty may be reached at any time from four to eight months after birth.

This makes it very difficult to determine the time of onset of puberty for an examination of the mammary gland. As the gland grows much faster after puberty, Ancel and Bouin found a decided variation in the size of the mammary gland in does of the same age.

DEVELOPMENT DURING PUBERTY

Normal, Continuous Estrous.—Upon reaching puberty the female rabbit remains in a state of estrus more or less indefinite in length. When copulation does not occur the stage of estrus may be followed by a brief rest period.

Unlike the mouse, rat, and guinea pig, Marshall (1922) states that the rabbit does not ovulate spontaneously. Neither does it ovulate during estrus as is found in the more common domestic animals, cow, mare, and sow. The follicles grow to maturity and remain in this condition until ovulation is induced, or degeneration takes place during the rest period.

The ripened and developing follicles are continually secreting hormone. Thus the gland development at this period is under the influence of the follicular hormone.

As the rabbit reaches puberty the gland begins to grow at a noticeable rate. The mammary ducts begin to develop and ramify into the subcutaneous tissue. At the height of puberty the gland has increased in size, measuring from 1.5 to 2.0 centimeters in width and from 2 to 3 centimeters in length. (Figure 25). At this time 8 or 10 mammary ducts branch out at the base of the teat terminating in numerous smaller branches. The glands are still separated by considerable space (4 or 5 centimeters) and measure about 3.5 centimeters in diameter.

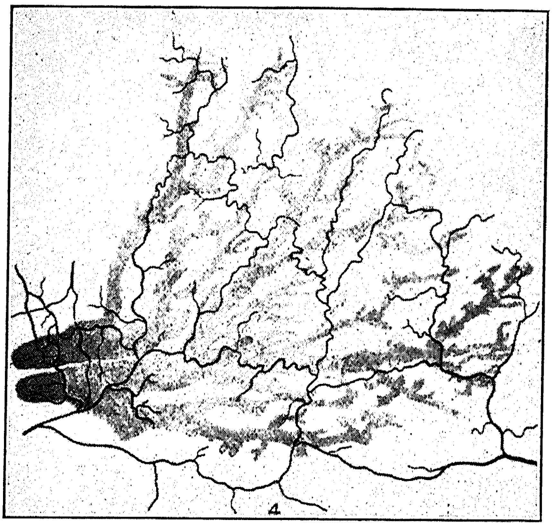

Fig. 25.—Two main ducts and their branches of a mammary gland of a female rabbit at puberty. A few of the arteries supplying the gland are shown. Diameter of gland about 2 centimeters. Wahl (1905).

At this stage a microscopic examination shows that the gland consists almost entirely of mammary ducts very thickly branched. The branching begins at the base of the teat with rather large ducts representing cisterns from which many smaller ducts branch out and terminate in still smaller branches toward the outer extremities. The ducts are lined with two cellular layers, the inner layer being cylindrical, while Rein described the primary sprouts having a single cellular layer. The few alveoli present are lined with a single layer of small cells. These make up the main sectory cells of the gland. Small fat globules were present in a few of the cells and a secretory exudate was present in the intercalary ducts. Ancel and Bouin found small amounts of secretion beginning at the earliest growth of the alveoli.

In the full grown virgin rabbits Wahl found the glands developed to a 2 to 3 centimeter diameter. At this stage the blood supply of the teats and ducts was essentially the same as at two months, except the blood vessels and capillaries supplying the outer extremities of the ducts were much better developed.

Abnormal, Pseudo-pregnancy.—The phenomena of pseudo-pregnancy follows when ovulation occurs without fertilization of the ova. As in normal pregnancy, active corpora lutea develop in the old follicle cavities. In the rabbit the corpora remain in an active condition over a period of 14 days. During this time development of the gland corresponds to that of normal pregnancy.

Various methods have been used to produce pseudo-pregnancy. Ancel and Bouin (1909) and Loeb (1928) produced pseudo-pregnancy by sterile matings. Either vasectomized bucks were used or the Fallopian tubes of the female were ligated. Ancel and Bouin (1911-12) and O'Donoghue (1913) were able to get formation of corpora lutea in a few cases by mechanically opening the ripened follicles. However, Walton and Hammond (1928) were unable to get the growth of corpora lutea by mechanically opening the ripened follicles. Parks (1929) produced the formation of corpora lutea by injecting alkaline extracts of the anterior lobe of the pituitary. Bellerby (1929) induced ovulation by an intravenous injection of an acid extract of the anterior pituitary.

Ancel and Bouin (1911) found that the phenomena producing the changes which take place in the mammary gland during pregnancy or pseudo-pregnancy begins the day after mating. These changes in morphological transformation are decidedly marked by the fifth day. The glands have increased in size, measuring from 6 to 8 centimeters in length and 4 to 5.5 centimeters in width. The ducts have increased in length until the gland extremities have come in contact. The mammary apparatus appears as a continuous gland from well up on the neck anteriorly to between the rear limbs posteriorly.

Thus what appears to be two continuous glands develop on either side of the median line. The teat pattern of a normal mature rabbit is shown in Figure 51 and the mature glands removed with the skin intact are shown in Figure 52.

The ducts have increased in length and ramified considerably with an increased number of alveoli developing on the smallest branches. (Figure 26).

The epithelial secretory cells lining the alveoli contain a large number of fat globules, while a secretion is observed in the lumen. As yet none of the fluid has entered the ducts of the lobules. The cells lining the ducts show no signs of secretion.

The connective tissue and ducts seem to be slower in developing than the alveoli, which consists of a single layer of epithelial cells. Cell division is actively taking place as different stages of mitosis can be distinguished in the lobules and ducts.

During the early part of pregnancy the mammary gland increases rapidly in extended growth of the ducts and their ramification. The lobules comprising the smaller compound units of structure appear at the ends and sides of the main ducts and their branches. Wahl (1915)

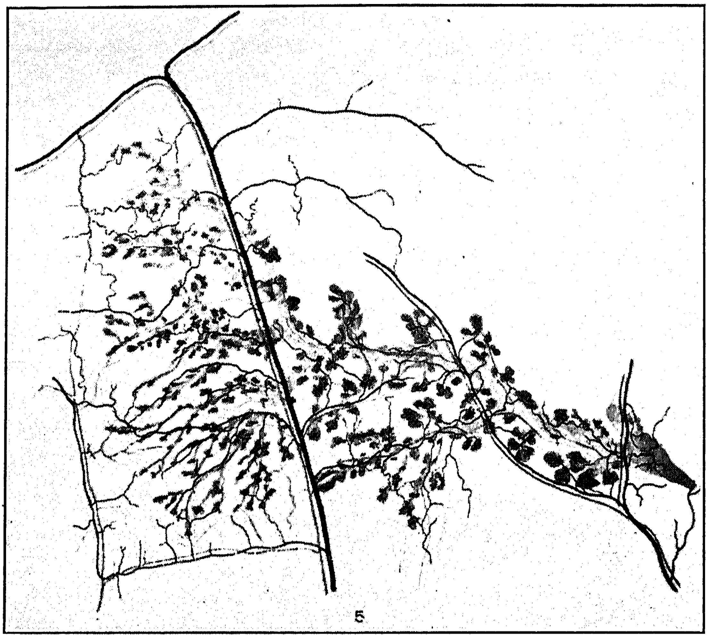

Fig. 26.—Mammary duct of rabbit during first few days of pregnancy showing development of lobules on the smaller branches. Magnified 8 diameters. Wahl (1905).

describes the first lobular ducts to appear on a plane perpendicular to the skin on both the superficial and deep surfaces of the ducts.

The vascular development proceeds essentially along the lines described in the growing animal. The capillary plexuses about the ducts become much more richly developed than before. Development of arterioles and venules in these plexuses also become more active. The differentiation of venules continues as before to exceed that of the arterioles. The subcutaneous vessels in the region of the gland and all the vessels supplied to the gland increase rapidly in size.

Gland 10 days Pseudo-pregnant.—On the tenth day of pseudo-pregnancy, as described by Ancel and Bouin, the lobules have increased in number and size, becoming crowded by adjoining lobules. There is less connective tissue which is very thoroughly vascularized with a development of the lymphatic ducts as well as venules and arterioles.

As the lobules enlarge they all manifest the same structure. They consist of secreting alveoli and ducts leading into the intralobular ducts. There is more secreted fluid in the lumen at this stage, with the presence of one or more fat globules in the distal end of each epithelial cell of the alveoli. The fat globules seem to push the nuclei back close to the basement membrane.

The intercalary ducts (mouths of alveoli) are variable in size, but all seem to be very restricted. No excretory products are found in the mammary ducts, which seem to be in a mitotic stage. The gland appears to be nearly at its limit of development.

Gland 14 days Pseudo-pregnant.—This stage is marked by a further increase in size of the lobules due to the increased size of the alveoli. The lumen of the developed alveoli is filled with an excretory exudate, while intercalary ducts have become larger and open. There are a few lobules in the developing process similar to the ten day period which are incapable of secreting because the cells are in a mitotic stage.

The vascular system has become further developed, with a decrease in the width of connective tissue.

Gland 16 days Pseudo-pregnant.—Ancel and Bouin found that at 16 days of pseudo-pregnancy there can be no further change noted in the gland macroscopically. While upon microscopic examination there has been a morphological change. The alveoli have shrunken and the secretory products have apparently been partially reabsorbed giving the lobule the appearance of a much smaller size. The vascular system is less pronounced, associated with a narrowing of the mammary ducts, thus causing a contraction of the connective tissue, giving it a much thicker appearance.

Up to this time they found that the pseudo-pregnant and normal pregnant development of the mammary gland was the same. But at this stage a distinct division of the two begins.

Gland 19 Days Pseudo-pregnant.—Upon the 19th day Ancel and Bouin found that further degeneration of the gland had taken place. The lobules appear to be fading away and the excretory products are almost absent. The alveoli are nearly indistinct (appearing as a shapeless, necrobiotic mass as described by Ancel and Bouin) with a decided increase or thickening of the connective tissue. The vascular system is very much reduced. The mammary ducts are shrunken with their walls touching in places.

Gland 25 days Pseudo-pregnant.—On the 25th day after sterile coitus the gland appeared the same as on the 14th day macroscopically. Microscopically, Ancel and Bouin found a further state of atrophy. The mammary ducts converge toward the teat, being almost indistinct where the fine ducts ramify into the lobules. Part of the lobes have almost disappeared while others have decreased in size, the alveoli being represented by a small mass of nuclei where degeneration has advanced. Ancel and Bouin state, "In a word the elements of the mammary gland are, in this state, well on the way to reabsorption."

Pseudo-pregnancy Prolonged to Normal Pregnancy.—Parks (1929) was able to produce full growth of the mammary gland similar to that of full time pregnancy by prolonging the duration of pseudo-pregnancy in the rabbit. The corpora lutea were kept in an active condition by continued injections of alkaline anterior lobe extracts. Thus the fetal factor appears unnecessary for complete development of the mammary gland. Only prolonged luteal action is necessary. Glands were photographed showing the comparative amounts of growth produced by pseudo-pregnancy and prolonged pseudo-pregnancy.

Pseudo-pregnancy in Normal and Hysterectomized Rabbits.—Hypertrophy of the mammary gland in rabbits that have never been pregnant may be so great as to lead to milk secretion. Hammond and Marshall (1914) report secretion of milk in pseudo-pregnant rabbits as early as the 19th day. Pseudo-pregnant rabbits were killed at 3, 5, 9, 12, 14, and 24 days after sterile copulation. Growth of the lobules had begun at 3 days, with nearly full growth of the gland at 9, 12, and 14 days, while the ducts were full of milk at 24 days after sterile coitus. Old corpora lutea persisted at 24 days.

Equal mammary growth was produced in hysterectomized rabbits during pseudo-pregnancy. The persistency of one ovary was sufficient for full development of the mammary gland. The corpora lutea were in good condition at 28 days after sterile coitus.

Pseudo-pregnancy Prolonged Experimentally.—By mechanically stimulating the reproductive apparatus of virgin female rabbits Ancel and Bouin (1912) were able to induce development of the mammary gland. The growth of the corpora lutea was mechanically induced. Upon the 8th day (time at which the egg is normally embedded in the uterine wall) the wall of the uterus was sectioned.

As a result of the stimulation of the corpora lutea a maternal placenta quickly grows along the sectioned uterus. This in turn caused the development of the myometrial gland which Ancel and Bouin believed to be responsible for the development of the gland during the latter stages of pregnancy.

This gland became visible about the 20th day of normal pregnancy. It appeared about the 7th day after sectioning the uterus of a pseudo-pregnant rabbit. The maternal placenta disappeared almost as soon as it was developed.

Following the appearance of the myometrial gland the alveoli and glandular ducts were filled with a secretory fluid, which could be drawn from the gland. Thus Ancel and Bouin advanced the hypothesis that mammary development during the last half of pregnancy may be due to secretions of the myometrial gland.

Hammond and Marshall (1914), Hammond (1917), and Parks (1929) were unable to find the myometrial gland in all pregnant rabbits or in other pregnant animals.

DEVELOPMENT DURING PREGNANCY

The Vascular System During the Last Half of Pregnancy.—During the later stages of pregnancy Wahl (1915) states that the chief development of the vascular system is centered in the capillaries supplying the lobules. At first the anlage of the lobule is surrounded by a network of capillaries continuous from the capillaries of the ducts. As the alveolar ducts develop at the periphery of the lobular ducts the same process is repeated, venules and arterioles for the alveoli being developed between the capillary plexus about the alveoli and the ducts of the lobule.

Persistence of Corpora Lutea During the Last Half of Pregnancy.—Hammond (1917) states that contrary to the commonly accepted opinion, the corpus luteum of the rabbit does not reach a state of atrophy during the last part of the gestation, but maintains its full size late in gestation and even into the period of lactation. The cause of the corpora lutea persisting during this period originates in the fetus. The changes in the mammary gland during the last half of pregnancy are still due to the persistency of the corpora lutea. Thus the influences causing the growth during the first and last half of pregnancy are the same. The secretion of milk apparently results whenever the factors causing the mammary growth are removed, provided the gland is sufficiently developed.

DEVELOPMENT DURING LACTATION FOLLOWED BY REGRESSION

Appearance of the Mammary Apparatus and Vascular Supply During Lactation.—During lactation Wahl found the gland further increased in size owing to the expansion of existing alveoli and the formation of new ones. The connective tissue between the expanded alveoli becomes very thin and slight in amount. (Figure 27.)

There is a great elaboration and enrichment of the vascular supply of the gland, but the essential features are as described during the first half of pregnancy.

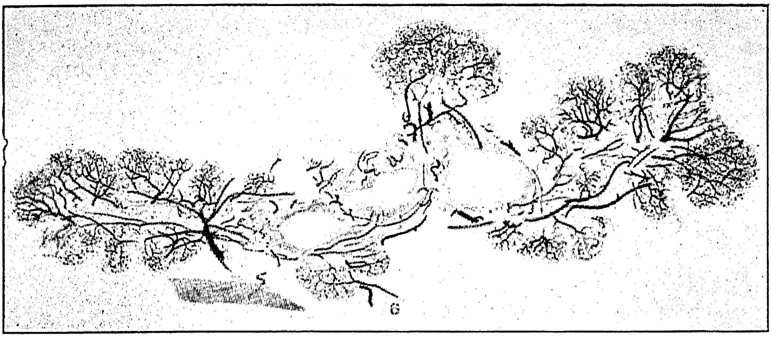

Fig. 27.—Cross-section through a duct and its branches of a mammary gland of a rabbit during lactation. Magnified 17 diameters. Wahl (1915).

The Mammary Apparatus and Vascular Supply During Regression.—Within two weeks after weaning, the gland as described by Wahl (1915) becomes much thinner as the milk disappears from the ducts. The alveoli first shrink in size, then the alveolar epithelial cells degenerate and are resorbed leaving a duct system and a small group of epithelial cells with merely a small lumen or no lumen. The connective tissue appears greatly increased as the alveoli disappear. The walls of the ducts become collapsed as the milk is absorbed.

As the alveoli are absorbed the surrounding capillaries disappear. The venules and arterioles appear disproportionately large compared with the capillary field which they supplied. The appearance is similar to the virgin rabbit gland.

In general the blood supply of the gland during growth and rest appears in the main to be secondary to the skin and muscle. During functional activity it becomes more independent.

Studies of the Physiological Development of the Mammary Gland

From the time of puberty there are obvious changes taking place in the mammary glands of the female, whereas in the male no further development ordinarily occurs. The source of the stimulation which produces such profound changes in the female mammary gland, especially during pregnancy, has long been sought. The earlier theories referred the source of the stimuli to reflexes originating in the nervous system. The experimental work of that period was chiefly concerned with the possibilities of normal growth of the mammary gland taking place if severed from the usual nervous connections.

In recent years with the development of the "hormone" theory, the work has shifted to a study of the source of hormones which may activate the mammary glands. This work may be divided into two phases.

I. From 1906, when Lane-Claypon and Starling began their work, until 1912, only aqueous extracts were used from which only very slight activation of the glands could be produced. Solutions causing activation were from organs which have later been shown to contain the estrus producing hormone by the rat unit test. About 1912 a new and vigorous impetus to experimenters followed the introduction of lipoid solvent extracts. With these extracts it was possible to get very potent solutions which produced marked activation of the mammary glands and reproductive organs.

II. The establishment of the rat unit test for the potency of extracts introduced a new interest into the field of hormonal research. With this simple and easy test quantitative results could be obtained with live animals. During the first few years following the rat unit test, extracts were used which were essentially the same as those introduced in 1912. However, purified forms of crystaline and aqueous solutions or emulsions of very great potency have been produced during the last few years. These have very little superiority over the weaker extracts in the development of the mammary gland, because the weaker solutions, when injected over a long period of time seem to produce the same amount of growth as the more potent solutions over the same length of time.

Aside from injections of potent extracts, activation of the glands have been produced from operative technique performed upon the reproductive organs. The persistency of well preserved corpora lutea produces the development of the lobules along the ducts of the mammary gland.

EXPERIMENTS PREVIOUS TO THE INTRODUCTION OF THE ALLEN AND DOISY TEST

Mammary Growth from Aqueous Fetal and Ovarian Extracts.—Lane-Claypon and Starling (1906) obtained growth of the mammary gland in virgin rabbits, similar to that occurring during the height of estrus, from injections of aqueous extracts of rabbit fetuses. The largest amount of growth was obtained when repeated injections were made over a long period of time. They failed to produce growth from extracts of ovaries, placentas and uterin material from pregnant rabbits.

Later, Lane-Claypon and Starling's experiments were confirmed by Foa (1908) using extracts of cow fetuses. Biedl and Konigstein (1910) and Aschner and Grigoriu (1911) also repeated their work using rabbits.

Frank and Unger (1911) in repeating Lane-Claypon and Starling's experiments were unable to produce growth of the mammary gland in rabbits as well as rats by injections of fetal extracts. However, they produced growth from ovarian extracts. They also reported that the growth of the mammary gland during estrus was greater than that produced from injections. From these results they conclude that intra-uterin, prepubertal, and pubertal growth of the breasts are directly dependent on ovarian function. In their work they emphasize the necessity of using castrates.

Schickele (1912) produced growth of the mammary glands in castrated and normal virgin bitches and does. Aschner (1913) produced growth of the mammary gland in virgin castrate and normal female guinea pigs. Both workers were able to produce premature growth of the mammary gland in very young animals. Aqueous extracts of corpora lutea and placentas were used for injections.

It is interesting to know that the various sources of extracts used by these early workers which produce mammary development have been shown to contain the estrus producing hormone by the rat unit test.

Activation of the Mammary Gland from Injections and Operations.—Basch (1909) advanced the theory that hyperplasia of the breast and the secretion of milk was brought about by an ovarian-placental activation. He based his theory upon two types of experiments. In the first type multiparous rabbits were injected with placental extracts. In these, various degrees of milk secretion were obtained. Also milk secretion resulted from subcutaneous implants of placental fragments. In the second type, a virgin bitch was brought to active secretion of milk sufficient to suckle puppies. Ovaries from a pregnant bitch were implanted subcutaneously and followed by placental extract injections. Breasts excised at intervals during the experiments showed a gradual change from virginal to fully secreting breasts.

Frank and Unger (1911) were unable to produce in rats and rabbits the changes described by Basch. They relate cases where multiparous rabbits have been brought into active milk secretion when there were infections in the region of the breasts.

Mammary Growth Produced by Persistent Corpora Lutea.—Lane-Claypon and Starling (1906) state that Heape and Kehrer recorded cases of bitches which had not been impregnated at the normal time and after two months not only made a bed for young but showed swelling of the mammary glands with actual milk secretion in some cases.

O'Donaghue (1912) and Hill and O'Donaghue (1913) reported getting mammary development equal to that of pregnancy in the Dasyurus after sterile coitus. It is possible that the production of a partial development of the mammary gland followed by a light milk flow was mistaken for full development of the mammary gland equal to that during the first half of pregnancy.

In the pseudo-pregnant bitch Marshall and Halman (1916) observed a rapid development of the lobules and ducts a few days after estrus. The corpora lutea persisted about 30 days, after which regression of the reproductive organs and mammary glands began.

Loeb (1927) prolonged the life of the corpora lutea by hysterectomy in normal guinea pigs. The functional activity of the corpus luteum prevented the typical cyclic activity of the ovary and other reproductive organs bringing about essential characteristics of pregnancy. The effects varied quantitatively with the amount of uterus removed. Deciduomata was present at the lower stumps of the uterus where incomplete hysterectomy resulted. In all cases where the corpora lutea were actively functioning the mammary glands were in a state of proliferation. The similarity in the effects of hysterectomy and pregnancy on the corpus luteum suggests the possibility that a functional activity of the mucosa of the pregnant uterus is responsible for the prolonged functional activity of the corpus luteum. Very slight proliferation of the mammary gland was observed by Loeb and Kountz (1927) during the normal short persistency of the corpora of estrus, while the larger, more persistent corpora of hysterectomized animals caused a proliferation of the gland.

Mammary Growth from Lipoid Extracts.—Up to this time the reactions from aqueous extracts of various sources were very weak. Physiological changes on test animals were so slight that they were hardly noticeable. However, following the publications of Iscovesco (1912) on lipoid extracts of the ovary, corpus luteum and placenta from which very noticeable changes were produced on the female genital organs and mammary glands, a new impetus was introduced in the study of the estrus producing hormone.

The most complete and extensive early experiments upon the mammary gland growth of the rabbit were performed by Fellner (1913). He was able to produce growth of the gland equal to that during the height of estrus; but was unable to get milk secretion in any case. Injections were made with alcohol and ether extracts of placentas, membranes of ovum and ovaries containing corpora lutea. He concluded that the ovary secreted the hormone first and that the placenta either collected the secretion or became a secreting organ after being stimulated by the ovary.

Hermann (1913-15) reported getting maturation of the follicles as well as activation of the mammary glands and reproductive organs; however, ovulation did not occur from lipoid extract injections. Both castrate and normal virgin female rabbits were used. Frank and Rosenbloom (1915) produced activation of the mammary glands in castrate rabbits and rats with 11 to 22 injections of placental lipoid extracts.

Mammary Growth in Normal Guinea Pigs.—Loeb and Hesselberg (1916) found that a definite cycle in the mammary gland of non-pregnant guinea pigs corresponded to the cycle of the ovary and uterus. The height of the cycle is reached during estrus and ovulation after which it gradually falls until another heat period. Two factors seemed to favor proliferation of the gland, (1) The presence of well preserved corpora lutea accompanied by experimentally produced deciduomata, and (2) the imminence of a new period of estrus.

With injections of aqueous extracts of corpora lutea they failed to produce any increased growth of the mammary gland. This is possibly due to the fact that corpus luteum hormone may not be water soluble.

EXPERIMENTS FOLLOWING THE INTRODUCTION OF THE ALLEN AND DOISY TEST

With the introduction of the rat unit test established by Allen and Doisy (1923-24) there followed a second renewed interest in the field of hormonal research. Aside from the knowledge of the potency of the extracts used, no material change in experimental technique has been introduced.

Follicular Liquor Injections Into Rabbits.—By repeated injections of liquor folliculi from cows' ovaries Vintemberger (1925) produced an extended growth of the ducts of the mammary gland in normal virgin female rabbits as well as in young castrate males and females. He was unable to produce an increased number of lobules and alveoli.

The injections produced more rapid results in females than in males. The growth action was marked for a short time and then seemed to lag. The increase in growth was much greater when the gland was already partially developed. A growth of the ducts similar to that of a long term of puberty in the normal female was produced.

Vintemberger reports Tsu-Zong-Yung (1924) as producing mammary gland growth in 3 month old rabbits with 8 injections of liquor folliculi over a period of 20 days.

Gland Growth from Placental and Ovarian Extracts.—Allen (1927) injected 113 to 218 rat units of ovarian and placental extracts into spayed monkeys. The left mammary gland was removed as a control. The ducts of the control glands were small and shrunken exhibiting only a few small alveoli. The right gland was removed at the end of a series of injections. The whole mounts showed marked growth and branching of the ducts with some increase in the number of alveoli.

Laquer (1927) produced mammary gland growth in immature male, female and mature male rats with 18 to 19 injections of Menformon. A total of 72 to 76 mouse units were injected subcutaneously as well as peritoneally.

Champy and Keller (1927) produced mammary growth equal to that of estrus in the normal guinea pig with 5 continuous daily injections of ovarian hormone. In 10 to 25 days uterine development was comparable to that of pregnancy while mammary growth reached its maximum after 15 to 18 daily injections.

Mammary Growth in Guinea Pigs from Placental Extracts.—Haterius (1928) injected 4 adult castrated male guinea pigs twice daily with one-half c.c. doses of Estrogen. The Estrogen* was prepared from human placenta. From 20 to 25 rat units were injected daily. Three controls were given saline solution.

After 8 days a swelling of the mammary gland was very noticeable. Enlargement continued over three weeks after which continued injections produced no further change. In the injected animals the teats were three times larger than the controls. There was a decided growth and branching of the ducts. The lobules increased in size considerably but there was no increase in their actual number.

Following injections of Menformon* into castrated male and virgin females as well as into normal male guinea pigs Laquer, Borchardt, Dingemanse and DeJongh (1928) state that proliferation of the gland and growth in size of the teat were equal to that produced by pregnancy. In part of the experiments 30 to 300 mouse units were injected daily for 24 to 26 days. It was found that the smaller quantity gave nearly equal results in gland growth to that of larger quantities. They did not distinguish between the growth of the ducts and lobules of the gland.

Mammary Growth and Milk Secretion in Castrate Guinea Pigs.—Steinach, Dohrn, Schoeller, Hahliveg and Faure (1928) report that

*Trade names for estrus producing hormone.

development of the mammary glands in castrate male and female guinea pigs followed by milk secretion was obtained from injections of a purified aqueous solution of estrus producing hormone. An average of 25 mouse units were injected daily. The teats increased from 1 millimeter in length at the beginning to 7 millimeters in length and 3. to 3.5 millimeters thick at the base by the end of 3 weeks of injections. At this time the teats and glands were equal in size to that of 17 weeks old normal females. A slight secretion of milk was obtained at the end of 4 weeks followed by full milk secretion at the end of 7 to 8 weeks of injections. From 1400 to 2000 mouse units were required for full milk secretion.

SUMMARY OF PRECEDING WORK

It may readily be seen from the preceding discussion that there is a very great difference of opinion as to the factors involved in causing the complete development of the mammary gland during pregnancy. The most far reaching claims have been made in connection with the experimental development of the mammary gland in the rat and guinea pig. In these animals it is claimed that gland development equal to that of pregnancy has been produced by continued injections of estrus producing hormone. On the other hand in the case of the rabbit no such claims have been advanced.

These differences may be explained by the difficulty of making an examination of the mammary gland in the guinea pig and the rat. In these animals, especially the guinea pig, there is present a fairly thick compact gland growing deep into the tissue. In the rabbit the gland is very thin, and is spread over a large surface, and can easily be examined macroscopically.

The fact that milk secretion is obtained does not necessarily indicate that the gland has developed to the extent observed at parturition. As a limited number of end tubules are present a small amount of milk may be secreted accompanying the formation of the end tubules.

In an attempt to make a microscopic examination of the gland it is difficult to differentiate between true alveoli and developing end tubes. Thus it is possible that investigations with these animals have led to confusion because of the difficulty of differentiating between the development of the duct system and the proliferation of the alveoli.

The presence of milk has been clearly shown to be inconclusive proof of extensive alveolar development. In the rabbit it is possible macroscopically to clearly differentiate between growth of the duct system of the gland and the growth of the lobules which begin to appear on the ends and sides of the main ducts and their branches in planes perpendicular to the skin on both the superficial and deep surfaces of the ducts. For this reason we have used the rabbit in our experiments.

RABBITS USED FOR CONTROLS

As a preliminary it is necessary to have a complete picture of the mammary gland development in the normal rabbit. It has been shown that there is progressive development of the mammary gland during puberty and pregnancy, but there may be an abnormal condition of pseudo-pregnancy produced during puberty. Thus the controls may be divided into 3 groups.

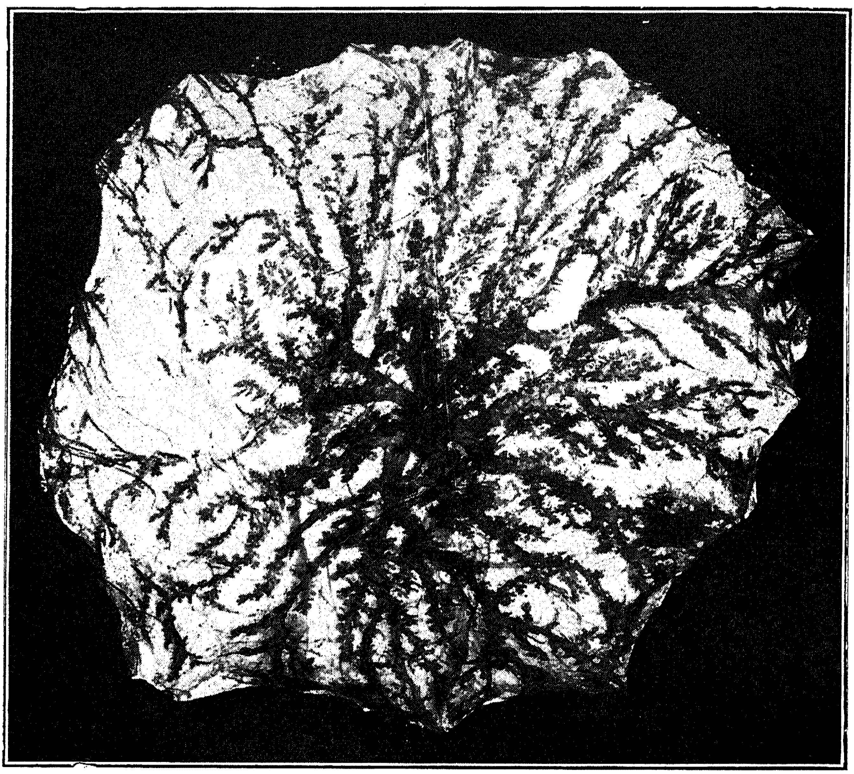

Fig. 28.—Mammary gland of virgin female rabbit in an advanced state of estrus. The ducts have extended to full length terminating in end tubules. Enlarged 2¼ times.

(1) Due to the fact that ovulation does not occur in the rabbit during normal estrus, as previously described, there is present in the ovaries mature follicles which undoubtedly are secreting the estrus producing hormone. It seems reasonable therefore that the development of the mammary gland during normal estrus comes as a result of the

activity of this hormone. Control glands for the development during estrus were taken from mature virgin female rabbits. In these, various degrees of development of the ducts were found. In advanced cases the duct extremities were in contact with the adjacent glands. The ducts terminate in end tubules thus resembling the branches of a tree (Fig. 28).

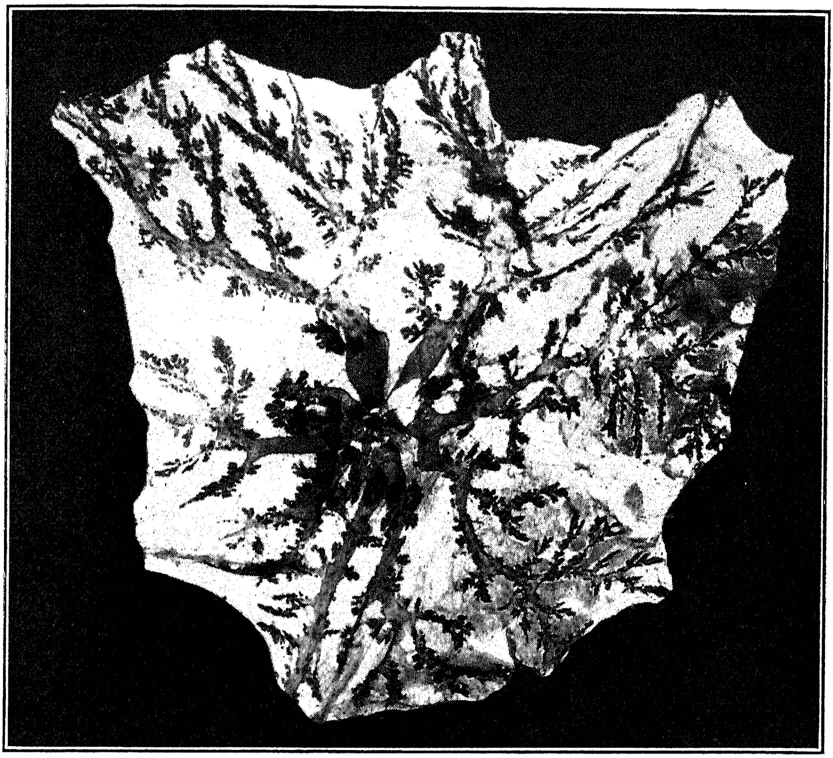

Fig. 29.—Control gland removed before producing the pseudo-pregnant condition in the rabbit. The extended growth was produced during estrus. Enlarged 2¼ times.

(2) If during normal estrus, pseudo-pregnancy (ovulation and corpora lutea production in the ovary) is produced by a sterile coitus further development of the mammary gland ensues. This development may be due to several factors. First, it may be due to the direct effect of a hormone produced by the corpora lutea. Second, it may be due to the combined effect of the estrus producing hormone and the corpus luteum hormone. Third, it may be due to either one or both of these hormones acting on the uterus which may in turn produce a hormone or hormones which may be the active agent. As a control a gland was removed at the time of mating (Fig. 29) and at ten day intervals for 30

days. The appearance of the lobules, previously described, developing along the ducts at ten days pseudo-pregnancy resembled the budding of leaves from the smaller branches. The total number of lobules appeared to be developed (Fig. 30). Macroscopically the gland appeared the same at 20 days as at 10 days (Fig. 31). At 30 days the lobules appeared to be slightly shrunken (Fig. 32).

Fig. 30.—Mammary gland of rabbit 10 days after producing pseudo-pregnancy. The full number of lobules appear to be developing along the mammary ducts. Enlarged 2¼ times.

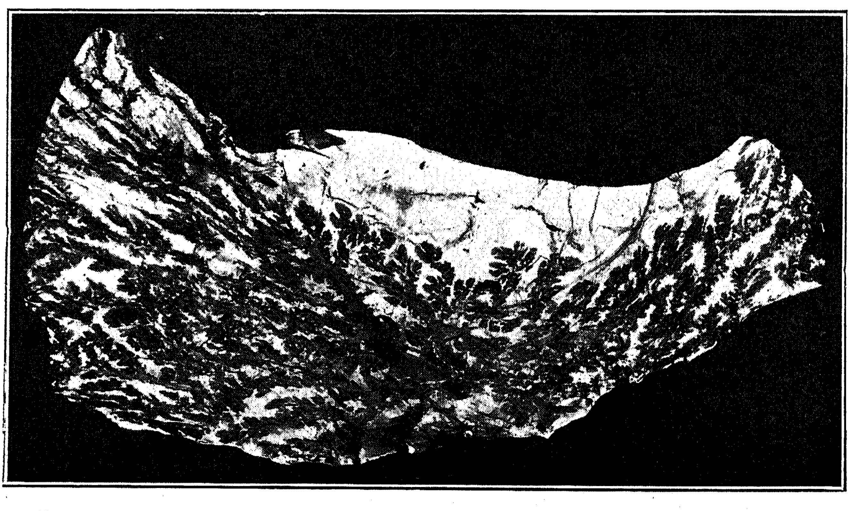

Fig. 31.—Mammary gland of rabbit 20 days after producing pseudo-pregnancy. Enlarged 2¼ times.

(3) During pregnancy the gland is completely developed. The agent producing this development may be due to any or all three factors mentioned during pseudo-pregnancy with the addition of others.

Fourth, it may be due to a hormone or hormones secreted by the placenta. Fifth, it may be due to a secretion from the fetus or fetal membrane. Sixth, it may be due to either the placenta or fetus, or both, causing the persistence of the corpora lutea during the last half of pregnancy which continued producing a corpus luteum hormone directly influencing the mammary gland growth. Two mature virgin rabbits were used as controls. A gland was removed at the time of breeding (Figure 33) and at ten day intervals for 30 days. The appearance of the glands, also previously described, resembled those of pseudo-pregnancy up to 20 days (Figure 34). At 30 days the lobules were developed to their largest size, which resembles the maturing of the leaves. Not only is there growth in a horizontal plane but there is considerable development in thickness. When filled with milk the glands appeared much thicker (Figure 35) than when the milk had been removed. (Figure 36).

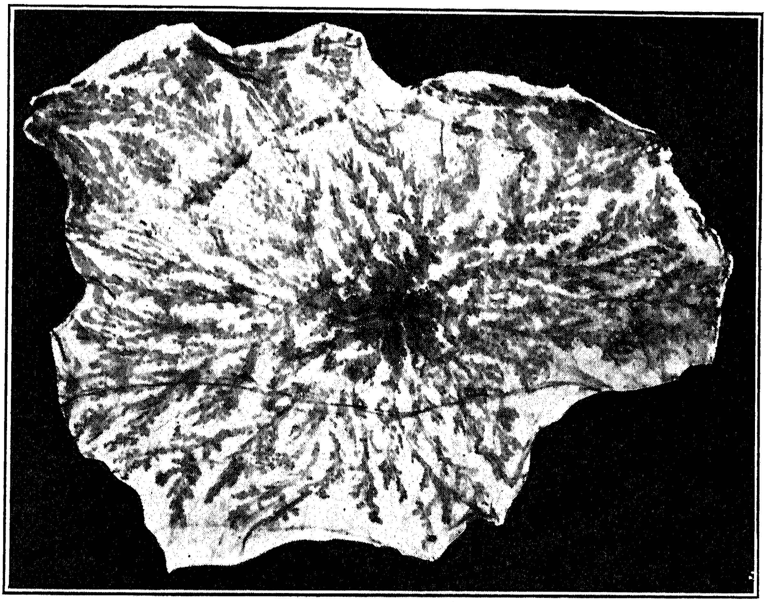

Fig. 32.—Mammary gland of rabbit 30 days after producing pseudo-pregnancy. The lobules are beginning to shrink. Natural size.

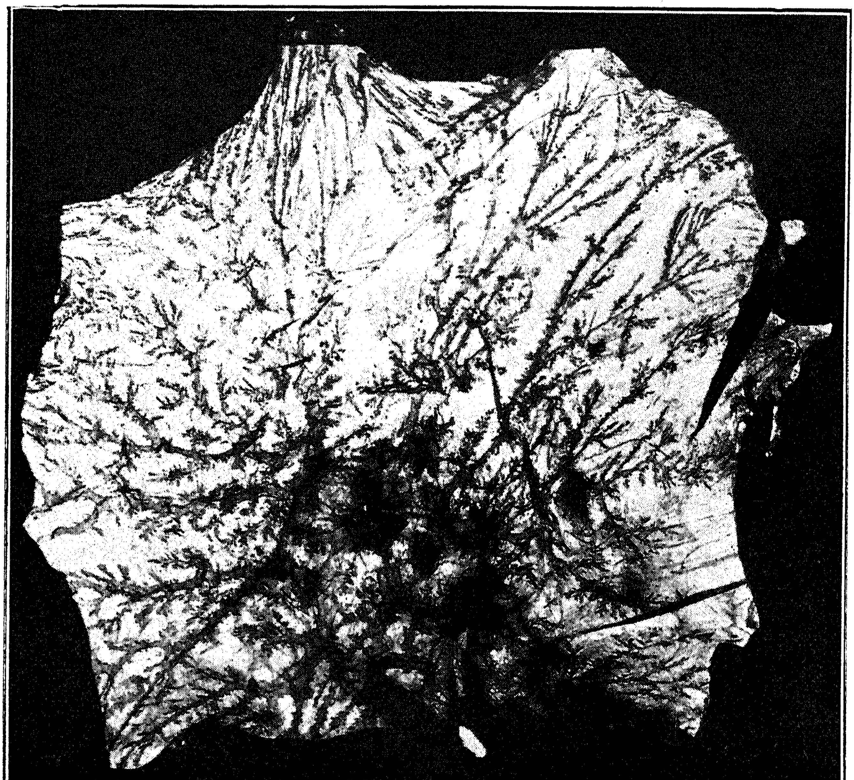

Fig. 33.—Control gland removed at time of breeding. The gland shows a development equal to that of advanced estrus. Enlarged 2¼ times.

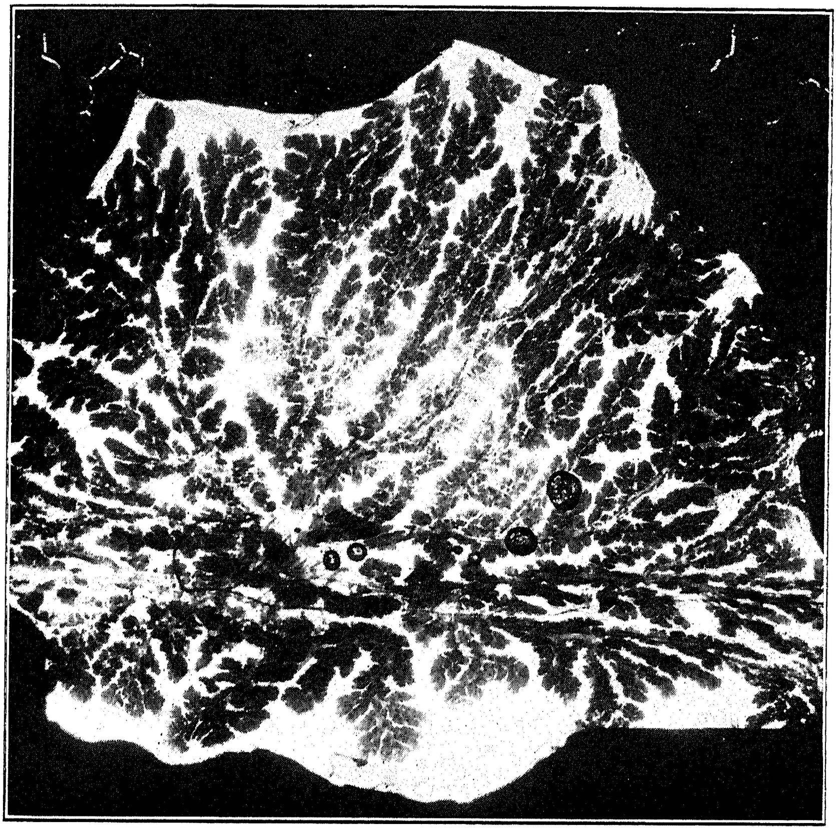

Fig. 34.—Mammary gland at 20 days pregnancy. The developing lobules are similar to the ten and twenty day condition of pseudo-pregnancy. Enlarged 2¼ times.

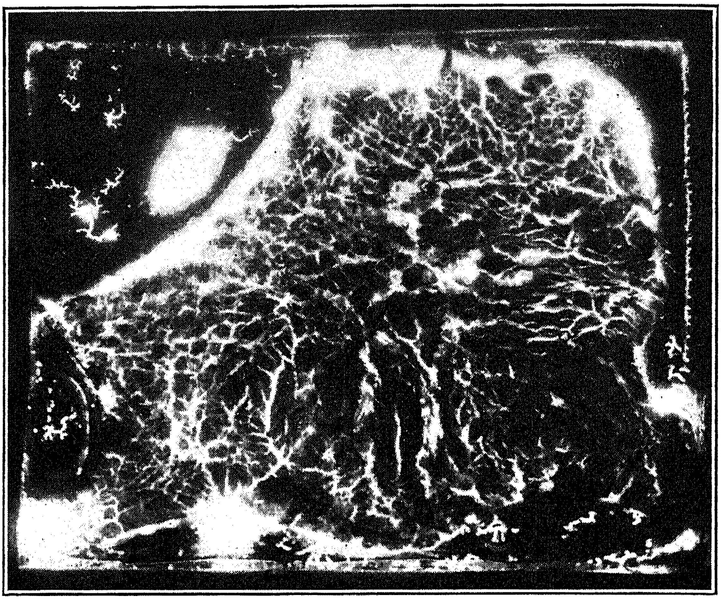

Fig. 35.—Mammary gland at time of parturition. The ducts and lobules are filled with milk. Natural size.

Fig. 36.—Mammary gland at time of parturition after removing the milk. The lobules are fully developed as in number 35, natural size.

INDIVIDUAL CONTROLS

A check gland was removed from each rabbit at the beginning of the first series. All glands were taken so that a parallel gland could be removed at the end of the series, thus eliminating as much individual variation in size as possible.

Rabbits used for the second series were continued from the first series so the last gland removed in the first series served as a control.

In the third series glands were not removed at the beginning for two reasons. First, it was considered unnecessary as only immature castrate males were used, and from previous work it had been shown that the glands were not advanced beyond an infantile state, the ducts scarcely reaching beyond the base of the teats. Second, to avoid infection in the region of the glands as this may influence the normal development (Frank and Unger 1911).

OILS USED AS CARRIERS OF THE HORMONE

The hormone or hormones used in the following experiments were extracted from pregnant cow's urine with olive oil. Just as the surplus estrus producing hormone present in the blood is excreted in the urine, so it seemed possible that all the other hormones produced would be eliminated in the urine. For this reason an attempt was made to determine if hormones other than the estrus producing hormone were taken up in the oil, by subjecting it to different treatments.

The potency of all oils was determined by the rat unit test. It was hoped to make a further assay by means of the mammary gland growth produced as a result of injecting a product of known potency. Thus the oils subjected to different treatments were standardized to the same number of rat units. Differences in the extent or type of growth due to the presence of other hormones, not detected by the rat unit test, could be determined. If complete development of the mammary gland was not produced by the estrus producing hormone it was hoped that this unknown hormone might be found in some of the oils.

The following oils were used:

Oil from Urine.—The oil containing the estrus producing hormone was used as it was taken from the urine.

In addition to the presence of the estrus producing hormone, previous experiments (Nibler and Turner 1929) indicated the possible presence of the corpus luteum hormone. If this were true it might be expected that greater growth of the mammary gland would result from the combined activity than from the estrus producing hormone alone.

Refractionated Oil.—The hormone in the oil from urine was extracted with 95 per cent ethyl alcohol, the alcohol decanted off, and evaporated to a small volume. The residue was taken up in a small amount of oil and the evaporation completed. It was thought possible that by this procedure the ovarian hormone might be removed leaving the corpus luteum hormone in the residual oil. This possibility was indicated by the fact that the estrus producing hormone was found to increase as a result of the above procedure.

Residual Oil.—The oil left after several extractions with alcohol. The extractions were continued until 4.5 cc. contained less than one rat unit. As indicated above it was thought possibly that this oil contained the corpus luteum hormone.

Purified Hormone.—Using Doisy, Veler and Thayer's (1929) method of purification a hormone of considerable purity was obtained.

Olive Oil.—This was used as a check to determine the effect of the injection of oil alone on the mammary gland.

Presentation of Experimental Data

The rabbits used for this experimental work on mammary gland development were of mixed breeding raised in the laboratory. All rabbits used were castrated either before or shortly after reaching puberty. A rather extended growth of the ducts was already established in most of the females*, but in no case had ovulation occurred. With the exception of two males used in Series I the rabbits had not received any previous treatment. All wounds from castration were completely healed before injections were begun.

As the mammary gland spreads out over a large surface instead of growing in thickness, the diameter can easily be measured for a comparison in size. Thus the diameter of all the glands was measured in centimeters. All glands were removed from 4 to 5 days after injections were stopped, allowing time for the last injection of hormone to be utilized. Three series of injections were made.

DETAILED DESCRIPTION OF EXPERIMENT (SERIES I)

In a preliminary experiment (series I) the object was to determine, first, the possible presence of a mammary gland stimulating hormone. Second, to determine the type and extent of growth resulting from the injection of three different oils (residual, refractionated, and oil from urine). Third, to determine the effect of increasing amounts of the estrus producing hormone (measured in rat units) on the growth of the mammary gland.

A total of 13 rabbits consisting of 6 females, 5 young males, and 2 previously injected males (numbers 61 and 62) were used. As there might be some factor present in the castrate females (uterus) which if activated by the injections of hormone would in turn induce mammary growth a male and female were run in duplicate, with one exception, male number 61. Daily injections were made over a period of 30 days.

RESULTS OF EXPERIMENT (SERIES I)

Male Number 61 (not run in duplicate) had previously received one cubic centimeter daily, of refractionated oil, for 16 days. The refractionated oil contained 100 rat units per cubic centimeter, thus a total of 1600 rat units were injected. There was a six months interval between the period of previous injections and Series I. During Series I a total of 1200 rat units, in oil from urine, was injected.

At the close of Series I streams of milk could be drawn from the teats. An extended growth of the ducts was produced reaching full

*The effect of the growth stimulating hormone would have been more effective if the females had been castrated before growth of the ducts had started.

length with the outer extremities coming in contact. However, the type of growth which can be seen macroscopically in the pseudo-pregnant gland consisting of development of the alveoli was not produced. Instead the ducts terminated in end tubules without extensive branching. During the experiment the gland developed from 2.8 centimeters at the beginning to 7 centimeters at the close.

Male No. 62 (run in duplicate) had previously received 3 cubic centimeters daily of residual oil for 16 days. A total of 48 cubic centimeters of oil were injected. These injections were made six months previously to Series I. During Series I a total of 2400 rat units of refractionated oil were injected.

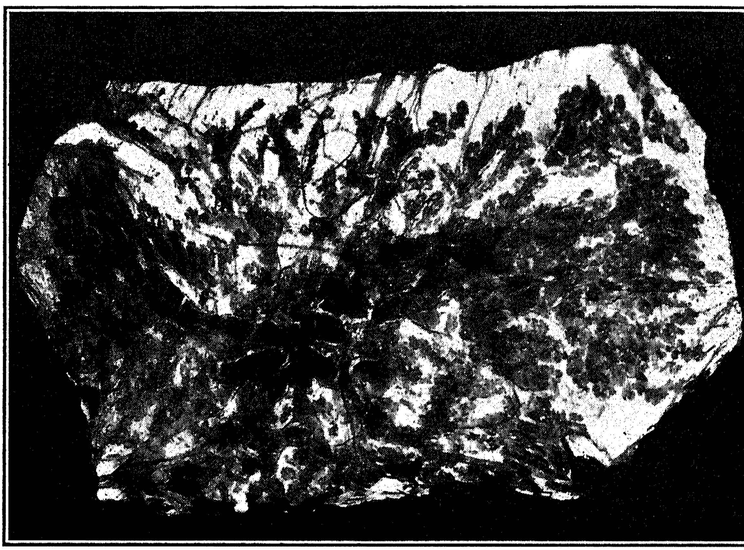

Fig. 37.—Mammary growth produced as a result of injecting 80 rat units of refractionated oil daily for 30 days. The ducts are large and thickly branching. Male Number 62 Enlarged 2 1/4 times.

At the close of the series milk could be expressed in streams, from the teats. An increased development of the ducts was produced. This development consisted mainly of an enlargement in diameter of the individual ducts with the development of numerous short branches terminating in end tubules. Thus the gland appeared thick (Fig. 37), more nearly approaching the appearance produced during the first few days of pregnancy, although alveoli were not produced. The gland increased in diameter of from 2.3 centimeters at the beginning to 4 centimeters at the close.

With the exception of female No. 25 an increase in growth of the ducts was produced in all cases. As this gland was poorly dissected and stained there could have been considerable error in measuring it. The extremities of all other glands taken at the close of the series were more easily distinguished.

The same type of growth was produced by all of the oils, which consisted of an increase in length and branching of the ducts. The type of ducts produced from the residual oil was much finer and more thickly branching, but less in diameter of gland growth, than that produced from the other oils. (Figures 38 and 39). The development of lobules

Fig. 38.—Control gland removed before injections were begun. Male Number 20. Enlarged 2¼ times.

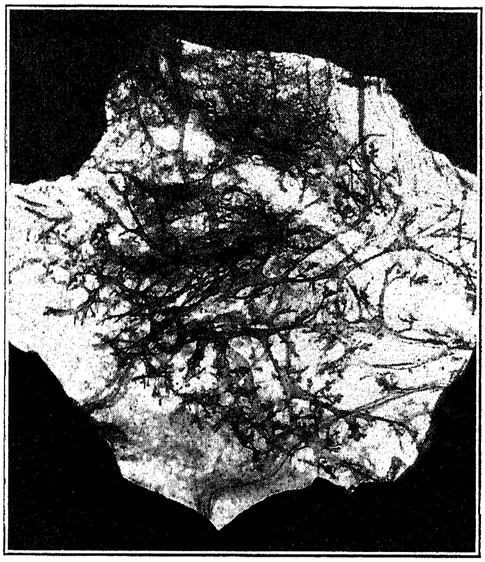

Fig. 39.—Mammary gland growth produced as a result of injecting 2 cubic centimeters of residual oil daily for 30 days. The ducts are of a fine type and thickly branching. Male Number 20. Enlarged 2¼ times.

as produced during pseudo-pregnancy were not produced in any case. Milk secretion was obtained in the two cases of the previously injected males. This secretion may be produced by the developing end tubules.

An average increase, including all rabbits, of 1.8 centimeters in gland diameter was produced. The average increase for the males was 2.3 centimeters and for the females 1.3 centimeters. This difference may be due to an increased number of branches produced in the female gland instead of an increase in length of the larger ducts. The method used for measuring the total diameter of the glands did not include the increased growth of branches. Thus the male gland developing from an infantile state of .2 centimeters in diameter would result in an increased growth of the ducts in length instead of extensive branching of the larger ducts as was produced in the female, which would account for the larger average increase in diameter of the male gland over that of the female. Details of the amount of hormone injected and increase in growth of the glands produced are given in Table 1. Figures 37 to 47 inclusive illustrate the type and amount of growth produced.

TABLE 1.—MAMMARY GLAND GROWTH OF RABBITS FROM VARIOUS OILS
Series I

Tattoo Number	Source of Hormone	Total Daily Injection in		Total Injection in		Diameter of Gland	
						Control	Experimental
		c. c.	*r. u.*	*c. c.*	*r. u.*	*cm.*	*cm.*
25 ♀	Residual Oil	2	0	60		2.5	2.5
20 ♂		2	0	60		0.3	2.5
24 ♀	Oil from Urine	2	20	60	600	2.4	3.5
19 ♂		2	20	60	600	0.3	2.5
61 ♂		4	40	120	1200	2.8	7.0
27 ♀	Refractionated Oil	1	20	30	600	2.0	3.0
60 ♂		1	20	30	600	0.2	2.8
26 ♀		2	40	60	1200	2.5	3.3
62 ♂		2	40	60	1200	0.2	2.0
22 ♀		3	60	90	1800	2.0	2.6
21 ♂		3	60	90	1800	0.3	2.2
23 ♀		4	80	120	2400	1.0	4.0
62 ♂		4	80	120	2400	2.3	4.0

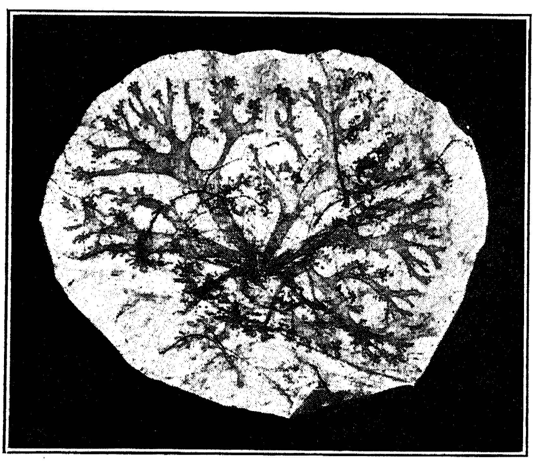

Fig. 40.—Control gland removed before injections were begun. The growth of the ducts was produced during estrus previous to castration. Female No. 27. Enlarged 2¼ times.

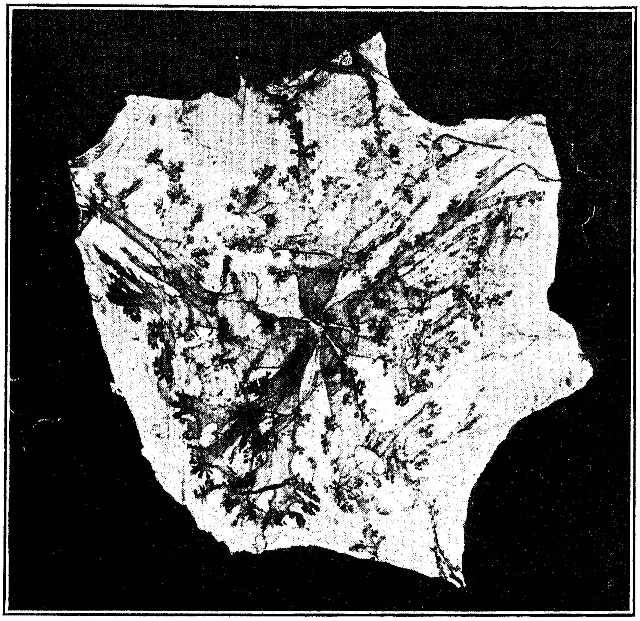

Fig. 41.—Increase in gland growth produced as a result of injecting 20 rat units of refractionated oil daily for 30 days. The same type of growth was produced by the hormone as was produced during estrus. Female No. 27. Enlarged 2¼ times.

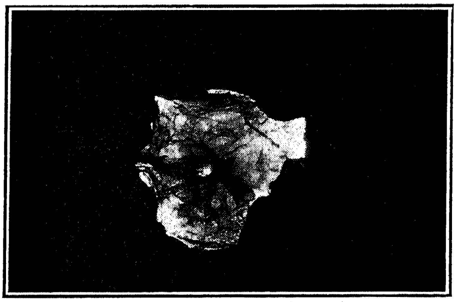

Fig. 42.—Control gland removed before injections were begun. Male No. 60. Enlarged 2¼ times.

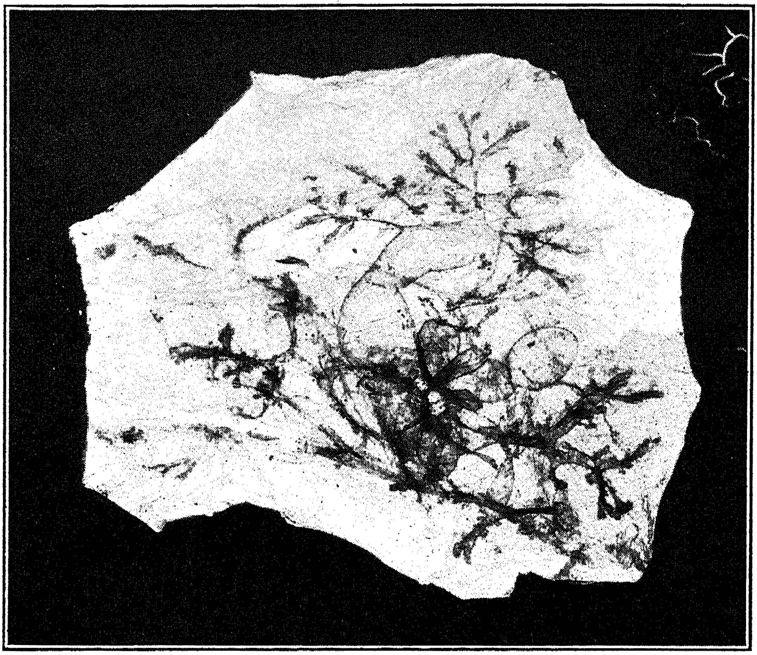

Fig. 43.—Mammary growth produced as a result of injecting 20 rat units of refractionated oil daily for 30 days. An increase in length and branching of the ducts similar to that occurring during estrus was produced. Male No. 60. Enlarged 2¼ times.

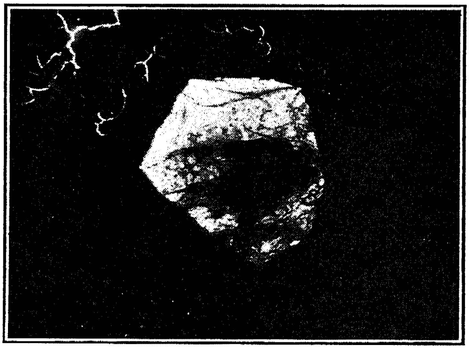

Fig. 44.—Control gland removed before injections were begun. Male No. 62. Enlarged 2¼ times.

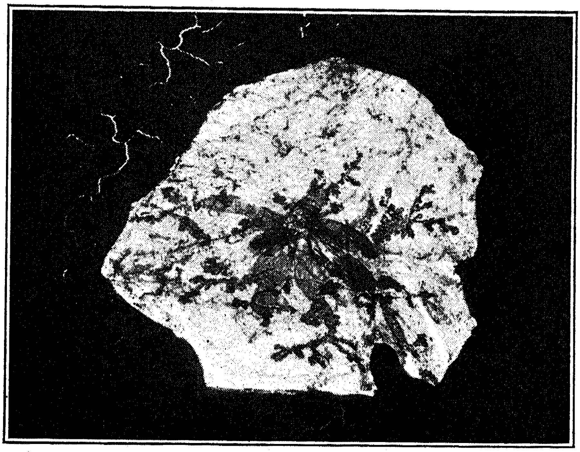

Fig. 45.—Mammary growth produced as a result of injecting 40 rat units daily of refractionated oil for 30 days. An increase in length and branching of the ducts similar to that occuring during estrus was produced. Male No. 62. Enlarged 2¼ times.

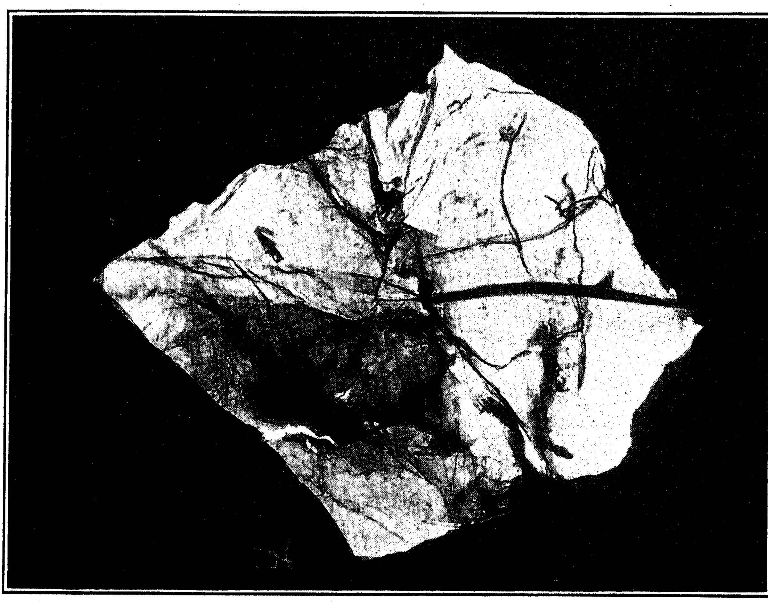

Figure 46.—Control gland removed before injections were begun. Female number 23. Enlarged 2¼ times.

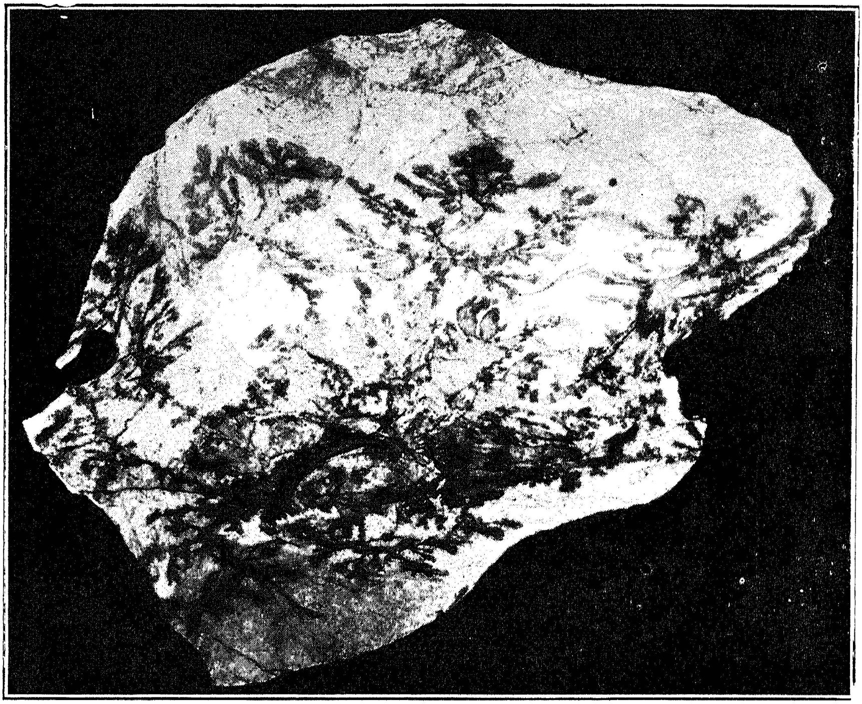

Fig. 47.—Increase in mammary growth produced as a result of injecting 80 rat units of refractionated oil daily for 30 days. Growth equal to that during advanced estrus was produced. Female No. 23. Enlarged 2¼ times.

DETAILED DESCRIPTION OF EXPERIMENTS (SERIES II)

Maximum growth of the mammary ducts, equal to that produced during continuous estrus in the normal female, was produced in castrate males by the injection of a small amount of the estrus producing hormone over a period of 30 days. The rapid growth of the gland during the first few days of pregnancy would indicate that either a large amount of the estrus producing hormone was secreted or other hormones were present. In continuing the experimental work (Series II) the object was to determine the extent and type of mammary growth produced as a result of a large amount of the estrus producing hormone injected over a short period of time.

Four male and two female rabbits from Series I were used in Series II. Very potent refractionated oils were used as a source of the hormone. A total of 800 rat units were injected daily into each rabbit for 10 days.

RESULTS OF EXPERIMENT (SERIES II)

In two cases, rabbits No. 22 and No. 60, there was an increase of 0.9 and 0.7 centimeters respectively, in the diameter of the gland.

In Rabbit No. 19 there was a decrease of 0.5 centimeters, while rabbits No. 20 and No. 23 were the same size as the checks.

Some difference in the size of the glands produced in comparison with the checks used might be due to individual variations in adjacent glands, as parallel glands were not removed. The type and extent of growth produced is shown in Figures 48, 49, and 50.

It was plainly shown that the increase in development of the glands was negligible. Details of the experiment are given in Table 2.

Table 2.—Mammary Gland Growth of Rabbits from Various Oils
Series II

Tattoo Number	Source of Hormone	Total Daily Injection in		Total Injection in		Diameter of Gland	
						Control	Experimental
		c. c.	r. u.	c. c.	r. u.	cm.	cm.
21♂	Refractionated Oil	1	800	10	8000	2.2	2.2
60♂		1	800	10	8000	2.8	3.5
20♂		1	800	10	8000	2.5	2.5
19♂		1	800	10	8000	2.5	2.0
22♀		1	800	10	8000	2.6	3.5
23♀		1	800	10	8000	4.0	4.0

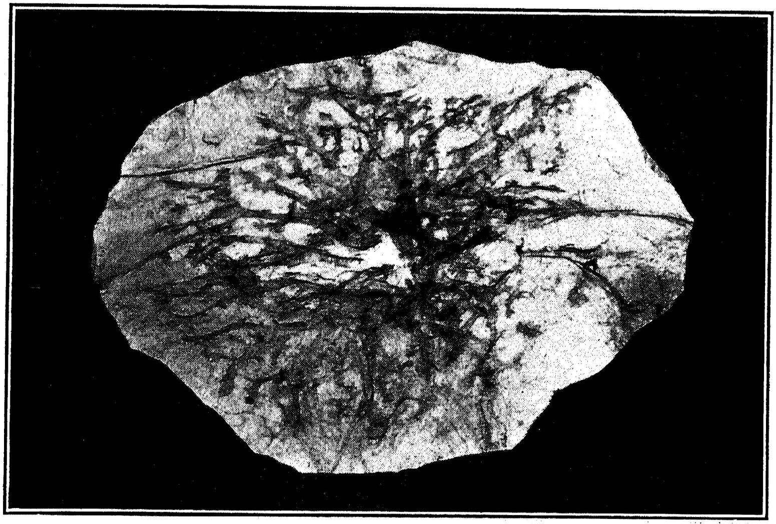

Fig. 48.—Gland removed after an injection of 800 rat units daily for 10 days. Control gland Figure 40. Male No. 20, Enlarged 2¼ times.

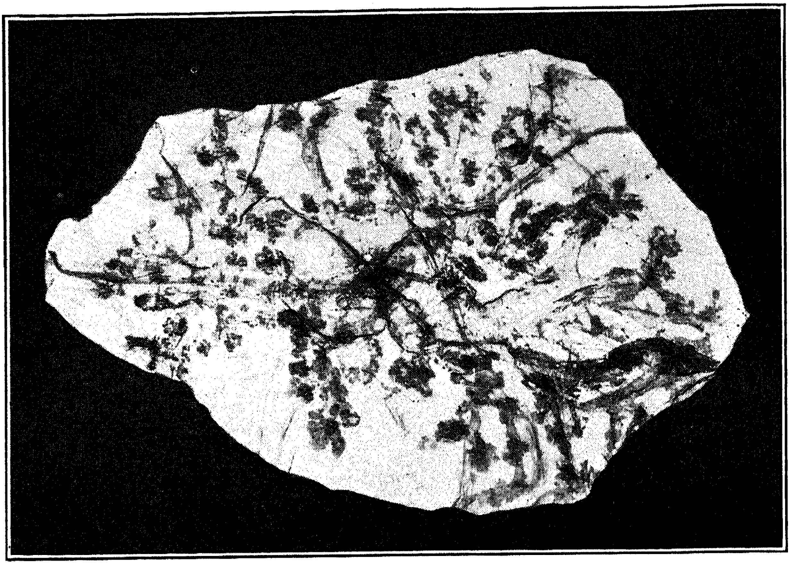

Fig. 49.—Gland removed after an injection of 800 rat units daily for 10 days. Control gland Figure 44. Male No. 60. Enlarged 2¼ times.

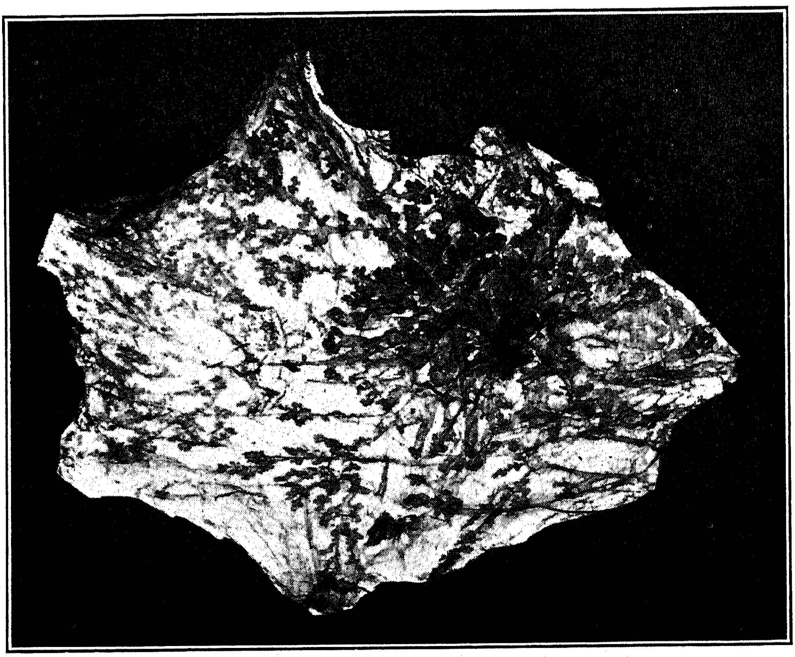

Fig. 50.—Gland removed after an injection of 800 rat units daily for ten days. Control gland Figure 48. Female number 23, enlarged 2¼ times.

DETAILED DESCRIPTION OF EXPERIMENT (SERIES III)

Growth of the ducts of the mammary gland resulted from injections of oils used which would indicate that the estrus producing hormone was the activating agent. Due to the fact that the oils used contained impurities from the urine, and that the residual oil produced a different type of duct growth, the question might arise as to whether the growth was entirely produced by the estrus producing hormone or some other activating agent. Cases of infection (resulting after the removal of glands in some cases) in the region of the mammary gland have been reported as activating the glands. In removing glands it was necessary to cut large blood vessels which might effect the future growth of adjacent glands by decreasing the circulation. Blood vessels supplying the gland are shown in Figure 52.

In taking control glands, if there is an individual variation in the size of the glands, the amount of growth measured in one gland would only be an estimate of the actual amount of growth produced in the other glands. For these reasons it seemed desirable to repeat the experiment to secure further observations, eliminating as far as possible the objections mentioned.

In further experimental work (Series III) the object was to determine the extent and type of growth produced by injections of purified hormone and olive oil using the other three oils (residual, oil from urine, and refractionated) as checks. The mammary glands in males do not normally develop beyond an infantile state, thus by using young males it was not necessary to take a check gland at the beginning, which would eliminate all chances of infection and impaired circulation.

Eighteen male rabbits were available for this experiment. Six different oils were used with three rabbits in each group. All rabbits in each group received an equal amount of oil. Two rabbits of each group were given daily injections during a period of 30 days, while the third rabbit received the same total amount during a period of 15 days.

All rabbits were killed at the close of the respective injection periods and the glands removed intact for a comparison of individual variation. As a check on the amount of growth produced in 15 days a gland was taken from each of six rabbits receiving the injection for 30 days. Thus 6 normal rabbits served as a check for the six from which glands were removed. The normal rabbits would serve as controls in case infection or impairment of the circulation resulted.

The details of the experiment including the kind and amounts of oil used and the results obtained are presented in Table 3.

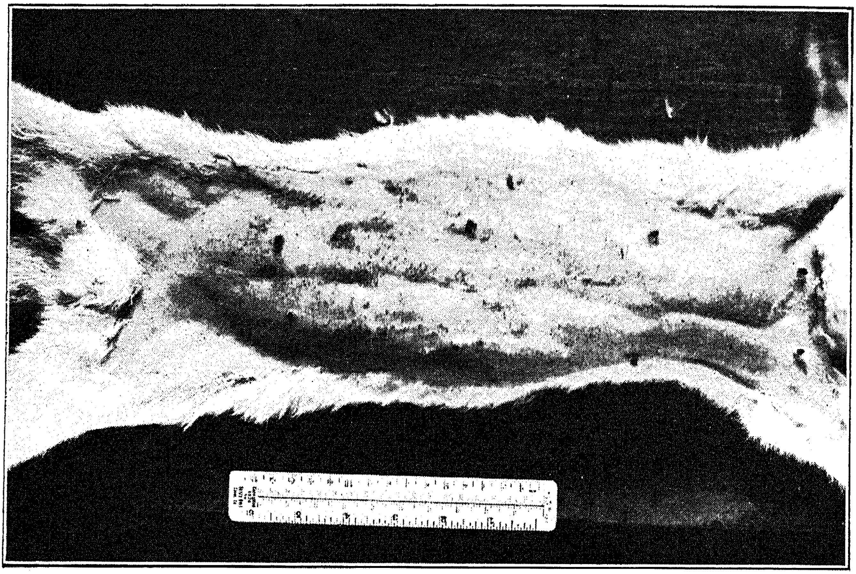

Fig 51.—Adult female rabbit after hair was removed from the abdomen, thorax and neck illustrating a normal teat pattern of eight teats extending from between the rear limbs to well up on the neck.

TABLE 3.—MAMMARY GLAND GROWTH OF RABBITS FROM VARIOUS OILS
Series III

Tattoo Number	Source of Hormone	Total Daily Injection in		Total Injections in			Diameter of Gland 15 Days	Diameter of Gland 30 Days
		c. c.	*r. u.*	*c. c.*	*r. u.*	*days*		
3♂	Olive Oil	4.0	0	60	0	15	1.5	
52♂	Olive Oil	2.0	0	60	0	30	.9	2.5
17♂	Olive Oil	2.0	0	60	0	30		1.0
10♂	Residual Oil	4.0	0	60		15	.8	
45♂	Residual Oil	2.0	0	60		30	.9	2.8
5A♂	Residual Oil	2.0	0	60		30		3.0
7A♂	Oil from Urine	1.2	40	18	600	15	1.7	
44♂	Oil from Urine	0.6	20	18	600	30	2.8	5.0
40A♂	Oil from Urine	0.6	20	18	600	30		4.0
34♂	Refractionated Oil	1.6	40	24	600	15	1.5	
40♂	Refractionated Oil	0.8	20	24	600	30	2.4	4.0
35♂	Refractionated Oil	0.8	20	24	600	30		3.0
47♂	Purified Oil	4.0	40	60	600	15	2.3	
6♂	Purified Oil	2.0	20	60	600	30	1.6	2.5
7♂	Purified Oil	2.0	20	60	600	30		4.5
41♂	Purified Oil	2.0	120	30	1800	15	2.5	
5♂	Purified Oil	1.0	60	30	1800	30	3.0	3.2
19♂	Purified Oil	1.0	60	30	1800	30		3.0

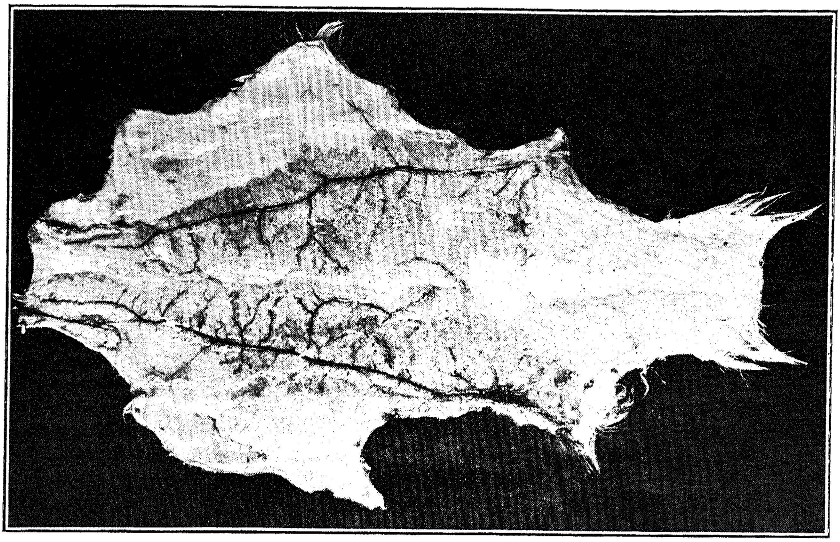

Fig. 52.—Vascular system of the mammary glands of a female rabbit 5 days after parturition. The glands are distended with milk. The blood vessels were injected.

RESULTS OF EXPERIMENT (SERIES III)

An increase in growth of the ducts was produced in all cases. The same type of growth as produced in Series I was produced by the oils in Series III. This consisted of an increase in length and branching of the ducts.

The olive oil produced a finer, more intensive branching type of growth similar to that produced by the residual oil. Neither of these oils produced a gland of large diameter. This would indicate that the finer type of growth produced by the residual oil was due to the action of the oil alone.

The purified hormone produced the same type and amount of growth as the other oils (oil from urine and refractionated) containing the estrus producing hormone. Thus the more extensive and coarser type of growth of the ducts was produced by the estrus producing hormone.

The average diameter of the glands produced as a result of injecting the total amount of oil in 15 days was 1.7 centimeters, while an increase of 3.25 centimeters was produced from the same amount of oil in 30 days.

The glands removed from the rabbits receiving half of the total amount of oil in 15 days showed an average diameter of 1.9 centimeters, as compared to 1.7 centimeters for those receiving the total amount of oil in 15 days.

The average diameter of the glands produced from injecting the total amount of oil in 30 days was 3.25 centimeters for the normal rabbits and 3.33 centimeters for those whose glands were removed.

The above experiments seem to indicate that the rate of growth of the ducts (estrous type of development) of the mammary gland is not influenced by the size of the dosage of the estrus producing hormone. These observations would indicate that either a uniform concentration of the hormone was maintained in the blood (surplus being quickly eliminated in the urine) or that the mammary growth takes place at a definite rate regardless of the concentration of hormone in the blood.

In cases where the glands were removed during the experiment, no noticeable difference was observed due to infections, or a decreased blood supply.

There was considerable variation in the size of individual glands. Thus a measurement of the gland diameter of any one gland would only be an approximation of the amount of growth produced in other glands. For comparative purposes in this work the measurements were satisfactory.

SUMMARY AND CONCLUSIONS

As a result of previous work at this Station indicating that the rate of excretion of the estrus producing hormone in the urine of pregnant cattle increases during the course of gestation, a study was made of the effect of the hormone on the growth of the mammary gland in the rabbit.

As a preliminary to this experimental study it became necessary to obtain an anatomical picture of the stages of development in the normal rabbit. The growth of gland was described under the following headings: I. Development during embryonic and fetal life. II. Development from birth to puberty. III. Development during puberty. IV. Development during pregnancy. V. Development during lactation followed by regression.

The review of the literature on the physiological development of the gland was divided into several periods. It was shown that from 1906 when Lane-Claypon and Starling began a study of the possible influence of hormones until 1912 aqueous extracts alone were used. However, with the report in 1912 by Iscovesco that lipoid extracts of the ovary produced noticeable changes in the female genital organs and mammary glands, a new impetus was introduced in the study of the physiology of the mammary gland. With the development of the method of the biological assay of the estrus producing hormone by the Allen and Doisy rat unit test in 1923 and 1924 there has followed a second renewed interest in this field which has continued up to the present time.

In the normal rabbit after continued estrus, the mammary glands show extreme extension of the duct systems resembling the naked branches of a tree. If pregnancy or even pseudo-pregnancy now ensues, the ducts develop lobules containing large numbers of alveoli, resembling the budding of leaves from the smaller branches. These two types of growth can be distinguished macroscopically.

It was found that the daily injection of 20 rat units of the estrus producing hormone recovered from pregnant cow's urine for 30 days in male castrate rabbits and in female rabbits castrated previous to puberty caused the growth of the duct system of the glands equal to that produced during continued estrus in the normal female. A slight milk secretion resulted in these cases. The injection of greatly increased amounts of the hormone did not carry the development beyond this stage.

The results obtained seemed to warrant the conclusion, that the oestrus producing hormone will cause the growth of the ducts equal to that produced during estrus, that the rate of development is not hastened by increasing the dosage, that there was present in none of the oils used a hormone or hormones which would produce the type of growth characteristic of pregnancy.

BIBLIOGRAPHY

Allen, E. and Doisy, E. A., *An ovarian hormone. Preliminary report on its localization, extraction, and partial purification, and action in test animals.* Jour. Am. Med. Assn. 81 (1923), pp. 819.

Allen, E. and Doisy, E. A., *The induction of a sexually mature condition in immature females by injection of the ovarian follicular hormone.* Am. Jour. Physiol. 69 (1924), pp. 577.

Allen, E., *The menstrual cycle of the monkey, Macacus Rhesus; observations on normal animals, the effects of removal of the ovaries and the effects of injections of ovarian and placental extracts into the spayed animals.* Carnegie Inst. Cont. to Embry. 19 (1927), pp. 1-44.

Ancel, P., and Bouin, P., *Action du corps jaune vrai sur la glande mammaire.* (*The function of the corpus luteum*). Compt. rend. Soc. d. Biol. 66 (1909), pp. 605.

Ancel, P., and Bouin, P., *Recherches sur les fonctions de corps jaune gestatif. II. Sur le determinisme du developpement de la glande mammaire au cours de la gestation.* (*Investigation of the functions of the corpus luteum*). Jour. de Physiol. et de. Path. Gen. 13 (1911) pp. 31-41.

Ancel, P., and Bouin, P., *Sur l'evolution de la glande mammaire pendant la gestation.* (*On the evolution of the mammary gland during gestation*). Compt. rend. Soc. de Biologie 72. (1912), pp. 129-131.

Aschner, B., *Ueber brunstartige erscheinungen* (*hyperamie und. harmorrhagie am weiblichen genitale*) *nach subkutaner Injektion von ovarialoder -plazentarextrakte.* (*The phenomenon of oestrus produced by subcutaneous injections of ovarian or placental extracts*). Arch. f. Gynak. 99 (1913), pp. 534-540.

Aschner, B., and Grigoriu, C., *Placenta, fötus u. keimdrüse in ihrer wirkung auf die milchsekretion.* (*Placenta, fetus, and ovary in their influence on milk secretion*). Arch. f. Gynak. 94 (1911), pp. 766-793.

Basch, K., *Über experimentelle auslösung von milchabsonderung.* (*Experimental secretion of milk*). Monatschr. f. Kinderh. 8 (1909-1910), pp. 513-524.

Bellerby, C. W., *The relation of the anterior lobe of the pituitary to ovulation.* Jour. Physiol. 67 (1929), pp. XXXIII.

Biedl, A., and Königstein, H., *Untersuchungen über das brustdrüsen-hormon der gra vidität.* (*Study of the mammary gland hormone of pregnancy*). Ztschr. f. Exper. Path. u. Therap. 8 (1910), pp. 358-373.

Brouha, Dr., *Recherches sur les diverses du developpement et de l'activite de la mamelle.* (*Investigations of the various phases of the development and activity of the breast*). Arch. de. Biol. 21 (1905), pp. 470.

Champy, C., and Keller, T., *Developpement utérin et mammaire par injection d'hormone ovarienne.* (*Uterine and mammary development following injections of the ovarian hormone*). Compt. rend. Acad. de. sci. 85 (1927), pp. 302-4.

Doisy, E. A., Veler, C. D., and Thayer, S., *Folliculin from Urine of pregnant women.* Abstracts of Comm., XIIIth International Physiological Congress Amer. Jour. Phys. 90 (1929), pp. 329-330.

Fellner, O. O., *Experimentelle untersuchungen über die wirkung von gewebsextrakten aus der plazenta und den weiblichen sexualorganen auf das genitale.* (*Experimental examinations of the effect of tissue extracts from the placenta and the female sexual organs on the genitals*). Archiv. f. Gyn. 100 (1913), pp. 641-719.

Foa, C., *Sui fattori che determinano l'accresimento e la funzione della ghiandola mammarie.* (*On the factors influencing the growth and function of the mammary gland*). Archiv. di fisiol. 5 (1908), pp. 520.

Frank, R. T., and Unger, A., *An experimental study of the causes which produce the growth of the mammary gland.* Archiv. Int. Med. 7 (1911), pp. 812-838.

Frank, R. T., and Rosenbloom, J., *Physiologically active substances contained in the placenta and in the corpus luteum.* Surg. Gynec. and Obst. 21 (1915), pp. 646.

Hammond, J., and Marshall, F. H. A., *Reproduction in the rabbit.* London, Oliver and Boyd, 1925.

Hammond, J., and Marshall, F. H. A., *The functional correlation between the ovaries, uterus, and mammary glands in the rabbir, with observations on the oestrus cycle.* Proc. Roy. Soc. B. 87 (1914), pp. 422-440.

Hammond, J., *On the cause responsible for the developmental progress of the mammary glands in the rabbit during the latter part of pregnancy.* Proc. Roy. Soc. B. 89 (1917), pp. 534-546.

Haterius, *Effect of placental extract on mammary glands of male guinea pigs.* Proc. Soc. for Exper. Biol. and Med. 25 (1928), p. 471.

Hermann, E., *Zur physiologie des corpus luteum.* (*On the physiology of the corpus luteum*). Zentrable f. Gynak. 37 (1913), p. 1563.

Hermann, E., *Über eine wirksame substanz im eierstocke u. in der placenta.* (*On an active substance in ovary and placenta*). Monatschr. f. Geburstch. u. Gynak. 41 (1915), pp. 1-50.

Iscovesco, H., *Le lipoide utéro-stimulant de l'ovarie; proprietes physiologiques.* (*The lipoid uterine stimulant from the ovary. Physiological properties*). Compt. rend. Soc. de Biol. 73 (1912), pp. 104-106.

Lane-Claypon, J. E., and Starling, E. H., *An experimental inquiry into the factors which determine the growth and activity of the mammary glands.* Proc. Roy. Soc. B. 77 (1906), pp. 505-522.

Laquer, E., DeJongh, S. E., and Tausch, M., *Über weibliches sexual-hormone, Menformon. V. Über den feminisierenden einfluss des Menformons auf de unentwickelte brustd rüse.* (*The effective influence of Menformon on the undeveloped mammary gland*). Deutsch. *med.* Wochenschr. 53 (1927), pp. 867.

Laquer, E., Borchardt, E., Dingemanse, E., and DeJongh, S. E., *Über weibliches* (*Sexual*) *hormon, Menformon* (*Female sexual hormone, Menformon*). Deutsche *med.* Wochen. 54 (1928), pp. 465-467.

Lewis, F. T., *The development of the lymphatic system in rabbits.* Am. Jour. Anat. 5 (1905-6), pp. 95-110.

Loeb, L., and Hesselberg, C., I. *The cyclic changes in the mammary gland under normal and pathological conditions.* Jour. Exp. Med. 25 (1917), pp. 285-304.

Loeb, L., and Hesselberg, C., II. *The cyclic changes in the mammary gland under normal and pathological conditions.* Jour. Exp. Med. 25 (1917), pp. 305-321.

Loeb, L., *The effects of hysterectomy on the system of the sex organs and on the periodicity of the sexual cycle in the guinea pig.* Am. Jour. Phys. 83 (1927), pp. 202-224.

Loeb, L., *Special Cytology.* Vol. 2 (ed. by Cowdry, E. V., New York, Hoeber Inc.,) 1928.

Loeb, L., and Kountz, W. B., *The effect of injection of follicular extract on the sex organs in the guinea pig and the interaction between the follicular substances given off by the corpus luteum.* Am. Jour. Physiol. 84 (1928), pp. 283-306.

Marshall, F. H. A., and Halnan, E. T., *On the post=estrus changes occurring in the generative organs and mammary glands of the non-pregnant dog.* Proc. Roy. Soc. B. 89, (1917), pp. 546.

Marshall, F. H. A., *The physiology of reproduction*, Ed. 2, London, Longmans, Green, and Company, 1922.

O'Donaghue, *The growth changes in the mammary apparatus of Dasyurus and the relation of the corpora lutea thereto.* Quart. Jour. Micro. Sci. 57, (1911), pp. 187-234.

O'Donaghue and Hill, *The reproductive cycle in the Marsupial.* Quart. Jour. Micr. Sci. 59 (1915), pp. 133-176.

Parkes, A. S., *The functions of the corpus luteum. III. The factors concerned in the development of the mammary gland.* Proc. Roy. Soc. B. 104 (1929), pp. 189.

Rein, G., *Untersuchungen über die embryonale entwicklungsgeschichte der milchdrüse.* (*An investigation concerning the embryonic development of the mammary gland*). Archiv. fur. Mikr. Anatomie 20 (1881), pp. 431, and 21 (1882), p. 678.

Rudolphi, *Einige bemerkungen über den bau, der brüste. Abhandlungen der konigal.* (*A note on the structure of the breast*). Berlin, Akademie der Wissenschaften, 1831.

Schickele, G., *Beitrage zur Physiologie und pathologie der ovarien.* (*Study of the physiology and pathology of the ovary*). Arch. f. Gynak. 97 (1912), pp. 409-473.

Schultze, O., *Ueber die erste anlage des milchdrüsen apparates.* (*The first anlage of the mammary gland apparatus*). Anat. Anz. 7 (1892), pp. 265-270.

Steinach, E., Dohrn, M., Schoeller, W., Hohlweg, W., and Faure, W., *Über die biologischen wirkungen des weiblichen sexualhormons.* (*Biological effect of female sexual hormones*). Archiv. fuer. Gesamte Physiol. 219 (1928), pp. 306-336.

Tsu-Zong-Lung, *Le rhythme vaginal chez la lapine et ses relations avec le cycle estrien de l'ovaire.* (*The rhythmic cycle of the rabbit vagina and its relation to oestrus cycle of the ovary*). These Medicine, Strassbourg (1924), (quoted from Vintemberger).

Vintemberger, P., *Action des injections de liquide folliculaire sur la gland mammaire.* (*Action of the injection of liquid folliculi on the mammary gland*). Arch. de Biol. 35 (1925), pp. 125-154.

Wahl, H. M., *Development of the blood vessels of the mammary gland in the rabbit.* Am. Jour. Anat. 18 (1915), pp. 515-524.

Walton, A., and Hammond, J., *Observations on ovulation in the rabbit.* Brit. Jour. Exper. Biol. 6 (1928), pp. 190-204.