UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION

Research Bulletin 207

The Normal and Experimental Development of the Mammary Gland

I. The Male and Female Domestic Cat

C. W. TURNER AND W. R. DEMOSS

II. The Male and Female Dog

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(Publication Authorized April 23, 1934)

COLUMBIA, MISSOURI
MAY, 1934

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TABLE OF CONTENTS

ABSTRACT

Experiments are reported which indicate that the estrogenic hormone will stimulate the growth of the duct system of the mammary gland of the domestic cat. The glands of the male and female are equipotential in their response to this hormone. Following the growth of the duct system in males, the growth of the lobule-alveolar system was stimulated by the daily simultaneous injection of one rabbit unit of corporin and 25 rat units of theelin for 20 days. The glands at the end of this treatment were similar to glands removed from pregnant animals at about mid-term. The injection of the lactogenic hormone, galactin, at this stage, caused the stimulation of milk secretion comparable to that observed in the glands of normal females after parturition.

Thus the mammary glands of the male cat have been stimulated experimentally by the proper sequence of hormones through the various stages of growth and the initiation of lactation.

ACKNOWLEDGMENT

The writers have profited from the constructive criticism, comment and suggestions of Dr. W. O. Nelson, Dept. of Anatomy, University of Missouri, and Dr. W. U. Gardner, National Research Council Fellow, Dept. of Anatomy, Yale University, who kindly consented to read the manuscripts of the papers in this bulletin. It is a pleasure to acknowledge our indebtedness to them.

The Normal and Experimental Development of the Mammary Gland

I. THE MALE AND FEMALE DOMESTIC CAT

C. W. TURNER AND W. R. DEMOSS

As an introduction to the study of the experimental development of the mammary gland of the domestic cat, it was planned to study the normal development during estrum, pregnancy and pseudo-pregnancy as well. However, the difficulty of breeding cats in confinement led to failure except in a few cases. As a consequence, such information as is available on the normal development of the mammary gland of the cat will be presented briefly in this paper.

The mammary gland complex of the cat consists of two parallel rows of glands, one on each side of the ventral mid-line extending from the thoracic to the abdominal region. There are normally four pairs of teats and glands which are about equidistantly spaced.

In a series of cats examined before the onset of puberty, the mammary glands were observed to consist of canalized primary sprouts with the duct system confined to an area not extending beyond the base of the teat. In removing control glands from such experimental animals, it was difficult to secure whole mounts of the glands. However, sections at the base of the teat showed that the duct development was limited. A similar condition of the duct system was observed in males of all ages (Fig. 5).

PUBERTY AND PREGNANCY

In females after reaching puberty, various degrees of duct development were observed. These observations were taken to indicate that following sexual maturity of the cat, the duct system gradually develops.

While we were unable to breed cats in confinement, two stages of gland development during pregnancy were observed, but the exact duration of pregnancy was not known. The type of gland development observed in these animals, namely, lobule-alveolar growth, was quite similar to that observed in the rabbit at the middle of pregnancy. It may reasonably be assumed that in the essentials the development of the mammary gland of the cat during pregnancy follows the same course as in other mammals that have been studied.

THE EXPERIMENTAL GROWTH OF THE DUCT SYSTEM

In previous work in this laboratory it has been shown that the growth of the duct system of the mammary gland of the rabbit (Turner and Frank, 1930), rat (Turner and Schultze, 1931), guinea pig (Turner and Gomez, 1934) and mouse (Turner and Gomez, 1934) may be stimulated to extensive growth as the result of the injection of the estrogenic hormone (theelin, estrin*, etc.). However, so far as is known, such studies have not been extended to the domestic cat, a carnivora. It seemed of interest, therefore, to extend the observations on the experimental stimulation of mammary development to the male and female of this family.

For a crucial test of the effectiveness of the estrogenic hormone it is necessary to use animals with a minimum extension of the duct system. For this purpose females castrated before puberty or males of all ages were found to be satisfactory.

In a preliminary experiment two immature females were castrated. One animal received 25 rat units once daily of the estrogenic hormone in oil, while the second animal received 50 rat units. The control gland showed that the ducts did not extend beyond the base of the teat. Glands removed at 15 day intervals showed rather slow growth. When the animal receiving 25 rat units died after 40 days of the treatment, the remaining glands had not developed beyond one cm. in diameter.

The control gland from the cat receiving the larger amounts of theelin had a duct system extending almost one cm. in diameter. Glands removed at 15 day intervals showed progressive growth of the duct system. After 43 days of treatment the glands had reached a diameter of 3 cm. The growth of the duct system did not seem complete since the smaller branches seemed to be growing as indicated by the presence of bulb-shaped end-buds.

These preliminary experiments demonstrated that theelin would stimulate the growth of the duct system of the immature castrated female cat. As would be expected, the larger dosage produced the most extensive growth, but in neither cat was the duct system developed to its maximum extent.

Two additional immaturely castrated female cats were next injected with 75 and 100 rat units of theelin once daily. The animal receiving 75 units showed a duct development of the glands to the extent of about 1 cm. after 26 days of injection. This gland was quite dense and thick, surrounding the base of the teat with numerous growing end-

*Strictly speaking *theelin* applies to a crystalline estrogenic substance of formula $C_{18}H_{22}O_2$ obtained from the urine of pregnant women. As our observations indicate that theelin, theelol, and a crude estrogenic extract from the urine of pregnant cows have identical physiological action on the mammary gland, the term theelin is used in a general sense to indicate the estrogenic hormone recovered from cattle urine. As used in the present paper it is synonymous with estrin, a term which is preferred by some.

buds. After 56 days of injections the duct system of the gland had reached a diameter of 2 cm. This development consisted of main ducts extending outward from the base of the teat with a rather dense growth of smaller ducts branching off from them. A number of bulb-shaped duct anlagen were noticed along the sides of the developed ducts, and also at their extremities (Fig. 3). After 86 days of injections the gland observed showed the same type of development as described above. However, much more growth had taken place, particularly in the extension of the main ducts.

The animal receiving 100 rat units showed a noticeable amount of duct development after 33 days of injection, but the gland was still quite small. After 54 days of treatment the gland observed was over 2 cm. in diameter. Along the sides of the main ducts bulb-shaped end-buds were observed thus indicating the continued growth of the gland. The experiment was terminated at this time by the death of the animal.

These observations indicated that theelin was capable of stimulating a very extensive mammary duct system in castrated female cats when injected at the rate of 75 to 100 rat units over a considerable period of time. It should be pointed out, however, that the growth of the lobule-alveolar system was not stimulated.

In continuing the experiment, the effect of theelin in stimulating the mammary glands of the normal male was determined. Control glands removed at the beginning of the experiment uniformly showed no duct development beyond the base of the teat. From 25 to 200 rat units were injected daily into these animals for periods varying from 15 to 95 days.

After 95 daily injections of 25 rat units of theelin daily in immature normal males, glands with a duct system of about 2 cm. in diameter were produced (Fig. 1). This gland showed that vigorous duct growth was in progress as indicated by the bud-like formations at the extremities of the main ducts and of the branch ducts.

The injection of 50 rat units of theelin daily for 90 days stimulated growth of the gland of a mature male to an area of 3.5 x 2.5 cm. (Fig. 2). This animal was then given twice the amount of theelin (100 rat units) for 33 days immediately following the above treatment. The gland removed at the end of that period showed increased development of ducts as compared with the one previously removed. Apparently the growth of the ducts was complete but the lobule-alveolar system had not developed (Fig. 6).

Next 100 rat units of theelin were tried. After 30 days of injection, the duct system of the glands of a mature male had developed to a diameter of about 1 cm. (Fig. 7). This gland showed development of the

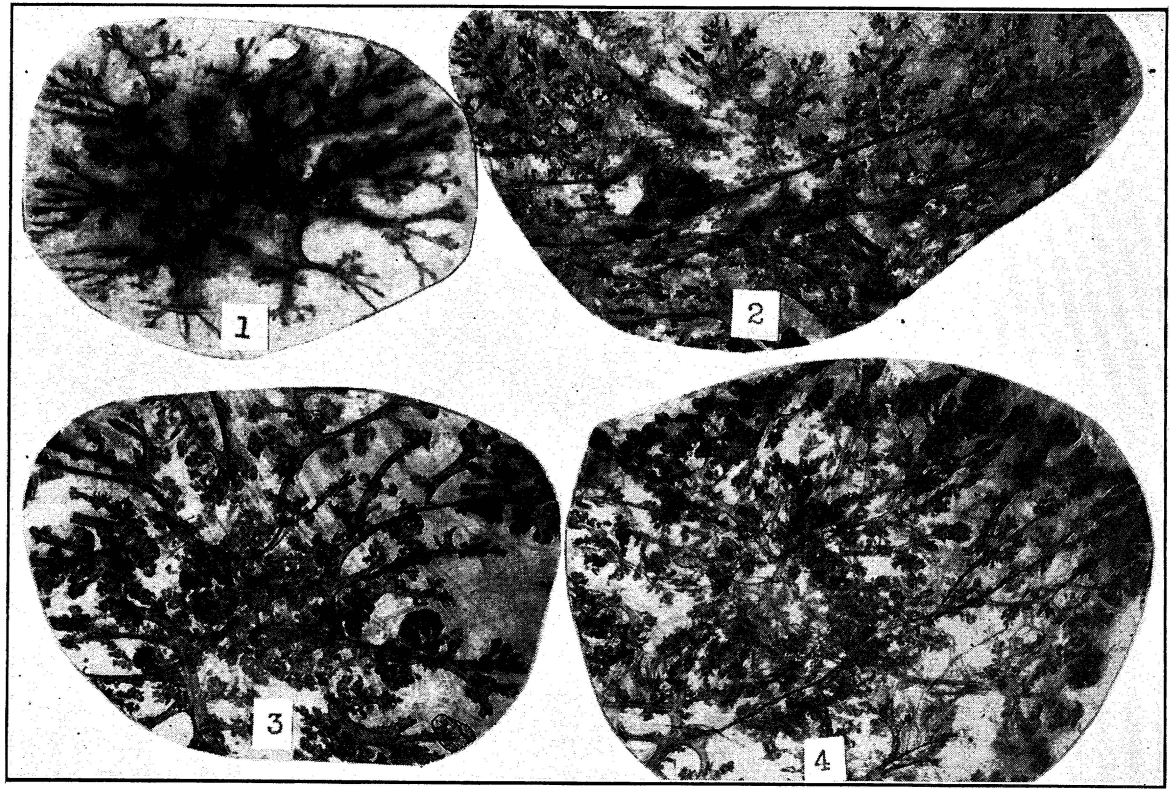

PLATE I

Fig. 1.—The mammary gland of an immature male cat which received 25 fat units of theelin daily for 95 days. x2.5

Fig. 2.—A portion of the mammary gland of an adult male cat which received 50 rat units of theelin daily for 90 days. x3.2

Fig. 3.—A portion of the mammary gland of an immature castrate female cat after 56 daily injections with 75 rat units of theelin. x2.5

Fig. 4.—A portion of the mammary gland of an adult male after 69 daily injections with 100 rat units of theelin daily. x2.5

main ducts with bulbous formations at their extremities and along the sides. After 69 days of injection the removed gland had increased five times in diameter over the previous gland. These glands seemed to have their duct systems fully developed but no lobules were present (Fig. 4).

The animals receiving 200 rat units daily did not survive for a period sufficient to note the rate or extent of growth of the mammary duct system.

These observations indicate that the estrogenic hormone, theelin, will stimulate the growth of the duct system of the mammary gland of the domestic cat. The glands of the male and female are equipotential in their response to this ovarian hormone. Rapid and extensive development of the duct system followed the daily injection of 100 rat units of theelin; however, smaller amounts of theelin stimulated to extensive growth but at a slower rate. It is inferred from these results that the growth of the mammary duct system of the normal female during the prepubertal and estrual periods is under the control of the estrogenic hormone secreted by the ovary.

THE EXPERIMENTAL GROWTH OF THE LOBULES AND ALVEOLI

With the initiation of pregnancy, the growth of the lobules and alveoli of the mammary gland takes place. In the case of the rabbit and mouse, the type of growth observed during that period was not stimulated by the long continued injection of theelin. This condition was also found true of the domestic cat as indicated by the previous experiments. In an effort to determine the hormones normally stimulating the lobule-alveolar growth, a second series of experiments was initiated using in part the animals with mammary duct systems previously stimulated with theelin.

In earlier experiments Turner and Frank (1931-32) observed in the rabbit the growth of the gland lobules following the simultaneous injection of corporin (or progestin) and theelin for 15 days. The best results were obtained with about one rabbit unit of corporin and 20 rat units of theelin. Similarly, the growth of the gland of the rat corresponding to that observed at the middle of pregnancy was stimulated with the same treatment by Turner and Schultze (1931). Recently Turner and Gomez (1934) confirmed these results in the albino mouse.

Four animals were available for the following experiment. Two animals were immaturely spayed females and two were mature normal males. At the initiation of the experiment, the duct system of the female glands was still incompletely developed, whereas in the males the ducts had been extensively grown by long continued theelin treatment.

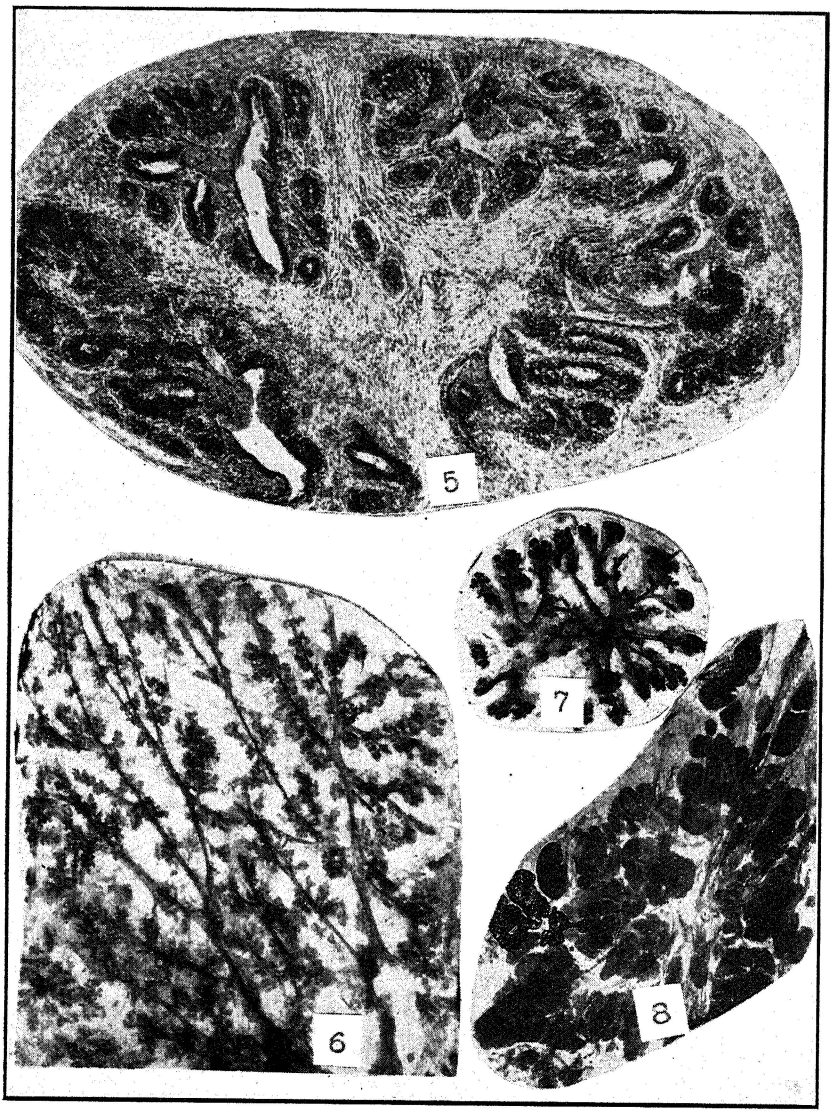

PLATE II

Fig. 5.—Microphotograph of a section of the mammary gland of a mature male cat. The duct system barely extends beyond the base of the teat. x94.

Fig. 6.—A portion of the mammary gland of a male cat which received 33 daily injections of 100 rat units of theelin following 90 daily injections of 50 rat units of theelin. x3.2

Fig. 7.—The mammary gland of an adult male cat which received 30 daily injection of 100 rat units of theelin. x2.5

Fig. 8.—The mammary gland of an adult male cat, which received simultaneous injections of one rabbit unit (0.35 cc.) of corporin and 25 rat units of theelin for 20 days following 60 daily injections of 100 rat units of theelin. The proliferation of the lobule-alveoli system was effected. x2.5

As the number of animals available was limited and our hope was to secure positive results if possible, the animals were given one rabbit unit of corporin and 25 rat units of theelin daily. The females were injected for 10 days. Examination of the gland showed continued growth of the duct system but no lobule development. In the case of the males, marked lobule development was observed in the gland of one animal after 13 days of treatment; whereas after 20 days the lobules appeared to be very markedly developed (Fig. 8).

The negative results obtained in the case of the females are difficult to understand. The growth of lobules in the two males, however, is believed to indicate that in the cat the growth of the mammary gland typical of the first half of pregnancy may be stimulated experimentally by the simultaneous injection of theelin and corporin in the proper proportions after the ducts have been developed as a result of theelin stimulation.

THE INITIATION OF MAMMARY SECRETION WITH GALACTIN

The final stage of mammary development during the course of pregnancy is the initiation of milk secretion that begins some time before parturition but is greatly increased shortly after. The hormone normally stimulating this phase of development has been located in the anterior pituitary. Extracts of this gland of internal secretion, called galactin, have been found to stimulate lactation in the rabbit, guinea pig, dog, and sow. For a review of the literature in this field the reader is referred to the recent study of Gardner and Turner (1933).

The first objective in this phase of the work was to determine whether the mammary glands of the two normal male cats whose mammary glands had been completely grown, as previously described, under the influence of the ovarian hormones—theelin and corporin—could be stimulated to lactation. These animals were injected subcutaneously for six days with 2 cc. of galactin daily. At that time the glands had become greatly distended with milk. Upon cross section the glands showed a thickness of about 3 to 4 mm. *These glands were judged to be in a stage of lactation the equal of normal females at the time of parturition.* As the glands were being removed from the animals a slight cut would allow a considerable loss of milk. Thus in male domestic cats the entire cycle of mammary gland growth and lactation has been experimentally stimulated. This is the second species (the rabbit was the first) in our laboratory and the only ones with which we are acquainted, in which the entire process has been stimulated by means of the proper sequence of hormones administered to male animals.

(See details in regard to lactation in guinea pig in Missouri Research Bulletin 206).

A number of mature female cats were also available for observation. One animal was given galactin beginning on the 15th day following parturition, the young having been removed at birth. At the time of the beginning of the experiment, the glands had involuted markedly, no milk being seen in a whole mount of the gland either in the lobules or ducts. After 9 days of injection of 2 cc. of galactin daily, a small amount of milk could be drawn from the teats. On the 10th day the glands were further extended with milk, and by the 15th day they appeared to be in full lactation, closely resembling those of the glands of a cat one day after parturition.

A second female was placed upon experiment 32 days after an abortion. The process of involution had extended to the point where only very slight lobule development could be observed. After 23 days of injection of 2 cc. of galactin daily, duct lactation was observed. The ducts were well filled with milk and remained so for 7 days with continued injections of galactin.

Four other mature multiparous cats with involuted glands of unknown duration were injected with galactin. After 8 days of injection one of the cats showed complete duct lactation. The injections were continued for 20 days but no further lactation was produced. Three of the cats did not show even duct lactation. In none of the animals was any evidence of growth of the gland being stimulated by galactin observed.

In certain species, preliminary experiments have indicated that the mammary duct system may be rendered responsive to galactin by preliminary treatment with theelin. In order to determine whether theelin would activate the duct epithelium so that galactin would initiate duct secretion, three cats, two females and a male, were given a preliminary treatment with theelin for two months in order to stimulate growth of the duct system. Then 5 cc. of galactin were injected for 6 days. Slight duct lactation was observed in one female but the other animals were negative.

These experiments with the cat indicate that the mammary glands of this species will respond to the lactation stimulating hormone, galactin. It is essential, however, that the gland be in proper condition if lactation corresponding to that observed at parturition is to result. This condition may be attained by the normal development of the lobule system during the first part of pregnancy or may be induced experimentally by the simultaneous injection of theelin and corporin in animals with a mammary duct system. Under such conditions the response of the gland to galactin is very striking.

During the process of involution following weaning, secretory activity may be re-initiated if involution has not progressed too far. In advanced involution, the lobule system largely if not entirely degenerates. Under such conditions, the glands are refractory to galactin stimulation.

While duct lactation can be stimulated occasionally by the preliminary treatment with theelin, it appears that other factors may be involved. Further study must be devoted to this phase of the problem.

The growth of the gland, that is, the extension of the duct or lobule system was not observed during the period of galactin stimulation.

INVOLUTION OF THE MAMMARY GLAND

Because of its relation to the re-initiation of lactation, the process of gland involution following lactation was studied in a series of cats. Immediately following weaning there was an accumulation of milk in the glands for about 24 to 48 hours. The presence of this milk gradually inhibited further secretory activity. By the fifth day, the glands have lost practically all of the milk from the lobules and much from the ducts. This gave the gland a much thinner appearance than during lactation (Fig. 9). The process of involution continued rapidly. The lobules of glands which had involuted 10 days are much reduced, having a shriveled, feathery appearance. In the glands involuted for 15 days there was quite a reduction in the size of the lobules and at 25 days, the main ducts were clearly outlined due to the collapse of the smaller branch ducts.

In the series observed there was a gradual loss of the lobule-alveolar system until at about 80 days of involution, only the duct system remained. The ducts appeared somewhat larger but more shrunken than the pubertal duct system and the lateral branches were finer and more feathery, lacking the bulbous appearance of the growing duct. In both cases, however, the lobule-alveolar system was lacking (Fig. 10, 11, and 12).

These observations indicate that the mammary gland of the cat passes through a complete regression of the lobule-alveolar system following each lactation period if there is a period of about 80 days or more between one lactation period and the initiation of another pregnancy. Consequently at each period of pregnancy, these structures are renewed in preparation for the new lactation period.

SUMMARY AND CONCLUSIONS

Experiments are reported which indicate that the estrogenic hormone will stimulate the growth of the duct system of the mammary gland of the domestic cat. The glands of the male and female are equi-

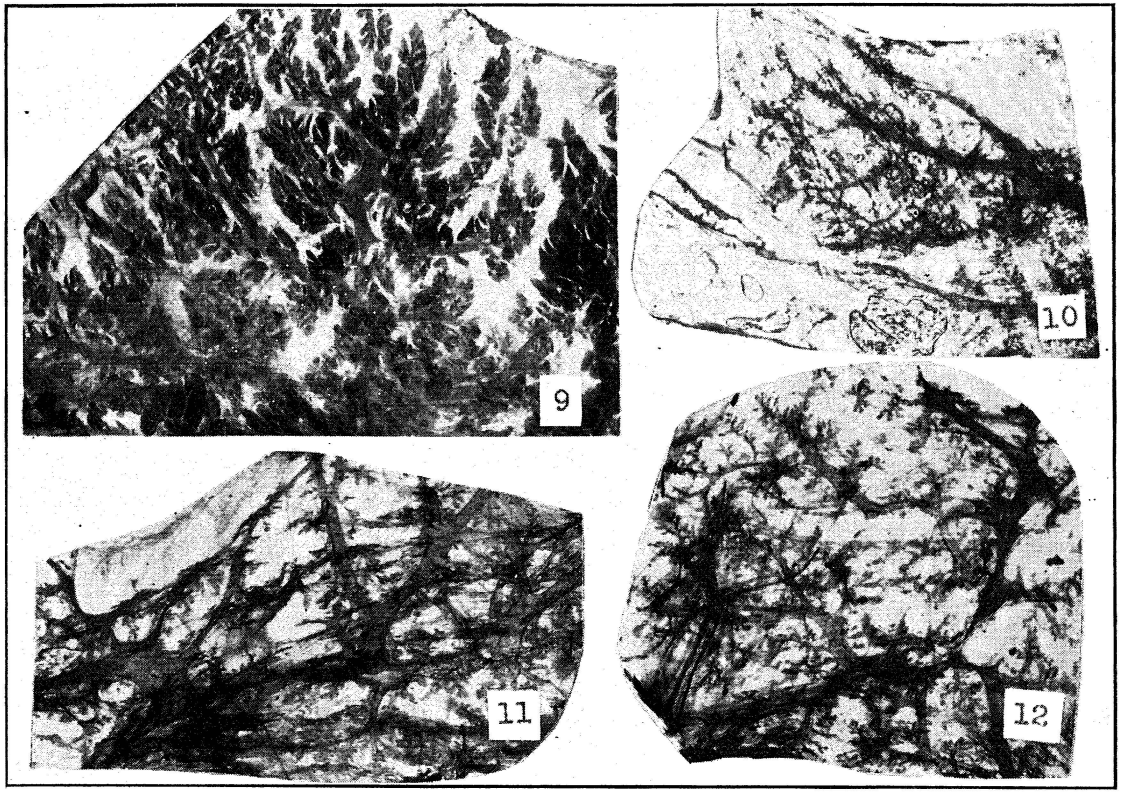

PLATE III

Fig. 9.—A portion of the mammary gland of a female cat five days after the young were removed. x2.5

Fig. 10.—A portion of the mammary gland of a female cat 50 days after the young were weaned. x2.5

Fig. 11.—A portion of the mammary gland of a female cat involuted 90 days. x2.5

Fig. 12.—A portion of the mammary gland of a female cat involuted 112 days. x2.5

potential in their response to this hormone. Rapid duct growth followed the injection of about 100 rat units of theelin daily; however, even the long continued injection of the hormone failed to carry the glands past the duct stage observed at estrum.

It was inferred from these results that the growth of the mammary duct system of the normal female during the prepubertal and estrual periods is under the control of the estrogenic hormone secreted by the ovary.

Two male cats with rather extensive duct systems previously developed as a result of theelin stimulation, were given daily one rabbit unit of corporin and 25 rat units of theelin simultaneously. After 20 days of injection, marked stimulation of the lobule-alveolar system was observed comparable with the glands of pregnant animals about mid-term.

At the above stage of gland development, the injection of 2 cc. of galactin daily for 6 days caused the stimulation of milk secretion to such an extent that they were judged to be in a stage of lactation equal to that of normal females at the time of parturition.

Thus the mammary glands of the male cat have been stimulated by the proper sequence of hormones through the various stages of growth and lactation normally occurring in the normal female at estrum, during pregnancy and parturition.

In this laboratory the male rabbit and now the male cat have been demonstrated to have equipotential mammary glands. Further, the kind and proper sequence of the hormones acting upon the mammary gland has been demonstrated.

Following weaning, lactation is quickly inhibited followed by a gradual involution of the lobule-alveolar system which continues until at about 80 days only the duct system remains.

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TABLE OF CONTENTS

ABSTRACT

The growth of the duct and lobule-alveolar system ot the mammary glands of the pregnant and non-pregnant bitch after estrum follows a similar course as indicated by the observation of glands removed at 10 day intervals from such animals. After parturition (about 60 days after conception) the pregnant dog comes into considerable lactation, while the pseudo-pregnant dog may come into lactation for a short time at about the same time after estrum, but as the milk is not removed resorption of the milk and gland involution takes place. Within 40 days after involution begins, the lobule-alveolar system has largely degenerated, leaving only the larger branches of the duct system.

II. THE MALE AND FEMALE DOG

C. W. TURNER AND E. T GOMEZ

Considering the wide use of the dog in experimental physiology it is rather surprising that the anatomy of the mammary glands and the course of growth and the initiation of lactation should have received so little attention. This is especially true considering the great interest in the ovarian and pituitary hormones which have a direct effect upon the mammary glands as well as the possible interrelation with other endocrine glands. No other mammal is subjected to such widespread observation under such a host of experimental conditions. Should not this condition be taken advantage of in determining the relation between the mammary gland and the thyroid and parathyroid, the adrenals, the pancreas, etc.?

Instead, as will be pointed out later in this paper, some questionable conclusions have been reached due to a lack of understanding of the normal changes occurring in the mammary glands of virgin dogs following estrum and the type of gland present during various sexual epochs.

The object of the present paper is to present the results of a study of the normal growth of the mammary gland of the dog. In addition a few preliminary experiments with the estrogenic and lactogenic hormones will be reported.

The mammary glands of the dog are arranged in two rows beginning in the thoracic region and extending over the abdomen into the inguinal region. Each row usually contains from four to six gland complexes, with five the modal number. In a few cases there may be more glands on one side than on the other. Kitt (1882) reported that five pairs of teats were present in females which he examined. In the males of the larger breeds of dogs, five pairs of teats were usually found, whereas in the males of the smaller species only four pairs occurred. Bonnet (1892) stated that the larger races of dogs had ten teats while the smaller races had only eight.

In dogs with ten normal teats, the normal teat pattern consists of two pairs of thoracic teats, two pairs of abdominal teats and one pair of inguinal teats.

METHOD

In the following study, the individual glands of the dogs were removed at 10 day intervals during pregnancy, metestrum, and involution, in order to have strictly comparable glands. When the development was rudimentary, the teat and gland was sectioned to show the type of development. As growth proceeded, it was found possible to isolate

layers of the gland for whole mount preparations to show the type of duct and extent of lobule development. At the approach of and following parturition it was found impossible to secure satisfactory whole mounts, so sections of the glands were prepared according to the usual method.

NORMAL DEVELOPMENT OF THE MAMMARY GLAND

The embryonic and fetal development of the mammary gland of the dog has not been investigated. From birth until the approach of estrum, the male and female glands consist of small primary ducts extending only slightly below the base of the teat (Fig. 24). The number of ducts in the dog's teat is quite variable. Christ (1905) reported that the number varied from 6 to 12, Martin (1910) from 8 to 12, and recently Käeppeli (1918) from 2 to 20. The writers examined the teats of two dogs. In the case of one dog, individual teats showed a variation of from 12 to 22 ducts. Counts made in the basal sections tended to approach 12 so that higher counts may be due to blind ducts described by Käeppeli. In the case of the second dog, sections at the base of the teat varied from 8 to 14 ducts (Figs. 1 to 3).

At the approach of the first estrum in the female there is stimulated slight growth of these ducts indicated by bulbous out-growths along the lateral walls and at the ends. However, the extent of duct development of dogs in their first estrum is very slight. Whole mounts of the glands at this time are difficult to separate from the teat. The most satisfactory method of examination of the extent of gland development is by means of cross sections at the base of the teat (Fig. 3).

The mammary glands of all male dogs examined were very rudimentary, the ducts of the teats comparing with those of the female before puberty was reached.

THE ESTRUS CYCLE AND PSEUDO-PREGNANCY

In animals which ovulate only after coitus as well as in certain mammals which have regular estrus cycles, a sterile coitus causes either ovulation and the formation of corpora lutea, or the lengthening of the luteal phase of the estrus cycle. This condition, first described by Ancel and Bouin (1911) in the rabbit, has been called pseudo-pregnancy.

In the rabbit pseudo-pregnancy extends for about one-half the normal period of pregnancy during which time the growth of the mammary gland takes place, but the initiation of lactation seldom if ever is seen corresponding to that observed after parturition. In the rat and mouse, the pseudo-pregnancy induced by coitus also extends for about one-half the period of pregnancy resulting in the growth of the mammary gland but not lactation.

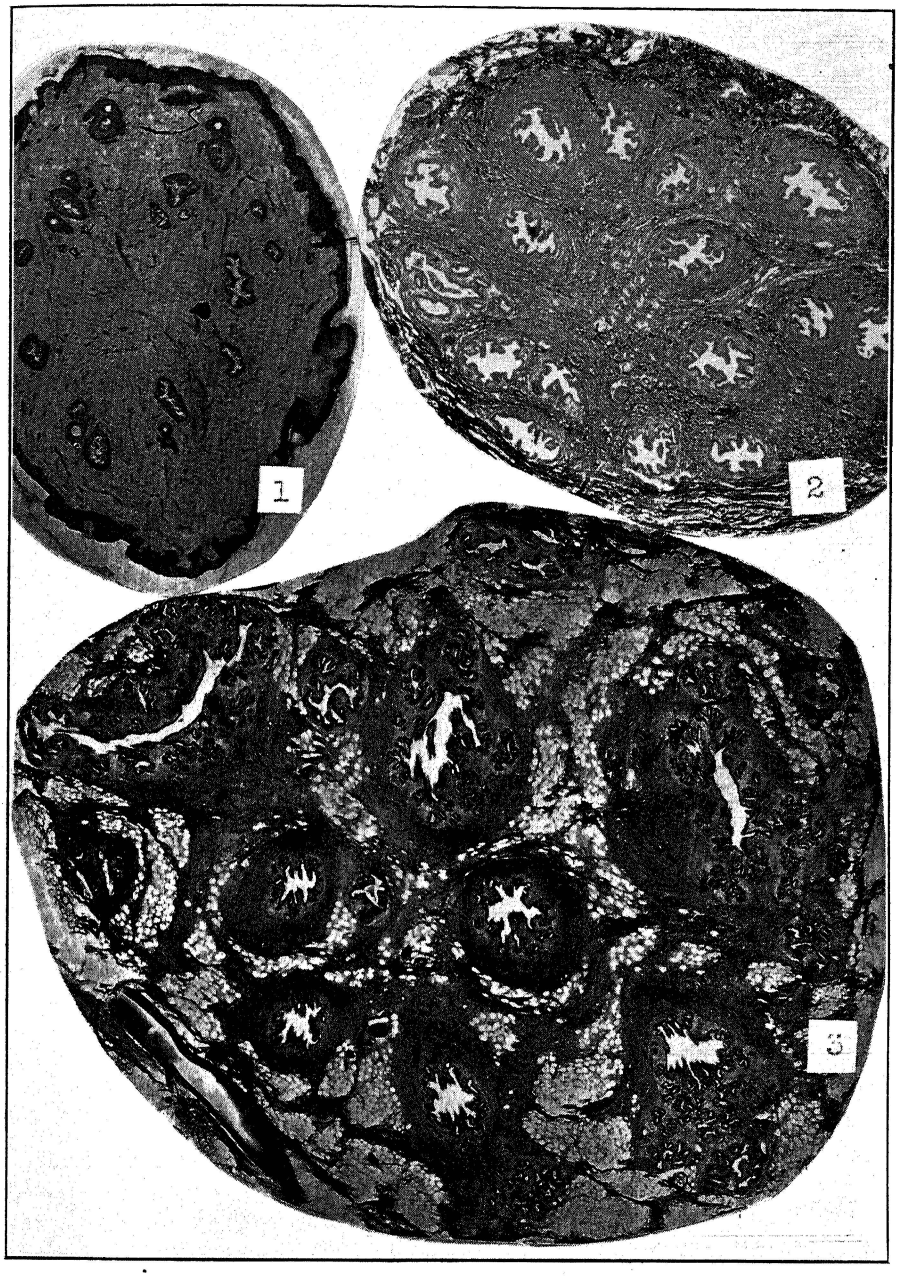

PLATE I

Fig. 1.—A cross section of the teat of a young bitch (in estrum) in the region of the streakcanal. Note the pavement epithelium lining the canals. X4

Fig. 2.—A cross section of the same teat through the mid-section of the cistern. Note the two-layered epithelium of the cistern wall. Sixteen individual ducts may be counted. X4

Fig. 3.—A cross section at the base of the teat showing the chief mammary ducts and the side branches. In the virgin female reaching puberty and in normal males, the ducts extend only slightly below the base of the teat (see also Fig. 24). X4

In the small marsupial, the Australian native cat, Dasyurus, viverrinus, Hill and O'Donoghue (1913) observed that ovulation was spontaneous. Studies of the mammary glands of pregnant and non-pregnant animals after estrum revealed not only equivalent growth but the initiation of secretion in the non-pregnant animals equivalent to that observed 36 hours after parturition.

A similar condition has been observed in the ferret. Hammond and Marshall (1930) observed that ovulation occurred only after coitus. In a series of pregnant and pseudo-pregnant ferrets killed at various stages it was impossible to distinguish between the two types of glands. Not only was growth uniform but after about 42 days (normal duration of pregnancy) a considerable amount of secretion appeared within the alveoli.

In the case of the dog, Evans and Cole (1931) in their study of the estrus cycle, observed that ovulation was spontaneous, but was followed by a long metestrum due to the persistence of the corpus luteum.

It will be seen from the above discussion that the term "pseudo-pregnancy" originally referred to changes of the female genital tract and mammary gland initiated by a sterile coitus in an animal (rabbit) which does not ovulate spontaneously. However, it was shown later that sterile copulation or mechanical stimulation of certain mammals (rat and mouse) which ovulate spontaneously, caused a lengthening of the luteal phase of the estrus cycle. In the dog still another difference was noted. Here, a long luteal phase of the estrus cycle followed spontaneous ovulation without coitus. It is obvious, therefore, that the condition cannot be defined or classified on the mode of production.

To classify the various types of pseudo-pregnancy, it is suggested that the duration of the condition and the changes in the mammary gland be used as an index. In the case of the rabbit, rat and mouse, pseudo-pregnancy normally extends for about one-half the normal period of pregnancy. In these animals the complete growth of the mammary gland occurs, but the gradual initiation of lactation which normally develops during the second half of pregnancy does not appear. It is proposed that this condition be called "incomplete pseudo-pregnancy".

In the marsupial, Dasyurus viverrinus, the ferret, and the dog pseudo-pregnancy extends for a period comparable to normal pregnancy and the development of the mammary gland includes the growth phase during the first half and the gradual initiation of lactation during the second half of the period. This condition is called "complete pseudo-pregnancy".

EVIDENCE OF "COMPLETE PSEUDO-PREGNANCY" IN THE DOG

Heape (1906) reported that huntsmen had observed that virgin hounds would produce sufficient milk to rear pups. The flow of milk occurred about the time when the bitches would have whelped if they had conceived during the previous estrum. Similarly bitches which had been bred but which had "missed" having pups came into milk secretion, at the time they were due to whelp, in sufficient quantity to enable them to rear litters of puppies belonging to other bitches.

The growth of the mammary gland of virgin and multiparous bitches following pro-estrus bleeding was observed by Marshall and Halnan (1917). They reported that in a virgin bitch shortly after estrus, the mammary gland tissue was limited almost entirely to a few ducts surrounding the nipple with no evidence of proliferation taking place in the tissue. After 7 days the mammary tissue showed very definite growth, being spread over considerable area, and not merely in the immediate neighborhood of the nipple. The lobes were constituted by numerous mammary acini separated by connective tissue. There was every indication of rapid development proceeding.

About 20 days after pro-estrus bleeding the mammary tissue was reported to have developed appreciably. There was a well marked growth for about ¾ inches around each nipple. Sections showed secretory acini in a further stage of development, and cellular proliferation was proceeding. After 31 days, development of mammary tissue was more prominent than in the preceding stage, and active proliferation was apparently still proceeding. Large numbers of acini had been formed.

In the multiparous bitch 38 days after pro-estrus bleeding had stopped, the mammary tissues were well developed and consequently unsuitable for comparison with the virgins described above. No secretion or fluid could be expressed from the nipples. In a second multiparous bitch examined 43 days after bleeding stopped, sections through the mammary glands showed that these were in a state of activity, the alveoli containing a quantity of fluid, but fat was not identified by staining with Sudan III. Since the bitch was multiparous the sections were not compared with the virgin animals, but it was believed to be significant that the glands must have undergone growth in order to be in a condition of secretory activity.

OBSERVATIONS OF GLAND GROWTH DURING METESTRUM (COMPLETE PSEUDO-PREGNANCY)

On the basis of the above observations, it seemed desirable to study the growth of the gland in individual dogs through the metestrus period

in the non-pregnant animal for comparison with similar stages during pregnancy.

While a young dog just reaching sexual maturity would have been preferred, the dog secured for the study upon inspection of a mammary gland showed a considerable duct system similar to the gland, to be described later, taken from a parous dog after 40 days of involution. In the light of our observations it would appear that the dog had been pregnant previously or if a virgin, had passed through a previous metestrus or complete pseudo-pregnancy period (Fig. 23).

Ten days after estrum* (indicated by a swollen vagina, bloody discharge and positive vaginal smear) the mammary duct system was distended with fluid and the branches showed evidence of proliferation as indicated by bulbous deeply staining ends. The feathery appearance of the shrunken ducts of the previous gland is in sharp contrast to the enlarged ducts and branches now present (Fig. 4).

Progressive development of the branch ducts and the anlage of the alveoli was seen in the gland removed at 20 days. At 30 days the alveoli were taking definite form and showed possibly a slightly more advanced stage of development than did the corresponding stage during pregnancy (Figs. 5-6).

Glands removed at 40 and 50 days showed completely developed lobule-alveolar systems with increasing evidence of secretory activity. These glands were quite comparable with glands removed at corresponding periods during pregnancy. In both glands the lumina of the alveoli were becoming increasingly distended with secretion (Figs. 7 to 9).

The gland removed at 60 days, however, had not developed secretory activity to as marked an extent as the gland from the pregnant animal (Fig. 10). In this case it appeared that the final stimulus of secretory activity was lacking in the non-pregnant animal. However, it is possible that maximum secretion may have occurred either slightly before or after the time of the examination of the gland. That it was fully capable of intense secretion was demonstrated by the use of galactin, which will be described in a later section.

These findings, in conjunction with previous observations, on the virgin dog, are believed to indicate clearly that the mammary gland passes through stages of development and initial secretory activity during metestrum, even without sterile coitus, entirely comparable to the development during pregnancy. The extent of secretory activity, however, is much more variable. In some, the extent of lactation is sufficient to nurse orphan puppies, but in others it may be very slight.

*Evans and Cole (1931) state that ovulation usually occurs within 24 hours of the first acceptance of coitus and probably occurs at the same time in the absence of coitus. Due to the long estrus period (6 days or more) the timing of the stage of the pregnant and pseudo-pregnant dogs may not exactly coincide.

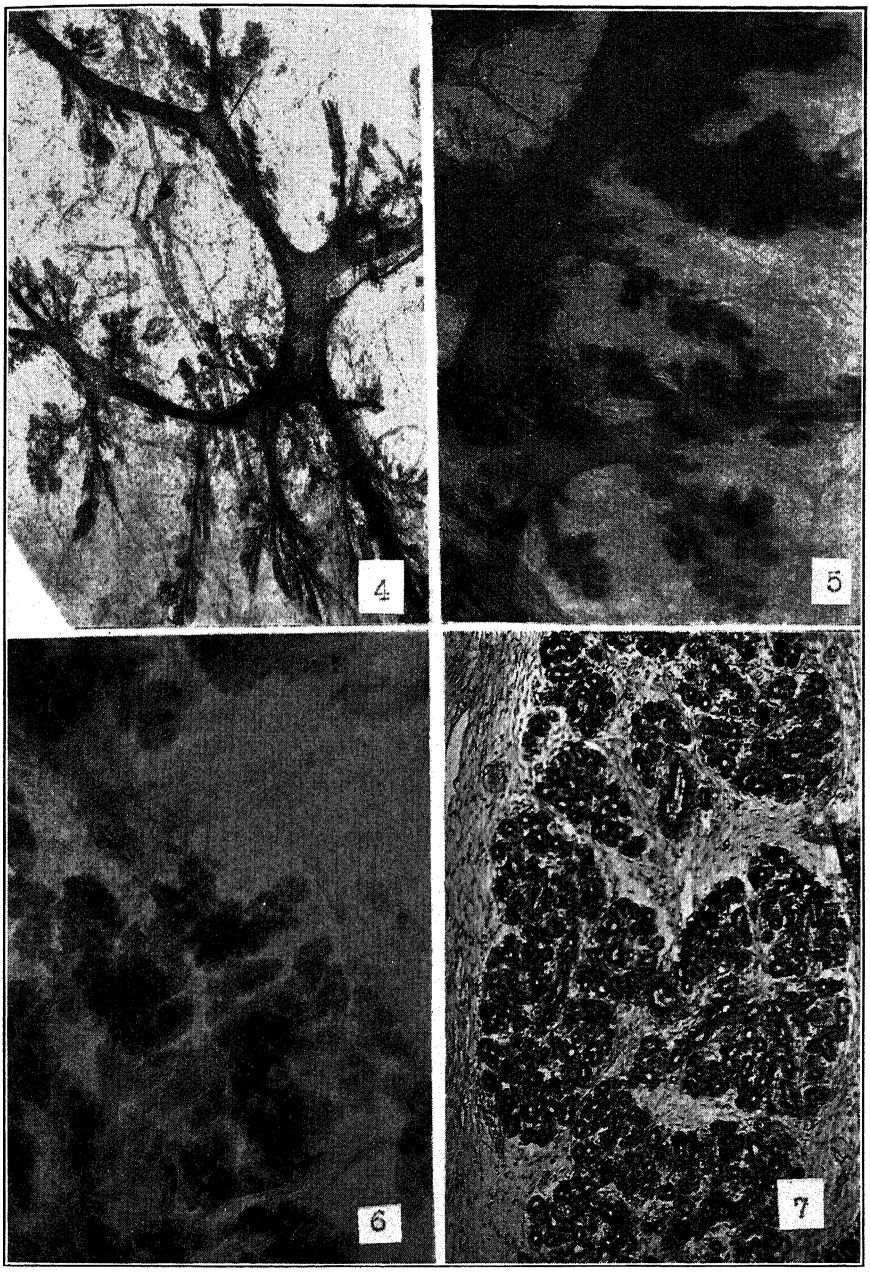

PLATE II

Fig. 4.—Photograph of a portion of a whole mount of an isolated layer of the mammary gland complex of a bitch 10 days following estrum (pseudo-pregnant 10 days). X4

Fig. 5.—Photograph of a portion of a whole mount of an isolated layer of the mammary gland complex of a bitch pseudo-pregnant for 20 days. X4

Fig. 6.—Photograph of a portion of a whole mount of an isolated layer of the mammary gland complex of a bitch, pseudo-pregnant for 30 days. X4

Fig. 7.—A microphotograph of a sectioned mammary gland of a bitch pseudo-pregnant for 40 days. X40

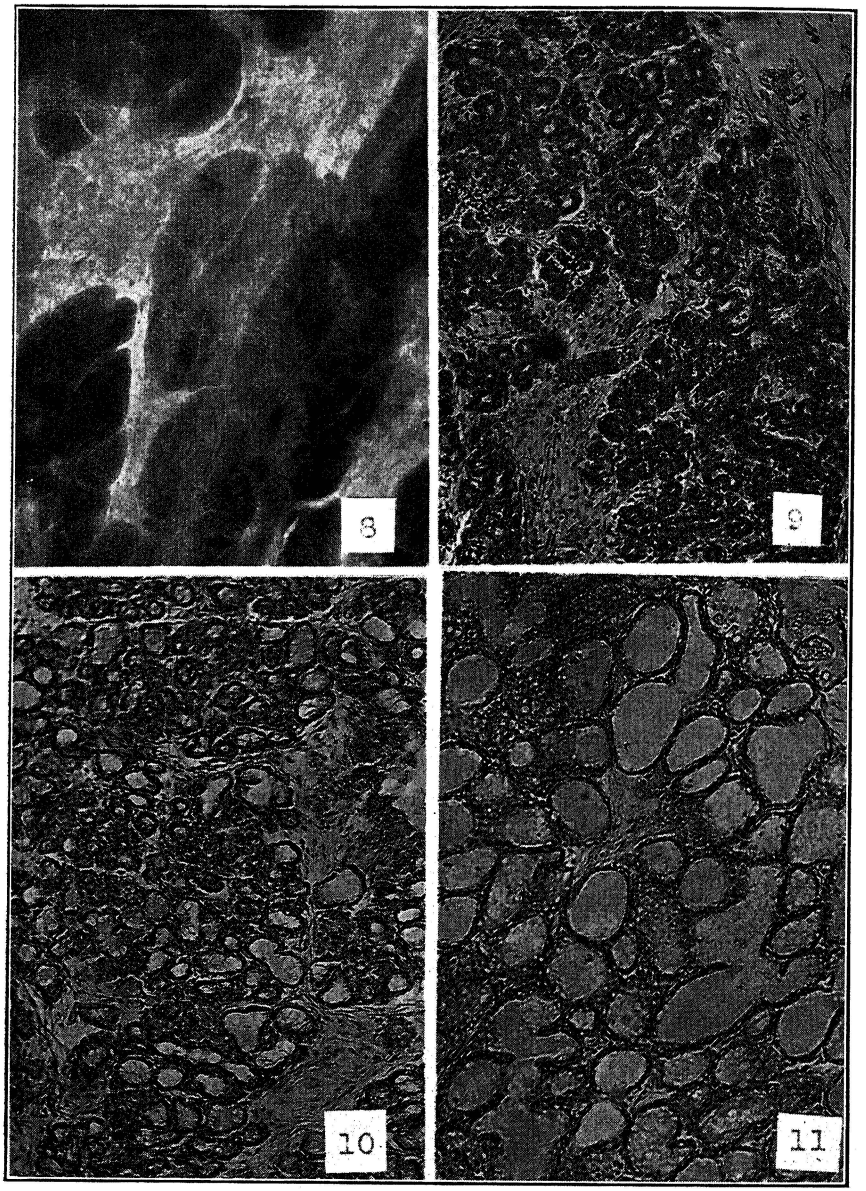

PLATE III

Fig. 8.—A portion of the whole mount of an isolated layer of the mammary gland complex of a bitch pseudo-pregnant for 50 days. X4

Fig. 9.—A microphotograph of a section of the above gland (Fig. 8). X40

Fig. 10.—A microphotograph of a sectioned mammary gland of a bitch pseudo-pregnant for 60 days. The gland was taken prior to the injection of galactin. X40

Fig. 11.—A microphotograph of a sectioned mammary gland which received five injections of galactin over a period of 10 days. X40

There can be little question that "complete pseudo-pregnancy" normally occurs following each estrum in the dog, causing the complete growth and the initiation of secretory activity of the mammary gland. It seems clear from these observations that the normal development of the mammary gland during pregnancy is not dependent either upon the fetus or fetal membranes. Rather, the maintenance of the corpus luteum in the ovary and the hormones secreted by the ovary play a major role in mammary gland growth. This being true, it should be possible to produce a condition of experimental pseudo-pregnancy in domestic animals by causing the production and retention of the corpora lutea for periods comparable to the normal duration of pregnancy. This would be important in animals which have become sterile.

PREGNANCY

For the study of the changes in the mammary gland during pregnancy, a young crossbred German shepherd dog was secured at the time of her first estrum. A section of gland removed shortly after coitus showed the presence of a fairly large teat but with the ducts extending only slightly below the base. Sections at the base of the teat showed numerous branches of the primary ducts (Fig. 3).

Ten days after conception, the growth of the gland was visible externally. Gland tissue measuring about 30 mm. in diameter around the teat was removed. After fixation, the gland measured about 9 mm. in thickness immediately below the teat and about 2 mm. at the periphery. Sections of the gland revealed a number of larger ducts with numerous small branches as satellites around them. The bud-like outgrowths of the ducts had in most cases canalized except at the growing ends. The lining consisted of two-layered columnar epithelium. Immediately surrounding the various groups of ducts, the connective tissue was rather dense and deeply stained. The remaining gland stroma was composed of a rather uniform connective tissue matrix in which the scattered branched ducts were embedded (Fig. 12).

After 20 days of pregnancy, the glands showed considerable further growth externally. The peripheral borders of the adjoining glands in each row were beginning to meet in the interspace between the teats and extending toward the midline.

Upon section, the connective tissue stroma which was so prominent in the previous stage had been reduced. Adipose tissue cells were present in the stroma also. The growth of the duct system was very marked. Not only had the main ducts increased in size but the number of secondary and tertiary branches had increased and further bud-like outgrowths along the lateral walls indicated that rapid proliferation of the gland was occurring (Fig. 13).

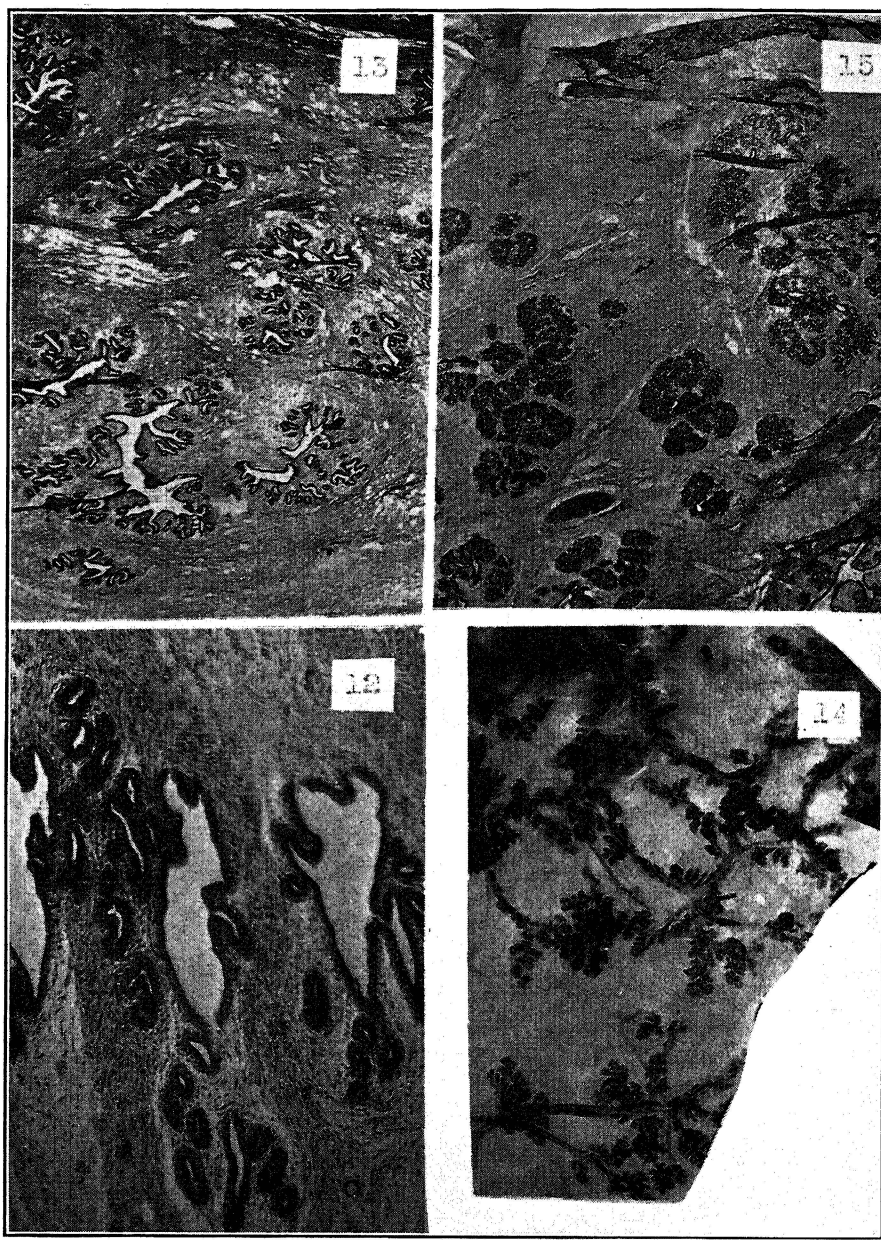

PLATE IV

Fig. 12.—A microphotograph of the sectioned mammary gland of a bitch pregnant 10 days. X60

Fig. 13.—A microphotograph of the sectioned mammary gland of a bitch pregnant 20 days. X40

Fig. 14.—Photograph of a portion of the whole mount of an isolated layer of the mammary gland complex of a bitch pregnant for 30 days. X4

Fig. 15.—A microphotograph of a section of the above mammary gland. (Fig. 14). X40

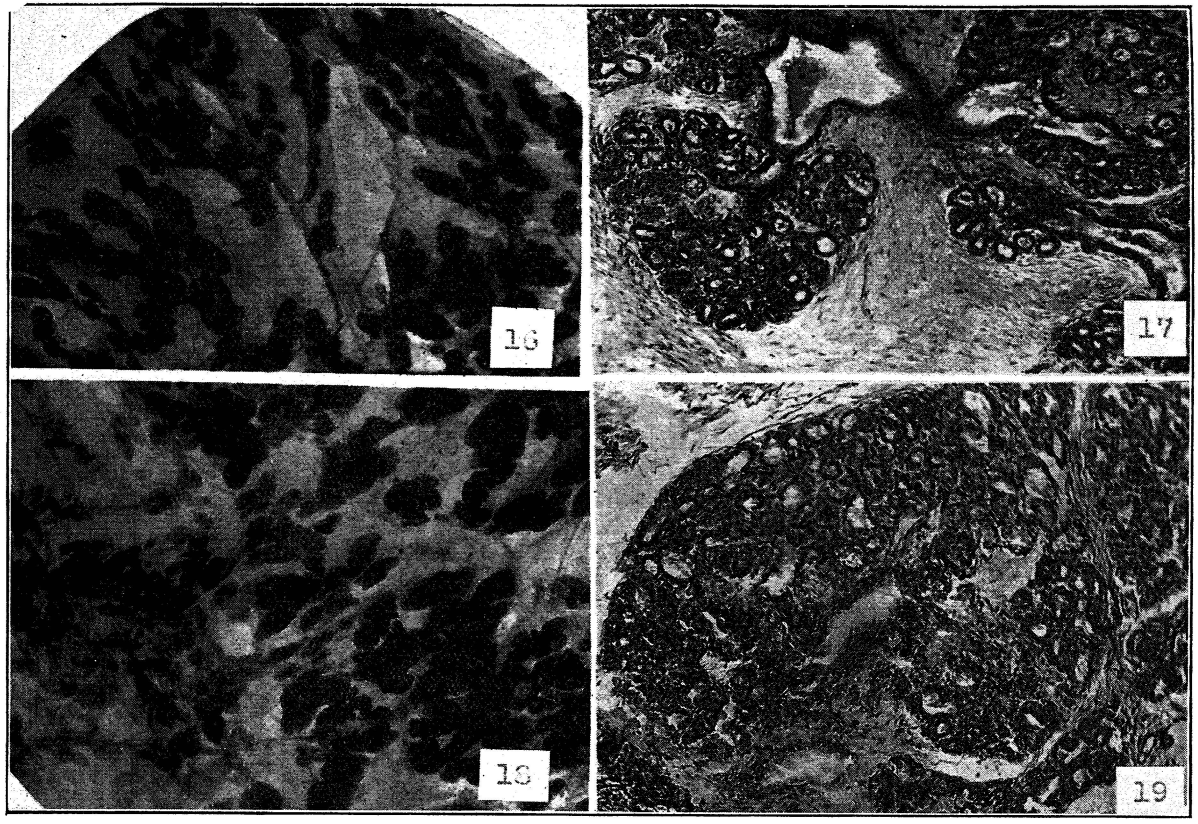

PLATE V

Fig. 16.—Photograph of a portion of a whole mount of an isolated layer of the mammary gland complex of a bitch pregnant 40 days. X4

Fig. 17.—Microphotograph of a section of the above mammary gland (Fig. 16). X40

Fig. 18.—Photograph of a portion of a whole mount of an isolated layer of the mammary gland of a bitch pregnant 50 days. X4

Fig. 19.—A microphotograph of a section of the above mammary gland (Fig. 18). X40

Examination of gross mounts of an isolated layer of the gland removed after 30 days of pregnancy revealed the nature of the typical duct and lobule system. Lobule growth was now quite extensive along the large ducts showing a dense arborization of small connecting ducts and anlage of alveoli. Upon section, the peripheral structures were observed to be lined by a two-layered epithelium and hence should be classed as ducts. In the region around the teat, where the older ducts were present, some alveoli were observed (Figs. 14 and 15).

At 40 days of pregnancy, whole mounts of the mammary gland revealed well grown lobule-alveolar structures. Long ducts could be seen with clusters of deeply staining lobules. The dissecting microscope revealed a honey comb appearance of these structures showing individual alveoli with small lumina. Sections of the gland showed in detail the structure of a lobe, lobule, and the individual alveoli. The lumina of the alveoli were just beginning to show.

In comparing the changes during the 30th and 40th days of pregnancy, it may be seen that the growth of the alveoli was completed during this period and the early stages of secretory activity of the epithelial cells lining the alveoli were initiated. Thus the growth phase of mammary gland development of the pregnant dog appears to be completed before the 40th day which is quite similar in duration to the total length of the growth period of the mouse, rat, rabbit, guinea pig, and sow which have been studied in this laboratory, namely, from about one-half to two-thirds of the period of pregnancy.

From the 40th day stage until parturition on the 60th day, the only change noted in the gland was the gradual enlargement of the gland due to the initiation of secretory activity of the epithelial cells lining the alveoli. Both whole mounts and sections of the gland taken after 50 days of pregnancy showed this enlargement. Considerable secretion was present in the lumina of the alveoli (Figs. 16-20).

One day after parturition, the sectioned gland showed great distension of the alveoli due to the accumulation of milk in the lumina. The gland stroma was greatly reduced in comparison to the parenchyma (Figs. 21 and 22). As the puppies were destroyed at birth, the development of lactation was not traced.

INVOLUTION

The changes in the mammary gland, when the milk was not removed from the glands following parturition, was traced in the dog observed during pregnancy. Ten days after parturition, the size of the gland was greatly reduced due to the resorption of milk. This was especially true of the lobule-alveolar structures as the ducts were still greatly distended with secretion (Fig. 22).

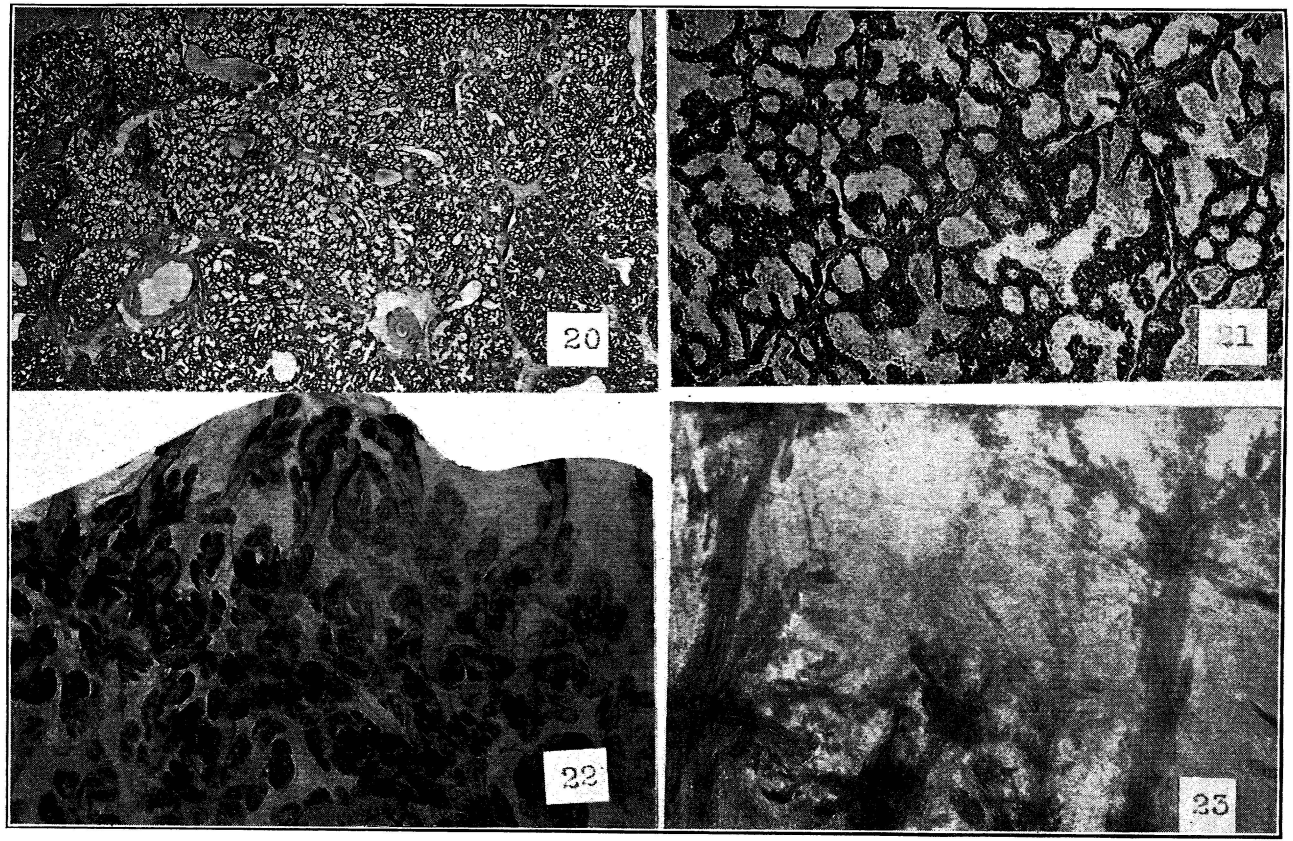

PLATE VI

Fig. 20.—A microphotograph of a section of the mammary gland of a bitch one day following parturition. X10

Fig. 21.—Same as Fig. 20, but magnified 60 times.

Fig. 22.—Portion of a whole mount of an isolated layer of the mammary gland of a bitch, 10 days after the puppies were removed. X4

Fig. 23.—Portion of a whole mount of an isolated layer of the mammary gland of a bitch involuted for 40 days. X4

Still further resorption of secretion from the gland was observed at the 20th day stage of involution. Some of the lobule-alveolar structures were becoming less prominent in relation to the ducts, while other alveoli still showed distinct lumina. In many ways this gland seemed comparable to the gland removed at 50 days of pregnancy.

Marked involution of the lobule-alveolar system occurred during the following 10-day period. Only a few of the more active lobules were still seen, while those that had undergone earlier regression were reduced to small ducts with feathery peripheral side branches.

Following 40 days of involution, the lobule-alveolar system had largely degenerated, only the shrunken duct system with a few remnants of the lobules remain. These observations are taken to indicate that the mammary gland of the dog soon regresses to a duct system when lactation is not sustained (Fig. 23).

EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND

Effect of Theelin

The daily injection of 100 mouse units of menformon for 14 days into a young male dog was reported by Laqueur et al. (1928) to have caused the enlargement of the mammary gland to such an extent that it was not difficult to distinguish the experimental animal from its control brother, which had received injections of a salt solution.

In connection with a study of the effect of theelin on the basal metabolism of the dog, Kunde, D'Armour, Carlson, and Gustavson (1930) report injecting from 100 to 200 rat units daily for periods up to 39 days into normal and castrate animals. Hyperplasia of the nipples was a constant finding in all dogs. Doubtful to pronounced hypertrophy of the mammary glands, resulting in no secretion of milk to a copious flow as occurs after the normal process of gestation and delivery, was reported. In a normal dog, the posterior and largest pair of mammary glands began secreting on the fifth day after beginning hormone injection. Within five days milk was expressed from all ten nipples. Six days later, two puppies were mothered and suckled for three weeks by this dog. However, this dog was in estrum approximately nine weeks (63 days) before beginning the theelin injections.

In the light of the evidence presented concerning the normal development of the gland following estrum, it seems probable that the theelin injected had little influence on the initiation of the lactation observed.

Only very limited observations have been made concerning the influence of the estrogenic hormone in stimulating the growth of the duct system of the dog. The first animal available for study was an ovariectomized female about 6 months old at the beginning of the ex-

periment. The gland removed as a control consisted of a very rudimentary duct system (Fig. 25). A daily injection of 200 rat units of hormone in oil was administered for 10 days, followed by a 10 day rest and then again a 10 day injection period. During the period of a month, 4000 rat units of the estrogenic hormone were injected. A gland removed at the end of the period showed little if any stimulation of the rudimentary duct system. Later 100 rat units of hormone were injected daily for a period of 35 days. A gland removed at the end of that period showed very slight extension of the duct system (Fig. 26).

A male dog, a litter mate of the female used for the previous experiment, was injected periodically with 100 rat units of the estrogenic hormone in oil for a period of six months. By this treatment it was hoped to stimulate the initial growth of the duct system and then by increasing the dosage determine the theelin requirements for extensive duct growth. However, due to conditions beyond our control, it became necessary to sacrifice the animal at the end of the preliminary treatment. The glands examined at that time exhibited only slight duct growth.

These observations indicate that 100 rat units daily of the estrogenic hormone stimulate the growth of the mammary duct system only very slightly. The animals used were rather large dogs weighing about 25 pounds.

Nelson (personal communication) has also observed the ineffectiveness of the estrogenic hormone (in oil) in stimulating the growth of the mammary duct system when injected at the rate of 100 rat units daily for 30 days. Only slight extension of the ducts was observed.

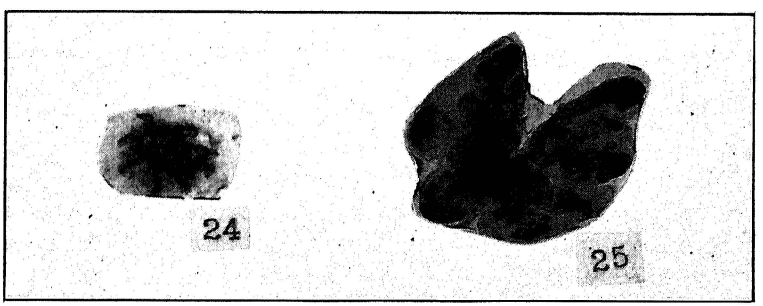

PLATE VII

Fig. 24.—The mammary gland of a young virgin bitch (about 6 months old) removed at the time of castration and prior to the initiation of theelin treatment. The gland consists of short rudimentary ducts which extend only slightly below the base of the teat. X4

Fig. 25.—The mammary gland taken from the animal described above (Fig. 24) after long continued injections with theelin. A slight growth of the duct system was effected. X4

Effect of Extracts of the Anterior Pituitary (the lactogenic hormone)

In connection with a study of the growth hormone of the anterior pituitary, Putnam, Benedict, and Teel (1929) injected a crude pituitary extract into one of two litter-mate female bull dogs holding the second one as a control. Beginning the daily intraperitoneal injection of 10 cc. of extract when the pup was about 7 weeks old, the amount was gradually increased until shortly before the close of the experiment 70 weeks later, 75 cc. were being injected.

When the dogs were about a year old, it was found possible to squeeze colostrum (verified microscopically) from the udders of the experimental animal which had reached an abnormally large size. At this time, the teats of the control animal were barely visible. The udders of the experimental animal ceased to secrete after about a month, but they continued to be larger than those of the control.

The control animal came into estrum when it was about 14 months old; whereas the animal that was given injections never came into heat. At autopsy, the ovary of the experimental animal showed the presence of corpora lutea whereas the control no longer showed signs of these structures (three months after the beginning of estrum).

The enlargement of the mammary glands of the experimental animal was probably due to the presence of the gonad-stimulating hormone in the extract. The luteinization of the ovaries would cause the growth of the gland similar to that observed during pseudo-pregnancy. The lactation that followed could have been due to the initiation of secretory activity observed during the latter part of pseudo-pregnancy or to the presence of the lactogenic hormone in the growth extract. In fact, probably both factors contributed to the lactation.

Gardner and Turner (1933) observed that lactation could be greatly augmented in a virgin dog at the end of metestrum by the injection of galactin every other day for a period of 10 days. Evans (1933) also mentioned the use of virgin dogs in studies on lacatation but gave no details.

Lyons, Chaikoff and Reichert (1933) reported that two subcutaneous injections representing a total of 20 mg. of crude lactogenic hormone sufficed to cause the secretion of milk in normal parous and non-parous mature bitches. Lactation was obtained in three bitches which had been ovariectomized one day previous to the administration of the hormone. A bitch which had been hypophysectomized one week prior to treatment was induced to lactate, but two other bitches failed, probably due to a lack of the proper mammary development. A mother that had been removed from her litter and had been dry for a week was brought back to full lactation to continue suckling.

Following pancreatectomy, Chaikoff and Lyons (1933) observed that five bitches maintained in good health by twice daily injection of insulin, failed to lactate despite the fact that the amount of hormone injected was greater, and the period of its injection much longer than that found necessary to induce lactation in the normal dogs. In a single depancreatized dog, lactation followed the administration of the hormone.

From a study of the protocols, the present writers are of the opinion that the negative results obtained by Chaikoff and Lyons with several animals were undoubtedly due to the fact that the glands of the animals were not in condition to lactate at the time the hormone was administered.

From our experience with the lactogenic hormone in the dog and other species and from the observations on the growth and involution of the mammary gland of the pseudo-pregnant bitch, the virgin dog would respond to galactin for a period from about 40 days until about 80 or 90 days after estrum (See Figs. 9, 10 and 11). At that time the lobule-alveolar system is complete. At about 60 days of pseudo-pregnancy, dogs frequently lactate spontaneously, so care must be taken to avoid the use of the hormone at that time or confusion will result. When virgin bitches are castrated it would be expected that involution of the gland would take place rapidly.

THE EFFECT OF HYSTERECTOMY ON MAMMARY GLAND GROWTH

In the dog the fact that normal growth of the mammary gland occurs during complete pseudo-pregnancy, rules out the fetus and fetal membranes as having a functional relationship to mammary gland growth. There is still the possibility that the uterus, during this period, functions as a gland of internal secretion supplementing the activity of the ovary. This possibility may be tested by removing the uterus prior to estrum and then noting the growth of the gland during metestrum.

A mature female dog weighing about 25 pounds was hysterectomized May 20, 1932. As she had not come into heat on June 14, we began the daily injection of 200 rat units of Antuitrin-S* for six days. These injections seemed to be without effect. During the summer this dog was farmed out with instructions to notify us if she came into estrum.

*Antuitrin-S is the trade name for a purified extract of gonad-stimulating hormone extracted from the urine of pregnant women. We are indebted to Dr. Oliver Kamm of the Research Laboratory of Parke, Davis and Company for a supply of the hormone used in this study.

On November 12, 1932, the vulva was swollen and she appeared to be coming into estrum. A gland removed on November 3, however, showed only very slight enlargement of the ducts. Another gland removed November 14th still showed only ducts without marked evidence of growth.

Beginning November 14, daily injections of 250 rat units of Antuitrin-S were made for 10 days. As no external evidence of estrum was induced it was decided that the Antuitrin-S had been ineffective, so the animal was sacrificed on December 22, 1932. To our surprise a gland removed at that time showed lobule development equivalent to that of pregnancy or pseudo-pregnancy of about 30-35 days. It would appear that the animal had ovulated about November 14 and gland development had begun at about that time.

These observations lead us to believe that the uterus is not necessary for the growth of the lobule-alveolar system which normally develops during the first 30 to 40 days of pregnancy or pseudo-pregnancy.

SUMMARY AND CONCLUSIONS

In the normal virgin bitch following spontaneous ovulation during estrum, there is a long luteal phase (metestrum) of the estrus cycle which has been designated as "complete pseudo-pregnancy". During this period, the duct system of the mammary gland rapidly proliferates, accompanied by the growth of the lobule-alveolar system. The growth phase of the gland development appeared to be completed between the 30th and 40th day after the beginning of estrum. Following this period, there is a gradual increase in the size of the gland due to the secretory activity of the epithelial cells of the alveoli, filling the lumina with secretion. At the time when parturition would occur if the bitch had been bred (about 60 days) more or less milk may be removed from the gland.

The mammary gland of the pregnant dog develops quite similarly to that of the virgin at comparable stages after estrum. They usually secrete milk more abundantly after parturition.

Following weaning, the mammary glands begin to involute. First, the milk present in the gland is resorbed, then there is a gradual degeneration of the lobule-alveolar system, leaving eventually only the larger branches of the duct system.

Only slight growth of the duct system has been stimulated in ovariectomized female and male dogs by the daily injection of from 100 to 200 rat units of the estrogenic hormone for periods of 20 to 35 days. These data are not believed to indicate that this hormone is not effective, but rather the amount injected was too low.

As complete lobule-alveolar growth in the virgin bitch occurs during pseudo-pregnancy, the mammary glands of such animals respond readily to the lactogenic hormone.

Hysterectomy was observed to interfere in no way with the growth of the lobule-alveolar system of the mammary gland during pseudo-pregnancy.

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