The Experimental Development of the Mammary Gland

I. The Male and Female Albino Mouse
II. The Male and Female Guinea Pig

C. W. TURNER AND E. T. GOMEZ

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I. The Male and Female Albino Mouse

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ABSTRACT

Crude extracts of the estrogenic hormone (estrin, theelin) recovered from the urine of pregnant dairy cattle and the purified crystalline hormone theelin $(C_{18}H_{22}O_2)$ and theelol $(C_{18}H_{24}O_3)$ were observed to stimulate the growth of the duct system of the mammary gland of the male mouse. However, even upon long continued injections, the growth of lobules characteristic of pregnancy was not stimulated.

The growth of lobules in normal male mice comparable to the growth observed during the first 10 or 12 days of pregnancy or pseudo-pregnancy was stimulated by the simultaneous injection of 0.15 cc. of corporin and 10 rat units of the estrogenic hormone following preliminary treatment to stimulate the growth of the duct system. Castration appeared to have little if any influence upon the rapidity or type of growth.

ACKNOWLEDGMENT

The Experimental Development of the Mammary Gland

I.   The Male and Female Albino Mouse*

C. W. TURNER AND E. T. GOMEZ

In a previous paper (Turner and Gomez, 1933) the course of normal development of the mammary gland of the albino mouse was described. It is the purpose of the present paper to describe further studies having as their object the determination of the hormones stimulating the development of the mammary gland comparable to that observed during each stage of normal growth.

As the question of the experimental development of the mammary gland has recently been reviewed by one of us (Turner, 1932) it will be unnecessary to describe the previous studies with animals other than the mouse.

In the studies to be described gross mounts of the entire glands were prepared according to the method described in the previous paper. The injection of all hormones was made once daily unless otherwise indicated.

THE GROWTH OF THE DUCT SYSTEM

In the normal female, the growth of the duct system during the prepubertal period was found to be rather slight but with the approach of puberty (50 days of age) and during the recurring estrus cycles the ducts begin to grow rapidly. After a number of estrus cycles the gland is characterized by the presence of long slender ducts with relatively few side branches. At the growing ends, the ducts are enlarged bulbous structures which have been designated end buds. During estrum the ducts appear enlarged with fluid, while the end buds stain more deeply indicating active growth.

The development of the mammary gland in the male mouse differs greatly from that observed in the female. As in the rat, teats are not associated with the gland ducts, but while in the rat there is an extensive development of the duct system, the duct system of the mouse is confined to a very rudimentary structure which appears to remain stationary throughout life (Fig. 1a, b, c).

When using female mice to study the influence of the estrogenic hormone (theelin) upon the growth of the duct system, it is important to ovariectomize them as early as possible (20 to 30 days of age) to stop the normal growth which occurs at this time.

*This study has been aided in part by a grant from the Committee on Grants-in-Aid of the National Research Council.

Because of the very rudimentary condition of the duct system in the male, they are excellent experimental animals to demonstrate the very positive stimulus of theelin. The absence of teats makes difficult though not impossible the removal of a check gland or a progressive series of glands during the course of hormone treatment.

The study of the experimental development of the duct system of the mouse has been very limited. In work on rats and mice, Allen et al. (1924) observed that during artificially induced (with ovarian follicular hormone) sexual cycles, changes occurred in the mammary glands. A preliminary report of the present study (Turner et al., 1932) was made to show that the response of the mammary gland to the two estrogenic hormones theelin and theelol was similar to that produced by a crude estrogenic hormone recovered from the urine of pregnant cattle. It was found that these extracts would induce extensive duct growth in both castrate male and female mice. The influence of theelin upon the growth of the duct (galactophore) system of mice has been confirmed by Bradbury (1932). No response of the mature female gland was noted after theelin treatment. Further, his observations were in agreement with our conclusion that the estrogenic hormone would not influence the mammae beyond the development of the galactophores.

Comparison of Theelin, Theelol and a Crude Estrogenic Extract

In the previous studies of the influence of the estrogenic hormone upon the mammary gland of the rabbit (Turner and Frank, 1930) and rat (Turner and Schultze, 1931) and preliminary studies on the mouse in this laboratory, a crude estrogenic extract obtained from the urine of pregnant cows was used.* With the crystallization of theelin by Doisy, Veler, and Thayer (1930) and of theelol by Doisy and Thayer (1931) it became of interest to determine whether our results with the crude extract which might contain other unidentified hormones would be comparable with the pure hormone.

Through the kindness of Dr. Oliver Kamm of the Research Laboratory of Parke, Davis and Company, a supply of theelin and theelol was made available for our use. The solution of theelin contained fifty rat units per cubic centimeter, while the theelol solution contained 1/5 mg. of pure crystalline material per cubic

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*The estrogenic hormone was extracted from the urine of pregnant cows by means of olive oil. The oil was then extracted with 95 per cent ethyl alcohol. Upon evaporation of the alcohol a highly concentrated extract in oil was recovered (see Turner et al. 1930).

centimeter dissolved in just sufficient alkali (0.003/N) to maintain
'it in solution.

Three adult male mice were each injected subcutaneously once
daily with 0.1 cc. (5 rat units) of theelin; and a second group of
three with 0.05 cc. daily of the theelol. On the 15th and 21st days
one animal from each group was sacrified. Examination of the
prepared mounts of the glands revealed no sign of growth. The re-
maining animal in each group was then given double the previous
dosage in three injections during the day for periods of 20 and 10
days, respectively. Upon examination, the glands showed that the
increased dosage had caused considerable growth of the duct sys-
tem (Figures 3 and 4). The glands showed prominent end buds
indicating that growth was still going on.

These preliminary comparisons indicated that the two purified
estrogenic hormones were capable of stimulating the growth of
the duct system of the mammary gland of the male mouse similar to
our results with the crude extract (Fig. 2). It was then decided
to compare the type and extent of growth of the duct system fol-
lowing long continued injection of these hormones.

A group* of seven normal male mice were divided into three
groups of two each with the extra one as a control. Two animals
were given injections of 10 rat units of theelin daily in a single in-
jection; two received 0.1 cc. of theelol; and two received 10 rat
units of the crude extract. One animal from each group was sacri-
ficed after 40 days of injection, while the second animal was ex-
amined after 90 days of treatment. The glands showed consider-
able variation in the extent of the duct system. No significant dif-
ferences in the effect of the three extracts were noticeable except
that theelin and theelol could be injected daily for long periods with
much less mortality and pathological effect upon the animals. The
40-day glands were less extensively developed than 90-day glands.
However, the enlarged end buds which are typical of growing ducts
were not very prominent. It would appear that growth of the duct
system was still in progress after 40 days of injection, but had
reached a maximum either before 90 days or at approximately that
time.

Three females were ovariectomized when 30 days of age. At
the approximate age of puberty (52 days), one animal was given
10 rat units of theelin daily, the second 10 rat units of the crude

*Because the mortality rate was rather high in the experimental animals, it was our
practice to include in each group a number of substitute animals which received the same
treatment, so that in case of loss of some of the animals others were available to take their
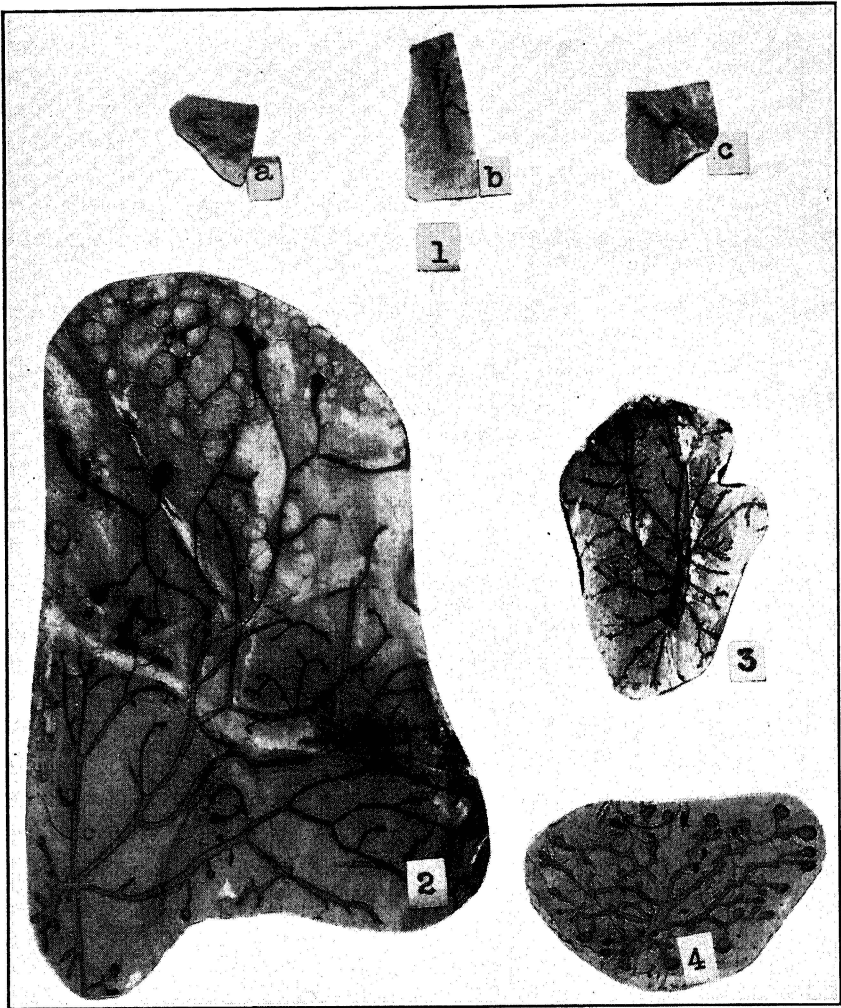place.

PLATE I

Fig. 1.—a, b, and c. Mammary glands of an adult albino mouse. The ducts remain rudimentary throughout life. x7.

Fig. 2.—The mammary gland of an adult male mouse which received 0.2 cc. (10 rat units) daily of a crude extract of the estrogenic hormone, for 30 days. x7.

Fig. 3.—The mammary gland of an adult male albino mouse which received 0.2 cc. (10 rat units) of theelin, administered once daily for 20 days. The growth of the duct system has been stimulated. x5.

Fig. 4.—The mammary gland of an adult male albino mouse which received 0.05 cc. of theelol administered once daily for 15 days followed by 0.1 cc. of theelol, for 10 days. x7.

extract, while the third was run as a control. A gland was removed from each of the three animals after 30, 60 and 100 days of injection. The glands removed showed progressive growth of the duct system to the 100th day. The latter gland, which received theelin, showed a few enlarged end buds at the extremity of the gland, indicating that slight growth was still in progress.

While these experiments are not as definite and precise as desired they are taken to indicate that the growth of the duct system of the mammary gland can be extensively stimulated by any of the estrogenic hormones. Growth apparently will continue for long periods although the question as to the ultimate growth that may be produced has not been settled. However, the duct system which is developed even by extensive hormone treatment shows no appearance of lobule growth. The ducts are not characterized by short side branches which may be confused with true lobules and alveoli such as are found in some other laboratory mammals.

In order to secure data on the question of the influence of the quantity of hormone injected daily on the growth of the duct system, groups of normal male mice were injected with 8, 10, and 20 rat units of theelin in a single daily injection. One animal from each group was sacrificed after 20 days and a second after 30 days. The glands of the animals which received 8 rat units of theelin daily showed relatively slight development even after 30 days. Considerable growth was produced in 30 days when 10 rat units were injected daily, but it did not appear nearly so extensive as when 10 rat units in oil (crude extract) were injected. It is believed that the difference is due to the slower and more gradual absorption of the hormone in oil. It is possible that comparable results would have been obtained if theelin (water solution) injected had been administered at intervals during the day. The daily injection of 20 rat units of hormone did not appear to stimulate the growth of the duct system to a greater extent than did 10 rat units.

It was concluded from these observations that about 10 rat units of the estrogenic hormone in oil administered once daily would stimulate the growth of the duct system of the mouse at an optimum rate. In comparison to these results it is interesting to note that in the rabbit (Turner and Frank, 1930) it was observed that 20 rat units daily for 30 days stimulated the growth of the duct system equal to that produced during continued estrus in the normal female. On the other hand, in the rat (Turner and Schultze, 1931) only one rat unit daily for 20 days of the estrogenic hormone caused extensive growth of the duct system.

In a normal sexually mature nullipara female, 60 daily injections with 10 rat units of theelin did not produce growth of the duct system beyond that observed in normal females of the same age in spite of the fact that the animal was in continuous estrum throughout the period.

Discussion

These experiments with the mouse furnish further evidence for the conclusion that the estrogenic hormone (theelin) secreted by the ovary stimulates the prepubertal and pubertal duct growth of the mammary gland. However, there were observed several species differences in comparison with the rat. It appears that the theelin requirement for duct growth in the rat is considerably less than in the mouse even though the body weight of the rat is much greater.* In this connection it is interesting to note that the mouse unit of antuitrin-S or follutein is five times that of the rat unit. The lack of growth of the duct system in the normal male mouse as compared to the extensive duct growth in the normal male rat is of considerable interest. Schultze and Turner (1933) observed that theelin was being eliminated in the urine and feces by groups of rats averaging 53 days of age as well as in more mature animals. From this evidence it was assumed that some organ in the male rat secretes theelin, causing the duct development. In the mouse, extracts of the urine and feces from two groups have failed to give positive results. It would appear that in some species of animals and in individuals of other species there may be a source of theelin other than ovarian which would account for duct development of the mammary gland in such species and individuals.

A second possible explanation of the lack of duct growth in the male mouse as compared with the male rat is the greater requirement of theelin in the mouse. A limited amount of theelin either formed in the organism or from the food consumed would stimulate greater duct development in the rat than in the mouse.

The duct system of the normal female and that grown with theelin in the male is unusually free of side branches or the appearance of the branch ducts which eventually grow to form lobules. For this reason it is possible to say without hesitation or reservation that the action of theelin even in large amounts and over long periods of time is confined entirely to the growth of the duct system. For this reason, the mouse, especially the male, is suitable for experimental work where a mammary duct system free of any suggestion of lobules is desired. As that is exactly the condition desired for the study of the stimuli required to grow the lobules and

*See addenda.

alveoli which normally develop during the first half of pregnancy, our interest in the use of the mouse in the experiments to be described was very great.

THE GROWTH OF THE GLAND LOBULES AND ALVEOLI

In the normal female, a new phase of mammary gland growth occurs during the first half of pregnancy or pseudo-pregnancy. Whole mount preparations of a series of glands during this period have been described (see Plate V, Mo. Res. Bul. No. 182) which are characterized by the rapid outgrowth or budding from the terminal ends and lateral walls of the mammary ducts and sub-branches. These buds represent the anlage of the lobes of the glands which eventually develop alveoli, the secretory portion of the gland.

The studies with theelin just described clearly indicated that it was impossible to initiate lobule development even with extended injections. The next step was to determine if the hormone of the corpus luteum of the ovary (corporin or progestin) which is probably being secreted during pregnancy played a role in the development of the gland during this period. In studies with the rabbit, Turner and Frank (1931-32) had observed that the injection of corporin alone was ineffective in stimulating lobule development whereas the simultaneous injection of 20 rat units of theelin and .3 cc. of corporin (1 rabbit unit) daily for 15 days caused the hyperplasia of the gland lobules corresponding to that observed during the first half of pregnancy or pseudo-pregnancy.

Similarly, the growth of the gland of the rat corresponding to that observed at the middle of pregnancy was stimulated by the simultaneous injection of 5 rat units of theelin and 0.2 cc. of corporin daily (Turner and Schultze, 1931).

Experimental Development of the Lobules

In connection with the study of the effect of theelin and corporin upon the growth of the mammary gland of the rabbit (Turner and Frank, 1932) it was reported that many of the crude extracts of the corpus luteum contained relatively little of the active principle. Some of these same extracts were used in our preliminary experiments with mice. As these experiments were uniformly negative, i. e. there was stimulated only an extension of the duct system following the injection of theelin and corpus luteum extract, it may be concluded in the light of later experiments that the corporin was inactive. It is needless to report these experiments in detail. With the improvement in the method of preparing cor-

porin described in that paper, the results described below were obtained.

Male mice were used in all experiments. It was necessary, therefore, to give the animals a preliminary treatment of theelin (in oil) to stimulate the duct system.

Mice were given 12 rat units of theelin daily for 10 days followed by the simultaneous injection of 12 rat units of theelin and 0.05 cc. of corporin for 10 days. Glands removed at the end of this period showed the presence of long slender ducts. Numerous lateral bud-like outgrowths were present on the secondary ducts close to the point of origin of the duct (Figures 5 and 6). These structures are believed to be the anlagen of the lobes representing the first step in lobule growth. (See Fig. A. Mo. Res. Bul. 182). After 20 days the lobe development had extended only slightly further.

The daily injection of 20 rat units of theelin for 10 days followed by the simultaneous injection of 20 rat units of theelin and 0.05 cc. of corporin produced mammary glands showing only the presence of ducts.

The next group of mice were given 10 rat units of theelin daily for 10 days.Then 10 rat units of theelin and 0.1 cc. of corporin were injected simultaneously. Animals were sacrified after 10, 20 and 30 daily injections. After 10 days of this treatment the duct system was quite extensive with occasional lateral bud-like outgrowths along the lateral walls of the ducts. After 20 days some of the duct ends showed end-buds typical of progressive duct growth but along the lateral walls of the secondary and tertiary ducts the secondary branches of the lobule anlagen had developed. This latter type of development had continued in the glands observed after 30 days of treatment (Fig. 8).

The next experiment was designed to determine the influence of an increased amount of corporin. Three animals were injected with 0.15 cc. of corporin in conjunction with 10 rat units of theelin. One animal died after 15 days of injection; a second after 30 days; and the third after 35 days of treatment. The corporin extract appeared rather toxic when given in this amount. The gland after 15 days treatment showed duct growth with true proliferating lobules comparable to a normal female pregnant about seven days. After 30 and 35 days of treatment, extensive lobule proliferation had taken place (Figures 7 and 9). The growth observed at this time compared favorably with the growth of the normal gland at about the middle of pregnancy (11 to 12 days) when it is believed that the growth phase is practically if not entirely completed.
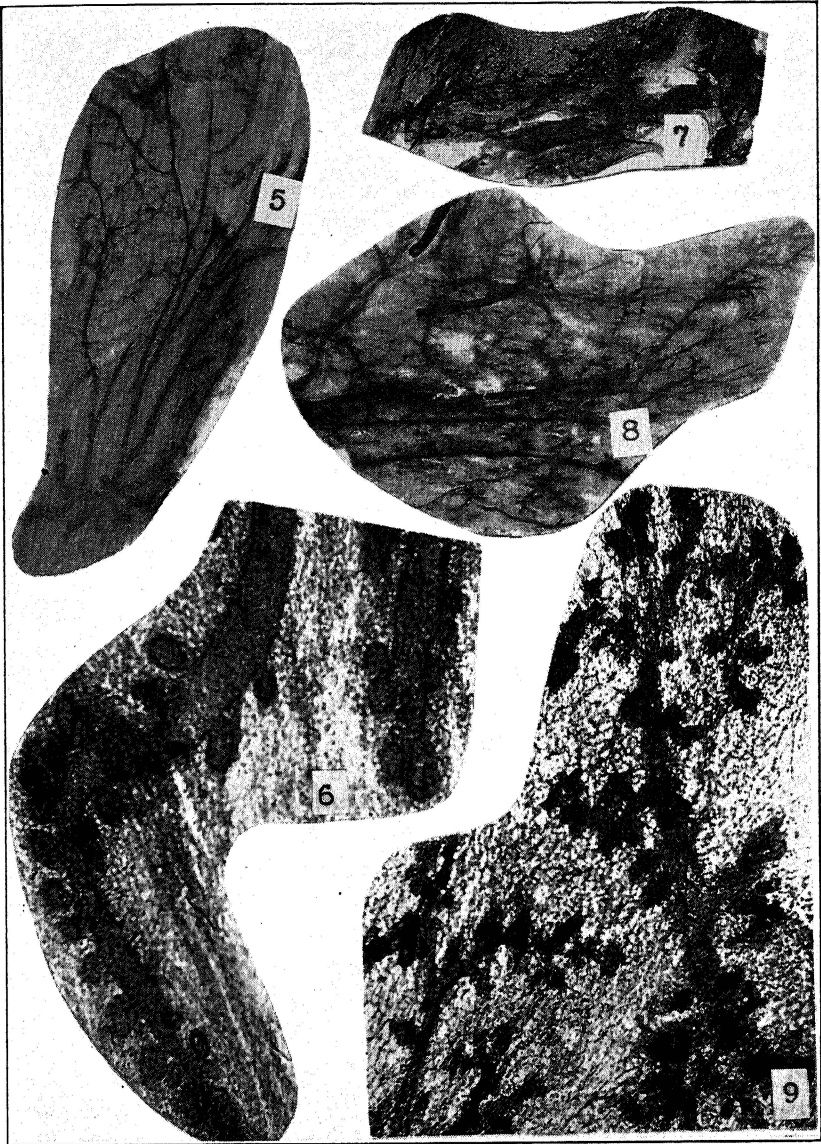
PLATE II

Fig. 5.—Mammary gland of an adult male albino mouse which received 12 rat units of theelin (crude preparation) daily for 10 days followed by 10 rat units of theelin and 0.05 cc. of corporin administered simultaneously once a day for 10 days. x7.

Fig. 6.—Microphotograph of a portion of a duct of the mammary gland shown in Fig. 5. The bud-like outgrowths (lobule anlagen) arise along the lateral walls and ends of the ducts. x60.

Fig. 7.—A portion of the mammary gland of an adult male albino mouse which received 10 rat units of theelin daily for 10 days followed by 0.15 cc. of corporin and 10 rat units of theelin, administered simultaeously once a day for 30 days. x7.

Fig. 8.—Mammary gland of an adult male albino mouse which received 10 rat units of theelin daily for 30 days followed by 0.1 cc. of corporin and 10 rat units of theelin, administered simultaneously daily for 30 days. x7.

Fig. 9.—Microphotograph of a portion of the gland parenchyma shown in Fig. 7. The development of the gland corresponds to that of a 12 day pregnant gland. x60.

Effect of Castration on the Growth of Lobules

In the previous experiments normal male mice were used. In order to determine the effect of castration on the growth of lobules, mice about 28 to 30 days of age were castrated. The duct system was developed with 10 rat units of theelin daily for 10 days. One group received 10 rat units of theelin and 0.1 cc. of corporin daily for 30 days, while the second group received the same amount of theelin and 0.15 cc. of corporin daily for 30 days. After 30 days the animals in both groups had mammary glands which compare favorably in the extent of lobule proliferation with normal females at the middle of pregnancy or the end of pseudo-pregnancy.

Discussion

The mouse duct system is especially favorable for the demonstration of the influence of theelin and corporin upon the growth of the lobule system which normally develops during pseudo-pregnancy or the first half of pregnancy due to the scarcity of lateral branches in the virgin female or the normal or castrate male following theelin injection. Theelin alone either for long periods or in large or small amounts has never been observed to stimulate the growth of the lateral duct buds which develop into the future lobes of the gland.

The results obtained with theelin and corporin injected simultaneously, however, is quite positive. With 0.05 cc. of corporin daily in conjunction with 10 or 12 rat units of theelin in oil the first indication of lateral bud (lobe anlage) growth was observed. If the theelin was increased then even this slight development was apparently inhibited.

With increasing amounts (0.1 and 0.15 cc.) of corporin injected daily with 10 rat units of theelin during a period of 30 days the growth of the mammary gland very similar to that observed at the end of pseudo-pregnancy or the middle of pregnancy was stimulated. It might be pointed out that the corporin extract used is still relatively impure. It undoubtedly contains small amounts of theelin as well as other foreign matter. The daily injection of this extract has not been well tolerated by the mouse and the mortality has been rather high. It seems clear from the above experiments that it has a distinct physiological effect upon the growth of the lobule-alveolar structure of the mammary gland.

Because of the impurity of the extract and the variation in the potency of extracts after holding for various intervals the amounts of corporin required and the exact relation to theelin at best can be

only a rough approximation. With highly purified extracts injected at shorter intervals, it should be possible to obtain the full lobule growth in approximately the time required to develop the gland normally.

SUMMARY AND CONCLUSIONS

Crude extracts of the estrogenic hormone recovered from the urine of pregnant dairy cattle and the purified crystalline hormone theelol were observed to stimulate the growth of the duct system of the mammary gland of the male mouse. However, even upon long continued injections, the growth of lobules characteristic of pregnancy were not stimulated. For optimum duct growth about 10 rat units daily in oil were required. This is considerably greater than the amount required to stimulate duct growth in the male rat.

The growth of lobules in normal male mice comparable to the growth observed during the first 10 or 12 days of pregnancy was stimulated by the simultaneous injection of 0.15 cc. of corporin and 10 rat units of theelin following preliminary treatment with theelin. Castration appeared to have little if any influence upon the rapidity or type of growth.

ADDENDA

In connection with the theelin requirement of male mice for optimum mammary duct growth, we are permitted to present briefly the results of a study by Dr. W. U. Gardner, National Research Council Fellow at Yale University, which will appear shortly in the Anatomical Record. Males of three different strains of mice received varying amounts of theelin (in saline solution) in two injections daily. One strain was resistant to mammary tumors in the female while in the other two strains from 80 to 90 per cent of the females die of tumor. Although from one to five rat units of theelin were injected no significant difference in the response of these animals could be noted. The injection of two rat units of theelin daily into the majority of the animals stimulated extensive duct growth during a period of 60 days or more.

Whether the difference in dosage of theelin apparently needed for optimum duct growth observed by Dr. Gardner and in this laboratory is due to variations in the strains of mice used, methods of assaying the hormone, frequency of administration, the carrier of the hormone (oil or water) or other causes must await further study.

It is interesting to note that in a few males of the tumor strain, small lateral buds appeared similar to the lobule anlage shown in Fig. 5. Further, in three males localized areas of "atypical" growth appeared which resembled normal lobules and were apparently functional as determined histologically.

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II.   The Male and Female Guinea Pig

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ABSTRACT

In the male and ovariectomized female guinea pig the estrogenic hormone (estrin, theelin) not only stimulates the growth of the duct system of the mammary gland similar to that observed in other species, but differs from several other species in that it stimulates the growth of the lobule-alveolar system as well.

The injection of corporin alone either with or without immediate preliminary treatment with theelin was ineffective in stimulating the growth of the mammary gland beyond that observed with theelin alone. The simultaneous injection of theelin and corporin appeared to initiate the early stages of secretory activity of the alveolar epithelium typical of the latter part of normal pregnancy.

Ovarian grafts in the experimentally cryptorchid testis of guinea pigs, when successful, stimulated rapid growth of the mammary gland corresponding to that observed at mid-pregnancy. Such animals come into lactation when the remaining testis containing the graft was removed.

The lactogenic hormone was found to stimulate lactation in animals which had a well grown gland, i. e., one with ducts and lobules which had been normally or experimentally stimulated immediately before with theelin.

II.   The Male and Female Guinea Pig*

C. W. TURNER AND E. T GOMEZ

The guinea pig has been used very extensively in studies of the experimental development of the mammary gland. This is true not only of the recent work which has been done since the ovarian and pituitary hormones have been available in a purified and standardized form, but there had grown up earlier a considerable literature dealing with the influence of ovarian, placental, and fetal extracts and of ovarian grafts on the growth and secretory activity of the mammary glands.

Since some of these reports differed considerably from the observations made with other experimental animals in our laboratory, it seemed desirable to extend our observations to include the normal and experimental development of the mammary gland of the guinea pig in order to determine whether the differences reported in this species were due to a lack of understanding of the normal changes in the mammary gland during successive stages of development or were in fact real species differences.

As a preliminary to the experimental work about to be reported, a study was made of the normal growth of the mammary gland of the male and female guinea pig, which has been reported (Turner and Gomez, 1933).

It will be unnecessary to present a detailed review of the literature concerning the hormones stimulating the growth of the mammary glands and the initiation of lactation as such a review has recently been published (Turner, 1932). A rather detailed review, however, will be made of the studies concerned with the guinea pig as an introduction to each phase of our experimental work.

PRESENTATION OF EXPERIMENTAL RESULTS

Unless otherwise reported, the animals used in the study of mammary gland growth were gonadectomized when quite young and before puberty so that the normal growth of the duct system would be held to a minimum. As pointed out previously (Turner and Gomez, 1933) even in the normal adult male there is considerable development of a mammary duct system.

The glands were prepared for microscopic study by the method described in the previous study. The sectioned materials were stained for the most part with Delafield's hematoxylin and eosin or

Mallory's triple stain, either of which proved satisfactory for the purpose .

The estrogenic hormone (in oil) used throughout the work was extracted from the urine of pregnant dairy cattle by a method described by Turner et al. (1930). This hormone will be referred to as theelin. The corpus luteum extract was prepared from swine ovaries by the method described by Turner and Frank (1932). Unless otherwise stated the hormones were administered subcutaneously once daily.

THE GROWTH OF THE MAMMARY GLAND

Early Studies with Ovarian and Placental Extracts

In 1911 Aschner and Gregoriu observed that water extracts of the placenta produced growth of the mammary gland of guinea pigs in both the male and female. In some cases even milk secretion was reported. Later Fellner (1913), using a lipoid ether-alcohol-acetone extract of corpus luteum and placenta, produced hypertrophy of the mammary glands in castrated and normal male and female guinea pigs. While a cloudy secretion was produced no real milk secretion was stimulated.

Growth of the Nipples and Duct System with Theelin

As early as 1925, Löewe, as well as Steinach, Heinlein and Wiesner, reported that there was some enlargement of the nipples of guinea pigs following the administration of crude estrogenic hormone preparations. Zondek (1926) also made a brief reference to the action of the estrogenic hormone. Negative results were reported by Laqueur, de Jongh and Tausk (1927) following the injection of small doses of menformon for as long as one-half year in castrate male guinea pigs. In young females the same negative results were obtained in the mammary gland, although distinct changes were noted in the uterus and vagina. Later, however, following the injection of 30 to 300 mouse units daily for 24 to 26 days, Laqueur, Borchardt, Dingemanse, and de Jongh (1928), were successful in securing proliferation of the gland and growth of the teat equal to that of pregnancy. Castrate and normal males and virgin females were used.

Champy and Keller (1927) produced mammary gland growth equal to that of estrus in castrate female and male animals by daily injections of estrogenic hormone obtained from ovaries and pla-

centa. By prolonged injections, development of the gland equal to that of pregnancy was believed to result.

Younge (1927) observed marked growth of the teats of castrate male guinea pigs following the daily injection of from ¼ to 1¼ rat units of "oestrin", a lipoid extract of whole sow ovaries. A lipoid extract of the follicular liquor of the sow, as well as of pregnant rabbit blood, also caused the growth of the teats.

Haterius (1928) injected four adult castrated male guinea pigs twice daily with 0.5 cc. of an estrogenic hormone which was prepared from human placenta and contained 20 to 25 rat units. Three controls were given saline solution. After eight days the swelling of the glands was very noticeable. Enlargement continued during a period of three weeks after which continued injections produced no further change. The nipples of the injected animals were three times larger than the controls. The lobules increased considerably in size but there was no increase in their actual number.

Loeb and Kountz (1928) injected follicular extracts into normal immature and mature female guinea pigs in various stages of the estrus cycle and pregnancy. In the immature animals the mammary glands showed early stages of proliferation. In all cases, whether the mammary gland consisted of the ducts only or complete lobules, mitotic activity was observed. These latter observations are rather difficult to evaluate because proliferation of the gland normally takes place during the sexual cycle.

Steinach et al. (1928) reported success in stimulating the growth of the mammary glands of castrate male and female guinea pigs with an aqueous solution of the estrogenic hormone. An average of 25 mouse units injected daily for three weeks caused the nipples to increase from 1 mm. to 7 mm. in length. At this time the teats and glands were equal in size to those of normal females 17 weeks of age.

That the crystalline estrogenic hormone, menformon, would stimulate the growth of the nipples of male guinea pigs was demonstrated by Dingemanse, de Jongh, Kober and Laqueur (1930). Within 12 days, the length of the nipples increased one and one-half times.

Nelson and Pfiffner (1931) caused an increase in the size of the nipple and gland area of castrate and normal male guinea pigs by the daily injection of 0.5 cc. of estrogen (12.5 rat units) for 10 to 15 days. Later Nelson and Smelser (1933) gave a gradually increasing dosage of theelin during a period of 11 weeks (total of

374.5 rat units) after which lactation was stimulated by means of a pituitary extract.

Smelser (1933) used the height of the nipple as a measure of gland growth. Daily injections of as little as 0.05 rat units of oestrin for 20 days gave a minimum response; with 0.125 rat units and 0.25 rat units, 30 and 70 per cent increases in size as compared to the control teats were obtained. In a comparison of the growth of the teats of gonadectomized male and female pigs receiving the same amounts of oestrin no differences could be observed. To determine whether the testis or male hormone would have an antagonistic effect on the female hormone, comparable groups of males and females, normal and castrate, were injected with oestrin alone or with oestrin plus 7 bird units of the testis hormone. The fact that the response of the teats was as great in the presence of effective doses of testis hormone as when oestrin alone was administered was believed to prove that the testis hormone is in no manner a deterrent to the responses of the glands to the homologous hormone stimulation.

Experiment I. Studies with Theelin.—The previous studies in which various types of the estrogenic hormone have been administered to guinea pigs have already clearly shown that the teat and gland parenchyma is stimulated to extensive growth. It is not so clear, however, whether the gland hyperplasia is confined to the duct system or whether the lobule-alveolar structures characteristic of the gland development during the first half of pregnancy may also be stimulated to growth by means of long continued theelin administration.

In a preliminary experiment, two adult males and two immaturely castrated females (about 150-170 days old) were stimulated daily with 10 rat units of theelin for 30 days. A gland was removed from each animal at the beginning of the experiment and the other at the close. As might be expected, the duct system of the females showed somewhat more extensive branches than did the males (Fig. 7). After 20 days of theelin stimulation, the glands

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LEGEND FOR PLATE I (See opposite page).

Fig. 1.—Microphotograph of a median longitudinal section of the teat of an adult male guinea pig. x10.

Fig. 2.—Microphotograph of a sectioned teat of an adult male guinea pig which received 1 rat unit of theelin daily for 20 days. x10.

Fig. 3.—Microphotograph of a sectioned teat of an adult male guinea pig which received 8 rat units of theelin daily for 20 days. x10.

Fig. 4.—Microphotograph of a sectioned teat of an adult male guinea pig which received 12 rat units of theelin daily for 20 days. x10.

Fig. 5.—Microphotograph of a sectioned teat of an adult male guinea pig which received 16 rat units of theelin daily for 20 days. x10.

Fig. 6.—Microphotograph of a sectioned teat of an adult male guinea pig which received 20 rat units of theelin daily for 20 days. x10.

Fig. 7.—Mammary gland duct system of a 90 day old male guinea pig. x7.
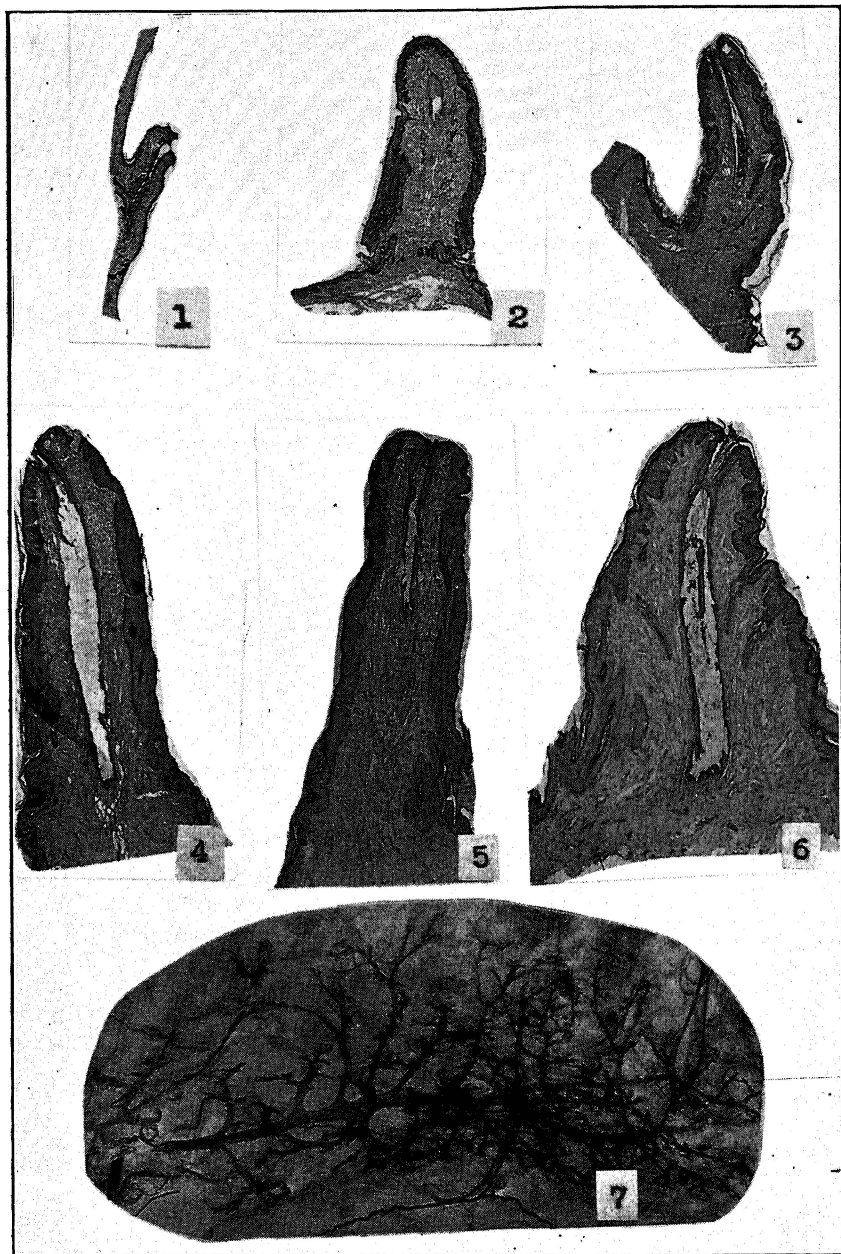
PLATE I

showed very evident growth and development both in extent and thickness. Microscopic examination of the gross mounts of the glands reveal what appeared to be not only an extensive development of the duct system, but at least partial development of alveoli. In order to further determine the extent of development of the gland complex, a thin layer of the gland was isolated from the complex mount showing a single branch of the duct system. This branch, however, showed only secondary and tertiary duct growth; no alveoli were present (Figures 8 and 9), yet sections of the gland showed typical lobule-alveolar structures in large numbers. Further evidence that the circular structures were alveoli rather than ducts, was seen in the single epithelial cell lining. An interpretation of these seemingly contrary observations was sought. It is probable that the growth of the ducts and lobules was still in progress and that the sections showing lobules were in that part of the gland close to the teat (Fig. 10), whereas the isolated duct system still in process of growth was out toward the periphery. Such a gradient in the growth of the lobule-alveolar system has been observed frequently in the rabbit.

In order to confirm the above assumption, an adult male guinea pig was given 10 rat units of theelin daily for a period of 90 days at which time a whole mount of an isolated layer from the periphery of the gland revealed the presence of a true lobule-alveolar system. Examination under high magnification showed a structure of honey-comb pattern. In two other animals a similar histological picture was observed in the gland after 40 daily injections with one and with 20 rat units of theelin (Fig. 11).

In continuing the work, the next question on which information was desired was the influence of varying amounts of the estrogenic hormone on the growth of the teat and mammary gland. Eight adult males of approximately the same age (about 120 days) were selected. They were injected with increasing amounts of theelin (in oil) as follows: 0 (control), 1, 2, 4, 8, 12, 16, and 20 rat units daily for a period of 40 days. The first gland was removed after 20 days of treatment and the second after 40 days. The teats were measured at the beginning of the experiment and after 20 and 40 days. (Table I). The teats of the animal receiving 1 rat unit of theelin daily showed the least response, while those receiving 2 and 4 units grew more rapidly. Those receiving 8 or more units appeared to grow more rapidly during the first 20 days but showed less difference at 40 days; however, the animals receiving 16 and 20 units for 40 days possessed teats of the greatest length (Figures 1 to 6).

PLATE II

Fig. 8.—Microphotograph of a whole mount of an isolated branch of the mammary gland of an adult male guinea pig which received 10 rat units of theelin daily for 30 days. x15.

Fig. 9.—Microphotograph of a portion of a sectioned mammary gland of an adult male guinea pig which received 12 rat units of theelin daily for 20 days. x150.

Fig. 10.—Microphotograph of a portion of a sectioned mammary gland (region near the base of the teat) of an adult male guinea pig which received 10 rat units of theelin daily for 30 days. From same gland as shown in Fig. 8. x460.

Fig. 11.—Microphotograph of a portion of a sectioned mammary gland of an adult male guinea pig which received 1 rat unit of theelin daily for 40 days. Lobule-alveolar system is evident along the periphery of the gland. x150.

TABLE 1.—EFFECT OF THEELIN ON THE HEIGHT OF THE TEAT

Amount of Theelin Daily	Length of the Teat				
	At start	After 20 days	Increase	After 40 days	Total Increase
r.u.	mm.	mm.	mm.	mm.	mm.
Control	1.5	1.5	0	1.5	0
1	2.0	3.5	1.5	4.0	2.0
2	1.5	4.0	2.5	5.5	4.0
4	1.0	3.5	2.5	5.5	4.5
8	1.0	4.0	3.0	5.5	4.5
12	1.5	4.5	3.0	6.0	4.5
16	2.0	5.0	3.0	7.0	5.0
20	1.5	4.5	3.0	7.5	5.5

Growth of the Teats and Duct System with Corporin

Aqueous extracts of dry bovine lutein were prepared by Loeb and Hesselberg (1917) in order to determine the possible presence of a hormone causing the proliferation of the mammary gland of the guinea pig. It was found that the injections of lutein extracts did not exert any noticeable influence on the mammary gland in animals in which the corpora lutea had been extirpated. Bencan, Champy and Keller (1927) injected lipoid extracts of the corpus luteum. These extracts were believed to have physiological actions differing from theelin; however, little or no effect was observed in the glands of guinea pigs following the administration of these extracts.

The above negative experiments may be assumed to be due to the use of extracts containing little if any of the active principle. During the past few years, extracts of the corpus luteum have been prepared by Corner and Allen (1929) (called "progestin"); Hisaw, Fevold and Meyer (1930) (called "corporin"); and others, which produce changes in the uterus similar to those occurring during early pregnancy. Using an extract prepared according to these methods, believed to be practically free of theelin, Nelson and Pfiffner)1930-31), following daily injections, reported the production of marked hypertrophy of the lobule-alveolar system of the gland and nipples of immature male and gonadectomized female guinea pigs.

Experiment II. Studies with Corporin or Progestin.—As Nelson and Pfiffner (1930-31) reported the production of marked hypertrophy of the glands and nipples of immature male and gonadectomized female guinea pigs with a corporin extract believed to be practically free of theelin, two guinea pigs (one adult male and one castrate female) were given 0.1 cc. (1/3 rabbit unit) of cor-

porin daily for a period of 40 days. The check glands and those removed at the end of the period of injection were similar in structure, thus indicating the ineffectiveness of corporin in stimulating either ducts or lobules.

It might be thought that the mammary gland, similar to the uterus, requires preliminary treatment with theelin for the corporin to be effective. To try this experiment two adult males and castrate females were given a preliminary treatment with theelin for 20 days. Comparing the control glands removed at the beginning of treatment with those removed at the close of the experiment, there was revealed no additional growth of the ducts or lobules. In fact, in the female, slight regression of the gland was observed as indicated by a shrunken appearance of the duct ends.

Two other adult males were given 10 rat units of theelin for 10 days followed by 0.2 cc. of corporin daily for 10 days. The glands showed only the theelin effect at the end of the treatment.

The injection of corporin alone either with or without immediate preliminary treatment with theelin, was ineffective in stimulating the growth of the mammary gland beyond that observed with theelin alone. Neither was secretory activity induced in the ducts or lobules present (Fig. 12).

The only explanation for the positive results obtained by Nelson and Pfiffner in comparison with the above observations that may be offered is that their corporin extracts contained considerable amounts of theelin.

Growth of the Mammary Gland with Theelin and Corporin

Nelson and Pfiffner (1931) injected four young male guinea pigs with theelin alone for a time followed by corporin alone. They reported that while in one animal the glandular growth was the best observed in any of the theelin or corporin treated animals, the general response was the same. Then three young castrates were administered 0.5 cc. of theelin (12.5 rat units) and 0.5 cc. of corporin for 15 days. This treatment was no more successful than corporin alone.

While Riddle, Bates, and Dykshorn (1932-33) were not studying the hormones concerned with the growth of the mammary gland, they observed that normal and castrate male guinea pigs given two to three weeks of preparatory treatment with theelin (2 to 5 rat units daily) and corporin (0.5 cc. daily) prepared the glands so that they would respond to the lactogenic hormone which they call prolactin.

Experiment III. Studies with Theelin and Corporin.—The experiments just reported show that theelin stimulates not only the growth of the duct system but the lobule system as well. Corporin alone did not seem to be effective in carrying the development of the gland beyond that observed with theelin alone. The next step was to determine if the simultaneous injection of theelin and corporin would have an effect that could be distinguished from theelin alone.

Two adult male guinea pigs were given 10 rat units of theelin daily for 10 days. Then one was given 10 rat units of theelin and 0.1 cc. (1/3 rabbit unit) of corporin daily for 20 days. The other animal was continued with 10 rat units of theelin as a control. Isolated parts of the mammary gland of the animal receiving theelin and corporin showed that the gland had increased in thickness with the lobes enlarged, masking to some extent the divisions of gland structure. A distinct change was noted in comparison with the control gland but the exact morphological difference was not clear from the gross mounts. Upon section, however, the cause of the change was clearly revealed. In the control gland, the lumen of the alveoli were very small, indicating that the epithelial cells were not in secretion. In contrast, the theelin-corporin gland showed alveoli markedly distended with secretion (Fig. 13).

An adult female castrate before sexual maturity was given 10 rat units of theelin daily for 10 days, then 10 rat units of theelin and one rabbit unit (0.35 cc.) of corporin daily for 10 days. Whole mounts of isolated parts of the gland complex revealed a well developed lobule-alveolar system. Upon section, the alveoli showed some evidence of secretion but the process was not as far advanced as in the gland described above. Secretion was also noted in the larger ducts.

LEGEND FOR PLATE III (See opposite page).

Fig. 12.—Microphotograph of a portion of a sectioned mammary gland of an adult castrate female guinea pig which received 0.1 cc. of corporin daily for 20 days following stimulation with 10 rat units of theelin daily for 20 days. No corporin effect was observed. x150.

Fig. 13.—Microphotograph of a portion of a sectioned mammary gland of an adult male guinea pig which received simultaneous injections of 10 rat units of theelin and 0.1 cc. (1/3 rabbit unit) of corporin daily for 10 days following 20 daily injections of 10 rat units of theelin. x150.

Fig. 14.—Microphotograph of a portion of a sectioned mammary gland of a young virgin female guinea pig hysterectomized during anestrum. The gland was removed 60 days following the extirpation of the uterus. A complete development of the gland complex similar to that which occurred during pregnancy was produced. x460.

Fig. 15.—Microphotograph of a portion of a sectioned mammary gland of a unilaterally castrated and experimental cryptorchid adult male guinea pig carrying a 50 day old ovarian testicular graft which was brought to lactation by the administration of the lactogenic hormone, galactin. x460.
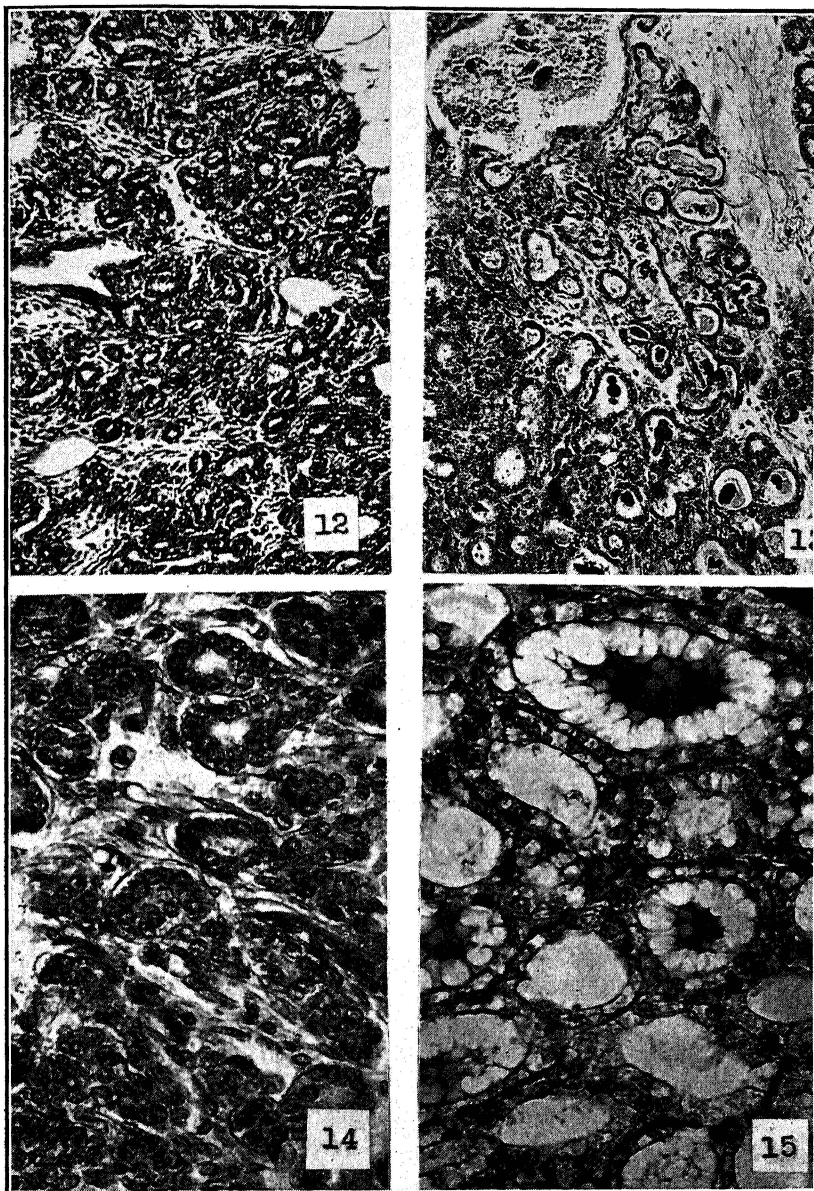
PLATE III

To determine the effect of increasing amounts of corporin upon the secretory process observed in the preceding experiments, three adult males were given preliminary treatment with 10 rat units of theelin for 20 days. Theelin was then continued at the same level but in addition .1 cc., 0.2 cc., and 0.3 cc. (one rabbit unit) of corporin was given. A gland was removed after the theelin treatment and the second after the combined treatment. The control glands showed the presence of compact lobules such as have been described after theelin treatment. In contrast, the glands after theelin and corporin treatment were enlarged, the lobules had increased in size and upon section the lumina of the alveoli were observed to be enlarged with secretion. The increasing amounts of corporin were not effective in increasing the extent of secretion, in fact, the largest amount appeared to induce less secretion than the smaller amounts.

While these observations are not extensive, the individual animals were quite consistent in their response to the combined injection of theelin and corporin. Glands previously stimulated with theelin to extensive lobule growth corresponding to the development at about the middle of pregnancy, were caused to enlarge and the epithelial cells of the alveoli to begin secretion. This latter process normally occurs during the latter stages of pregnancy.

The Effect of Ovarian Grafts upon the Mammary Gland

The removal of the ovaries of young animals stops the usual changes which occur in the uterus and mammary glands at puberty. However, it was found that the ovaries could be removed from their normal position and grafted successfully into other tissues. Thus Ribbert (1898) first produced in the guinea pig a successful ovarian as well as a mammary graft. With the ovarian graft present, mammary growth continued. In continuing this work Steinach (1912-13) was successful in grafting ovarian tissue into castrate male guinea pigs. Within a short time the growth of the nipple as well as the duct system began to develop faster than the normal female of the same age. Eventually milk was secreted sufficient to nurse young put to them.

Studies on the effect of ovarian grafts in castrate male guinea pigs were continued by Athias (1915-16). He was able to confirm the observations of Steinach that full growth and lactation followed the successful implantation of ovarian tissue. A careful histological study of a number of the ovarian grafts removed from animals shortly after they had come into lactation showed the pres-

ence of follicles in all stages of development as well as atretic follicles; however, no corpora lutea of recent development were ever observed. From these observations, Athias was led to the conclusion that the presence of the corpus luteum was not necessary for either the growth or secretory activity of the mammary gland. Sand (1919) confirmed the above findings noting that both growth and secretory activity was stimulated during a period of three months. Moore (1921) noted the growth of the nipples of male castrate guinea pigs after ovarian grafts had been made but did not examine the extent of mammary gland development. Lipschutz and Krause (1923) were successful in transplanting ovaries to the kidney of male guinea pigs. In castrate males, ovarian activity was observed in the growth of the teats in about two weeks. In normal males the grafts showed a longer period of latency (Lipschutz, 1925-27) unless the animals were castrated.

Lipschutz (1927) called attention to the observation that there was a distinct difference in the response of the mammary gland to ovarian grafts in immaturely castrate male and female guinea pigs. In the castrate male the growth of the nipple and gland is continuous, reaching maximum development considerably earlier than litter mate normal females. In the castrate female the graft does not stimulate as rapid development due to rhythmic alterations in the rate of growth. Eventually the mammary gland may approach the extent of development observed in the feminized male (Pettinari, 1925), but milk was never secreted (Lipschutz, 1925). These differences in the growth of the mammary gland are ascribed to the difference in the grafted ovary. In the feminized male the ovarian tissue showed only the presence of follicles, whereas in the female corpora lutea were observed. Thus in the male there would be a condition of continuous estrus whereas in the female the cyclic changes of the normal ovary would be continued in the ovarian graft. The larger amount of gonad stimulating hormone in the male pituitary may play a part in producing the differences observed.

The interesting observation that the implantation of pituitaries will hasten the action of the ovarian graft in stimulating the growth and milk secretion of the mammary gland has been made by Houssay et al. (1931).

Younge (1927) in cooperation with Dr. F. L. Hisaw, reported three successful ovarian grafts in castrate male guinea pigs. Growth of the teats of one animal continued until the 115th day, when the secretion of a milky white fluid commenced and lasted until the

128th day, after which the teat rapidly atrophied. The graft in the other two feminized males remained functional for only 30 to 35 days. No lactation was obtained.

Nelson and Smelser (1933) implanted ovaries in the kidney or testicle of castrate and experimentally cryptorchid male guinea pigs to stimulate the gland in preparation for tests of the lactogenic hormone of the anterior pituitary. These experiments are described later. They reported that although a large number of male guinea pigs with functional ovarian grafts have been examined only two have been observed which gave any indication of milk secretion. Nelson (personal communication) since has observed 5 additional cases. That the removal of the ovarian grafts of the above animals would induce immediate lactation had previously been reported by Nelson (1932).

Experiment IV. Studies with Ovarian Grafts.—A series of 14 mature unilateral castrated and experimentally cryptorchid male guinea pigs received half of an ovary in the form of a testicular graft, the donors being young virgins. Of the total, 9 or 64 per cent of the grafts were successful as indicated by the increase in the size of the nipples after two or three weeks. After 50 to 70 days the teats were well grown, but lactation was not observed. At this time the grafts were removed from three animals by removing the remaining testis. Within 36 to 48 hours following the extirpation of the testis a few drops of clear fluid secretion was expressed from the teats. The secretion gradually took on a milky appearance and increased in amount and consistency until about the fifth day. Lactation continued for a period of about 10 days.

Discussion

Because of our previous studies with the rabbit, rat, mouse, and cat in which the injection of theelin either in large or small amounts and over long periods of time stimulated only an extensive duct system and only a few lobules or lobule anlage if any at all, we were extremely skeptical of the reports in the literature concerning the growth of the mammary gland with theelin in the guinea pig. This was due in part to the general failure of the earlier investigators to follow the normal development of the mammary gland during puberty and the first pregnancy and note the development of the duct system characteristic of puberty and the growth of the lobule-alveolar system during the first part of pregnancy. As a result the type and extent of mammary gland development was generally loosely described. The compact nature of the guinea pig gland also made difficult the differentiation of ducts and lobules. ·

However, as a result of our observations, we are convinced that in the guinea pig there is a real species difference in the response of the male and female mammary gland to the estrogenic hormone. This hormone not only stimulates the growth of the duct system but of the lobule-alveolar system as well. Glands grown with 20 rat units of theelin injected daily for a period of 40 days are indistinguishable from those of an animal pregnant for 33 days.

The simultaneous injection of theelin and corporin appeared to initiate the early stages of secretory activity of the alveoli epithelium typical of the latter part of normal pregnancy.

The relation of the hormones of the ovary to the growth of the mammary gland during pregnancy still presents difficulties. The fact that pregnancy and mammary gland development proceeds uninterrupted even after ovariectomy indicates the probability that the uterus or the products of conception produce the necessary hormones. Whether theelin alone and/or theelin and corporin play functional roles in mammary gland growth during pregnancy in the guinea pig still awaits solution. Our observations indicate that theelin and corporin play a part in the changes normally occurring during the latter part of pregnancy.

LACTATION

Relation of Theelin to Milk Secretion

It is clear that the stimulation of the growth of the duct and lobule system normally precedes the stimulation of milk secretion. Similarly under experimental conditions the growth phase must be stimulated before the glands could be expected to respond to lactation stimuli. In this connection the observations of Steinach et al. (1928) are of interest. Histologic evidences of growth of the mammary gland were noted after three weeks of injection of a water soluble estrogenic hormone. Slight secretion was noted in the alveoli after four weeks of treatment, but not until 7 or 8 weeks of continuous injection was it possible to secure considerable milk which continued for about 25 days. At this time the lumina of the alveoli were reported to be filled with a secretion rich in fat and the epithelium lining the alveoli was higher than in the resting gland corresponding completely with the development of a normally lactating gland.

The initiation of secretion in castrate female guinea pigs after 14 days of "menformon" injection was also reported by Laqueur et al. (1928). The glands were examined three days after the last

injection and were reported as showing clearly the discharge of milk. This same phenomena was observed by Fellner (1931) in the case of mature virgin guinea pigs in which lactation was induced following three daily injections of "feminin." He advanced the theory that the estrogenic hormone prepared the gland for lactation, but that lactation was induced only upon the discontinuation of the hormone.

Experiments of a similar nature have been conducted by de Jongh and Dingemanse (1931) with male guinea pigs. After an extensive preliminary treatment with "menformon", the quantity of the hormone was greatly reduced, then alternately increased and decreased to stimulate lactation.

Inhibition of Lactation by Theelin

Nelson (1933-1934) injected five normal parturient guinea pigs with 100-125 rat units of theelin daily. Injections were begun within a few hours of delivery and continued for 5 to 6 days. In two instances a scanty lactation occurred the day following parturition but did not persist. In each case milk secretion was held in abeyance during the injection period but appeared within two days following cessation of treatment. Three parturient females were given 125 rat units of theelin daily for 6 days, but in addition were injected with 3 cc. of pituitary extract each day. In these animals lactation occurred normally. These experiments were taken to indicate that theelin suppresses the production of the lactation-stimulating hormone of the anterior pituitary.

In two other animals 300 rat units of theelin were administered together with 5 cc. of pituitary extract and only a scanty lactation occurred over a 5-day period. When the theelin was dropped to 100 rat units lactation increased. This experiment was taken to indicate that theelin in large amounts may inhibit lactation by acting directly upon the mammary glands.

Experiment V. Studies with Theelin.—It will be noted that several investigators reported that following long continued theelin injection or alternate stimulation, milk secretion could be induced. As we had failed to secure evidence of lactation by long continued injection of theelin in the rabbit (Turner and Frank, 1930-1932, Turner and Gardner, 1931) and rat (Turner and Schultze, 1931), the following experiment was tried. Three males and one castrate female were stimulated with 20 rat units of theelin daily for 55 days. Then one gland was removed from each animal for examination. The males were then injected with 400, 150, and 100 rat

units and the female with 100 rat units daily for 5 days. Injections were then discontinued for 5 days followed by 200, 150, 100 and 100 rat units respectively for two additional days. The animals were then rested for 5 days and the remaining glands removed on the sixth day. Throughout the course of the experiments, observations were made daily for the presence of milk in the glands.

During the first long continued injection of theelin, the nipples were observed to increase in height and size, but milk was never observed in the glands. The vagina of the female remained open and a slimy secretion was discharged from the vaginal orifice throughout the period of treatment. Gross mounts of the glands were too thick and opaque to determine definitely the type of growth of the mammary gland parenchyma. Upon sectioning, however, structures similar to the lobule-alveolar system were observed. No apparent difference in the extent or type of development was observed between the male and castrated female.

The cessation and periodic stimulation of the mammary gland with large doses of theelin failed to stimulate real milk secretion. It was possible, however, during the omission of the hormone to strip a few drops of a clear fluid secretion similar to that which is present in the ducts of the gland at the approach of and during estrum. Upon section, however, the alveoli of the lobules appeared very compact with very small lumina and little evidence of secretion.

The larger ducts appeared slightly distended with secretion. The gland stroma was reduced to fine connective tissue strands surrounding each lobule and the larger lobes.

It is not impossible that our negative results were due to the use of theelin in oil, which is absorbed slowly, thus continuing to supply theelin to the animal even during the interval between injections.

Effect of Pituitary Extracts on Milk Secretion

Following the work of Stricker and Grueter (1929) showing the lactation stimulating effect following the injection of extracts of the anterior pituitary in the pseudo-pregnant rabbit and of Corner (1930) using alkaline extracts of sheep pituitary on mature virgin rabbits, Nelson and Pfiffner (1930-31) reported that they were able to produce a copious flow of milk within three days using adult virgin guinea pigs which were gonadectomized during estrus, with extracts similar to those used by Corner. Normal adult females responded similarly. As would be expected, daily injections in im-

mature normal and gonadectomized animals of both sexes for as long as 20 days stimulated neither mammary hyperplasia nor milk secretion.

In the case of immature males or females the lactation response was obtained only when corporin was first injected for 10 to 15 days. Adult males responded similarly. On the other hand, when the estrogenic hormone was injected for a corresponding period, no secretion was stimulated with the pituitary extract. In later studies Nelson and Smelser (1933) injected gradually increasing amounts of theelin, totalling in all 374.5 rat units during a period of 11 weeks and secured a very marked response within 36 hours following the discontinuance of theelin and the initiation of pituitary treatment. They report that the response was quite similar to that induced in animals carrying ovarian grafts.

Riddle, Bates and Dykshorn (1932-33) using an extract of the anterior pituitary called prolactin, demonstrated that lactation may be stimulated in parous female guinea pigs and males previously prepared for lactation. This same extract was shown to be effective in stimulating the enlargement and functioning of the crop gland in doves and pigeons.

Lyons and Catchpole (1933) suggested the use of virgin guinea pigs ovariectomized in estrum for the assay of the lactogenic hormone. They reported that they never observed milk secretion in normal or ovariectomized virgin guinea pigs uninjected with the lactogenic hormone. They claim also to have induced a second lactation two weeks after an earlier positive reaction had subsided in ovariectomized virgins.

Nelson (1934) observed that lactation could not be stimulated in pregnant guinea pigs even though in advanced stages by the injection of pituitary extracts unless abortion occurred. However, abortion eventually occurred in these experiments and then lactation immediately followed. In cases of hysterectomy, spontaneous lactation did not occur but the injection of pituitary extracts later induced lactation in every case.

Nelson (personal communication) recently noted that purified pituitary extracts containing the lactogenic hormone do not always cause abortion. However, in the event that abortion was not induced, lactation was not observed until after parturition.

Effect of Pituitary Implants on Milk Secretion

Nelson and Pfiffner (1931) injected corporin into two castrated immature males and one female during a period of two weeks.

Each of the animals was then injected intramuscularly with four fresh rat pituitaries daily for four days. On the third day following the initiation of treatment, a watery fluid could be expressed from the nipples, and on the fourth day a few drops of milk were obtained which increased the next two days.

Experiment VI. Studies with the Lactogenic Hormone Galactin (Prolactin).—In the studies on the experimental initiation of lactation, various extracts of the anterior pituitary were used. Some of the earliest preparations were kindly supplied by Dr. Oliver Kamm of the Parke, Davis and Company research laboratory. They were prepared from whole sheep pituitaries. For the most part, the extracts were prepared in our laboratory by Dr. W. U. Gardner according to methods previously described (Turner and Gardner, 1931; Gardner and Turner 1933).

The object of the study was to determine the physiological state of the mammary gland when the response to the lactation promoting hormone was most marked. It may be said that in general the gland is very responsive to this hormone so that the guinea pig is suitable for qualitative tests of the hormone. In addition, the teats are of sufficient size to be stripped of milk so that the first sign of secretory activity may be detected. It is true, however, that glands producing a considerable amount of milk upon section show less extension of the alveoli systems with milk than do the glands of normal females after parturition (Fig. 15).

The detailed experiments are too numerous to mention, instead a summary of the observations will be reported.

1. Mature normal males and immaturely castrated females in all cases failed to respond to galactin. This would be expected because of the relatively slight development of the gland in such animals.

2. Mature males, either normal or castrate, with mammary glands stimulated with 20 rat units of theelin daily for 20 days or more responded readily to galactin.

3. Male guinea pigs carrying testicular ovarian grafts for 50 to 70 days lactated following the injection of galactin.

4. Adult normal virgins and multiparous females in anestrum failed to respond to galactin. When similar animals in estrum were injected with galactin, lactation followed within 48 hours. If castrated during anestrum, spontaneous lactation was not observed, neither would galactin stimulate lactation. However, if castrated when in estrum slight lactation followed which could be augmented by galactin treatment.

5. The above mentioned animals which responded to galactin were reinjected with galactin as soon as milk could no longer be removed from the teats. In all cases lactation could not be reinduced except in normal females when estrum occurred.

6. The injection of galactin into guinea pigs at various stages of pregnancy (from 30 to 55 days) invariably resulted in the death and expulsion of the fetuses within a few days. Following abortion lactation was always observed within 24 hours. Similar results have been noted in the case of mice, rabbits and in a single case in a goat. It is still unknown whether the effect noted above is due to the hormone or impurities present in the crude extract.

From a study of the histological appearance of the glands of the various types of guinea pigs, considerable variation was noted in the extent of the development of the lobule-alveolar system after treatment with galactin. Male glands developed with theelin and with ovarian grafts show extensive lobule development with alveoli and ducts greatly distended with secretion.

Adult virgins and involuted multiparous females have a less well developed lobule system. Consequently the ducts which are distended with milk are more prominent in relation to the alveoli.

These experiments indicate that the glands of females developed as a result of the recurrence of estrum are in a state immediately responsive to galactin stimulation at estrum. Male glands grown as a result of theelin stimulation will also respond. These results are taken to indicate that theelin not only is a stimulus to cell proliferation but increases the metabolic activity of the gland epithelium so that galactin will immediately cause these cells to begin the synthesis of milk. Thus theelin is believed to bear to galactin the same one-two relationship in the mammary gland that has been previously observed in the case of the theelin and corporin in the progestational proliferation of the uterus.

Ovariectomy During Pregnancy

In the guinea pig, Loeb and Hesselberg (1917), Herrick (1928), Courrier, Kehl and Raymond (1929), and Nelson (1934) have found that pregnancy may continue for some weeks after double ovariectomy. In reference to the mammary gland, Herrick reported that although the ovaries were removed in some cases before the mammary gland had begun to enlarge in preparation for lactation, they proceeded to enlarge and become functional in quite the normal manner after the operation. Nelson observed 9 of the 25 ovariectomized animals maintained a successful pregnancy

to term, gave birth to living viable young, and lactated normally. In no case did lactation occur until the uterus was emptied by delivery or abortion.

Ovariectomy at Beginning of Lactation

Kuramitsu and Loeb (1921) compared the changes in the mammary glands of normal and spayed guinea pigs during the progress of the lactation cycle. Lactation progressed normally in both groups. Similarly Nelson (1934) spayed two females the day of parturition. They suckled their young quite as successfully as did control females. Furthermore, a series of females were removed from their young a few days following parturition and milked twice daily by hand as completely as possible. Two of these were spayed and two served as controls. No difference was observed in the duration of lactation. Within two weeks the flow had practically ceased in all animals.

Hysterectomy During Pregnancy

Loeb (1923) observed that complete extirpation of the uterus was followed by long continued preservation and function of the corpus luteum. In such animals the mammary glands developed quite markedly and mitotic proliferation was observed in such glands as late as 74 days after the last ovulation. In one case 60 days after ovulation milk production in the mammary glands was observed.

In twelve pregnant animals Nelson (1934) removed the entire uterus, leaving the ovaries intact and took care to maintain the ovarian blood supply. No lactation occurred in these animals during periods ranging from 5 to 40 days after removal. However, when the entire uterus and both ovaries were removed lactation occurred within 40 hours. In four unilaterally ovariectomized pregnant animals, the remaining ovary and fertile horn of the uterus were removed after the 40th day of pregnancy, leaving the sterile horn intact. In each of these animals milk was secreted within 36 hours after the operation. In six similar animals, the remaining ovary was removed and the embryos shelled out of the fertile horn, care being taken not to disturb the placentae. The placentae were retained for periods varying from two to five days during which time no lactation was observed. Following expulsion of the placentae, lactation occurred within two days.

Three parturient guinea pigs which failed to lactate have been observed. In two cases, when autopsied after three days, bits

of placental tissue were observed. In a third case the female was injected with 6 cc. of pituitary extract after which lactation occurred.

Experiment VII. Studies on Hysterectomy.—In order to determine the relation of the uterus to the growth and secretory activity of the mammary gland several guinea pigs were hysterectomized during various stages of estrum and gestation.

In the case of the first two operations on 25- and 30-day pregnant animals, the ovarian blood supply was ligated. In these animals secretion gradually developed until the fifth day and continued for ten days. In the case of the remaining animals, consisting of two virgins hysterectomized during anestrum, two during estrum, one on the fifth day, two on the 20th day, and two on the 44th day of pregnancy, care was taken not to interfere with the ovarian blood supply. In none of these animals was milk secretion initiated. However, in the virgin animals there was a marked increase in the size of the teats and of the mammary glands which could be readily palpated in situ. Whole mounts and sections of these glands appeared similar to those removed from animals at the middle of pregnancy (Fig. 14).

SUMMARY AND CONCLUSIONS

The studies presented confirm and extend the previous observations that there is a real species difference in the response of the male and female guinea pig to the estrogenic hormone theelin. In the guinea pig, theelin not only stimulates the growth of the duct system similar to that observed in other species but differs from several other species in that it stimulates the growth of the lobule-alveolar system as well. Glands grown with 20 rat units of theelin injected daily for a period of 40 days were indistinguishable from those of an animal pregnant for 33 days.

The injection of corporin alone either with or without immediate preliminary treatment with theelin was ineffective in stimulating the growth of the mammary gland beyond that observed with theelin alone. Neither was secretory activity induced in the ducts or lobules present.

The injection of theelin and corporin simultaneously appeared to initiate the early stages of secretory activity of the alveolar epithelium typical of the latter part of normal pregnancy.

Ovarian grafts in the experimentally cryptorchid testis of guinea pigs, when successful, stimulated rapid growth of the mammary

gland corresponding to that observed at mid-pregnancy. Such animals come into lactation when the remaining testis and graft was removed.

Periodic or alternate injection of theelin in animals with a well developed gland failed to initiate milk secretion at all comparable to that induced by the lactogenic hormone, galactin.

The lactogenic hormone was found to stimulate lactation in animals which had a well grown gland, i. e., with ducts and lobule-alveoli, when normally or experimentally stimulated immediately before with theelin. These results are taken to indicate that theelin not only stimulates cell proliferation but increases the metabolism of the gland epithelium either through the vascular system or directly on the cells' activity, thus bringing or maintaining them in a condition to respond readily to galactin. When animals cease lactation, the lactogenic hormone was found incapable of restimulating lactation unless theelin was again administered.

In pregnant animals, the injection of galactin invariably caused the death and expulsion of the fetus within a few days. Whether this is due to the hormone or impurities carried with the extract is not known at present. The mammary glands of virgin guinea pigs hysterectomized with care to maintain the ovarian blood supply reached a stage comparable with animals at mid-pregnancy. Lactation was not observed unless the ovarian blood supply was occluded.

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