UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
RESEARCH BULLETIN 174

The Effect of the Ovarian Hormones Theelin and Corporin Upon the Growth of the Mammary Gland of the Rabbit

C. W. TURNER AND A. H. FRANK

(Publication Authorized August 15, 1932)

COLUMBIA, MISSOURI
SEPTEMBER, 1932

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

Agricultural Experiment Station

The Effect of the Ovarian Hormones Theelin and Corporin Upon the Growth of the Mammary Gland of the Rabbit*

C. W. Turner and A. H. Frank

Abstract.—Continuing a study of the hormones concerned in the development of the mammary gland, there is reported a series of experiments on the effect of the ovarian hormones theelin and corporin upon the growth of the mammary gland of the rabbit. The injection of theelin alone even for extended periods has been found to cause only the growth of the duct system and slight lobule proliferation comparable to that observed in the mature virgin rabbit after continuous estrum.

In an attempt to experimentally cause the proliferation of the lobules of the mammary gland observed during the first half of pregnancy, corporin was injected into gonadectomized rabbits either with or without a previously developed duct system without any observable effect. Upon the simultaneous injection of corporin and theelin, however,there was observed a hyperplasia of the gland lobules after 15 days similar to that found at the middle of pregnancy or pseudo-pregnancy.

Thus far lactation has not been observed to occur as a result of such treatment, however, in these experimental animals it has been found possible to stimulate the lactation phase normally observed during the second half of pregnancy by means of injections of extracts of the pituitary of sheep.

The mammary gland may be considered an integral part of the female reproductive system as it furnishes maternal nourishment for the young for a varying period during extra-uterine life. Consequently, it might be expected that the ovary would play a part in the growth and function of the mammary gland as well as of other parts of the reproductive system. The interrelationship of the ovaries and the mammary gland was undoubtedly observed at a very early time because with gonadectomy the gland no longer continued to develop. Not until the successful experiments of Grigorieff (1897), Halban (1900), Knauer (1900), and others, however, was it demonstrated that the reintroduction of ovarian tissue would cause the continuation of mammary gland growth.

From these observations it was only natural to infer that the ovary was the source of a hormone or hormones which stimulated the growth of the mammary gland. While some experimental work was done during the period from 1900 to 1920, it was the introduction of the rat unit test of the estrogenic hormones by Allen and Doisy (1923) and the subsequent determination of the tissues and fluids in which the hormone was present in greatest concentration which renewed interest in the experimental development of the gland.

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

Following the observation of the estrogenic hormone in the follicular fluid of the ovary, the placenta, blood, and urine of pregnant animals were shown to contain the hormone also. In an extensive study of the urine of pregnant cattle at this Station, Turner, Frank, Lomas, and Nibler (1930) observed that the estrogenic hormone was excreted at a low level during the early stages of pregnancy, but gradually increased until the time of parturition. Following parturition the excretion of the hormone declined rapidly.

These observations in connection with the fact that the growth of the mammary gland occurs during the same period caused us to initiate a series of experiments to determine the influence of the estrogenic hormone recovered from the urine of pregnant cattle on the immature mammary gland of the rabbit (Turner and Frank, 1930).

As an introduction to such studies, it became necessary to trace the normal growth of the mammary gland during successive sexual epochs. The careful observations of Ancel and Bouin (1909) were of great help in this study. It was observed that with the beginning of estrus, the duct system of the gland was quite restricted but with continued estrus the ducts lengthened and branched freely. In mature virgin rabbits the gland consisted almost entirely of thickly branching mammary ducts with terminal enlargements and a few lobules. In some animals the lobules may appear quite extensive but not comparable to the development observed at the end of pseudo-pregnancy.

In the rabbit, which ovulates only after coitus, a condition of pseudo-pregnancy is initiated by a sterile coitus. Under these conditions ovulation and the formation of corpora lutea occurs. With the luteinization of the ovary, the growth of the duct system of the mammary gland continues until the gland extremities come in contact. At the same time lobules of the gland now begin to appear at the ends and sides of the main ducts and their branches. This latter type of growth is very rapid during the period from the tenth to the fifteenth day. After the sixteenth or seventeenth days little further growth of the gland occurs. A small amount of milk may appear in the ducts. Soon the lobule and duct systems begin to show regression.

From these observations it was concluded by Ancel and Bouin (1911) and Hammond and Marshall (1914) that the growth of the mammary gland during the first half of pregnancy was caused by the presence of corpora lutea in the ovary.

During the first half of normal pregnancy the changes in the gland are similar to those occurring during pseudo-pregnancy. In either condition the growth of the gland is completed. In normal pregnancy, however, following the growth of the lobules the secretory activity of

the epithelial cells of the alveoli begins to appear. The secretory cells gradually enlarge and the lumina of the alveoli increase in size with the filling of the space with secretion. Gradually the ducts also become filled with secretion. Thus the growth phase of development is gradually superseded by secretory activity, which causes the enlargement and thickening of the gland observed at parturition.

Because of our interest in the use of male rabbits in the experimental growth of the gland, a large number of males have been examined. With a single exception, the mammary gland of the male has been confined to a very limited type of duct development extending only slightly from the base of the teat. In only a single case of the large number of males from which check glands have been removed has a rather extensive duct system been observed. This animal was normal in appearance and had been used as a breeding animal for several years. The exact age was unknown but it was probably at least three years old at the time the extensive duct development was observed. As practically all of our experimental animals were under one year of age, it is possible that the duct system may develop more frequently in old animals than our observations would indicate.

In the study of the hormones which stimulate the growth of the mammary duct system, the observation of the course of normal development indicated the desirability of spaying the females to be used some time before the approach of puberty so that the duct system would still show only limited growth. In the male the experimental animals may be castrated or not. No difference has been noted in the use of castrate or normal male animals in so far as the growth of the mammary gland is concerned. As the castrate animals are more easily kept together it has been our usual practice to castrate the males previous to their use.

EXPERIMENTAL GROWTH OF THE MAMMARY DUCT SYSTEM

With a knowledge of the type of growth of the mammary gland occurring in the rabbit during estrum and the changes that occur during the first half of pregnancy and during pseudo-pregnancy, a series of experiments were initiated to determine if it were possible to stimulate the growth of the duct system and then the lobule growth with the estrogenic hormone.

In the first series of experiments already reported (Turner and Frank, 1930), it was found that the daily injection for 30 days of 20 rat units of the estrogenic hormone in normal or castrated male and in females castrated previous to puberty stimulated the growth of the duct system equal to that produced during continued estrum in the normal female. The injection of larger amounts of the hormone during a similar period of time did not carry the development of the gland beyond this stage.

It has also been reported (Turner et al, 1932) that the two estrogenic hormones theelin and theelol, stimulate the growth of the duct system of the gland similar to that obtained with the crude extract from the urine of pregnant cattle.

In continuing the studies of the estrogenic hormone, it seemed desirable to determine the effect of long continued injection upon the growth of the mammary gland. Two castrate male rabbits were given 20 rat units daily for 120 days. During the first 30 days of the period the ducts grew rapidly as in the previous experiments and approached their maximum extension (Fig. 1). At the end of 60 days the glands removed appeared to indicate a further slight duct growth both in length and number of branches (Fig. 2). The ends of the ducts continued to show a deep staining appearance indicative of continued proliferation of the end buds. The glands removed at 90 days appeared to indicate that growth had ceased (Fig. 3), while at 120 days the ducts were shrunken, indicating that involution had set in (Fig. 4). These observations are taken to indicate that the growth of the mammary duct system under the stimulus of the estrogenic hormone is at first very rapid but that the rate of growth gradually declines until growth ceases at about 90 days. Following this period the involution of the gland may take place even in the presence of the hormone.

As a result of the experimental work with the estrogenic hormones in the rabbit, there appears to be little doubt that the initial growth of the mammary gland complex, including the teat and duct system, is stimulated by a hormone or hormones formed in the ovary at the approach of and during puberty. The continued stimulation of the glands by these hormones eventually causes, in mature virgin animals, the growth of an extensive duct system with slight lobule formation. A similar degree of development of the duct system and lobule growth may be produced experimentally by injections of the estrogenic hormones from whatever source obtained (follicle, placenta, amniotic fluid, or urine of pregnancy). Even in large amounts and over long intervals the estrogenic hormones have not been found effective in stimulating the extensive growth of lobules observed to develop during the first half of normal pregnancy or during pseudo-pregnancy.

Growth of the Lobules of the Mammary Gland

While slight lobule growth is induced in the rabbit with the estrogenic hormones, the rapid and extensive hyperplasia of the lobules of the mammary gland which occurs during the first half of pregnancy is believed to require an additional stimulus. The theory of the functional relation between the corpora lutea of pregnancy and the growth of the lobules of the gland was advanced by Ancel and Bouin (1911), and

Hammond and Marshall (1914) in the rabbit. The first attempt to stimulate the growth of the lobules of the gland experimentally with an extract of the corpus luteum was made by Loeb and Hesselberg (1917) using guinea pigs. Aqueous extracts of dry bovine lutein were found ineffective in causing the lobule growth in animals in which the corpora lutea had been extirpated. Interest in the further study of the experimental development of the mammary gland with corpus luteum extracts appears to have been held in abeyance awaiting the extraction of a physiologically active extract. In 1929 Corner and Allen reported the successful extraction of a hormone from sow corpora lutea which caused changes in the uterus (progestational proliferation) similar to those occurring during early pregnancy. They called their extract "progestin". Hisaw, Fevold, and Meyer (1930) working upon the same problem, prepared a physiologically active extract which they called "corporin". A number of modifications in the methods of extraction have been made since, by these and other workers.

At the time of the appearance of Corner and Allen's paper the writers, working with the estrogenic hormone had reached the stage where it was clearly apparent that extensive lobule proliferation could not be stimulated with that hormone alone in the rabbit. Consequently, it became of great interest to determine whether the extract "progestin" would cause the growth of the lobules of the gland similar to that observed during pregnancy and pseudo-pregnancy.

In our preliminary work castrated male rabbits were injected with sufficient estrogenic hormone to cause the growth of the mammary duct system. Then "progestin" prepared from sow corpora lutea was injected for a variable period of time. The glands removed were a disappointment in that they showed neither additional growth of the ducts nor the growth of lobules.

Considering the fact that considerable amounts of the estrogenic hormone were being secreted during pregnancy in the cow, we were led to believe that the non-effect of the lutein extract "progestin" was due to the fact that the presence of the estrogenic hormone was essential for the proper function of the lutein hormone.

Upon the simultaneous injection of the estrogenic hormone and progestin, the glands (both ducts and lobules) of male castrated rabbits developed in a manner strikingly similar to the normal development during the first half of pregnancy or pseudo-pregnancy (Fig. 12). However, before the preliminary report of our success was published (Turner and Frank, 1931), Corner (1930) published a paper reporting negative results with progestin in growing the mammary glands of spayed rabbits. He concluded that the corpus luteum acting alone in the absence of a

pregnancy does not induce proliferation of the mammary gland or lactation.

Later Nelson and Pfiffner (1931) reported experiments using a lutein extract prepared according to a modified method of Allen and Corner (1929). Injections for about 2 weeks in mature ovariectomized females and young male castrated and normal rabbits were reported to cause the development of the gland to the point where they secreted milk after pituitary treatment. In the case of the mature ovariectomized females, the glands may have reached a stage of development sufficient to respond slightly to the lactation hormone of the pituitary, but in the case of the young males it would appear that the extracts had stimulated the growth of the gland although they do not report having examined the glands to determine the type or extent of growth.

The differences in the response of the corpus luteum extracts alone on the growth of the lobules of the gland of the rabbit reported by Corner (1930) and Turner and Frank (1931) in comparison with those reported by Nelson and Pfiffner are difficult to hormonize unless it is assumed that the lutein extract used by the latter contained the estrogenic hormone.

EXPERIMENTAL PROCEDURE

The object of the present paper is to give a detailed report of observations further confirming the theory that the growth of the lobules of the mammary gland may be stimulated by the simultaneous injection of theelin* and corporin** and further to indicate the quantitative relationship between the two hormones and maximum growth of the mammary gland.

The rabbit was the experimental animal used in these studies. Both normal and castrate males and immature ovariectomized females were used. Sections of the mammary gland were taken before the initiation of each experimental period and at the close of the period. The glands were fixed and prepared for examination according to a method already described (Turner et al, 1932).

Various methods of preparing the extracts of the corpora lutea were tried during the progress of our work. These methods will be referred to in connection with the discussion of the various experiments. The corpora lutea tissue was secured from Armour and Company to whom our best thanks are due for their cooperation in furnishing this material at cost. At first the tissue was shipped to us in two volumes of acid alcohol (98 c.c. of alcohol plus 2 c.c. HCl) to each gram of tissue.

*For the sake of simplicity the term theelin will be used for the estrogenic hormone, whatever the source, as our observations indicate a similarity in their physiologic effect upon the mammary gland.

**The term corporin will be used for extracts of the corpus luteum capable of stimulating progestational proliferation of the rabbit uterus.

Later the corpora lutea were removed from the ovaries, then frozen and shipped to us by express. Both cow and sow corpora were used. Unless indicated the extracts were prepared from sow corpora.

In the early work, a crude extract of the corpus luteum was prepared by a method described by Allen (1930) except that acid alcohol was used as the first extractive. This extract was found effective in stimulating progestational proliferation of the rabbit uterus (Corner and Allen, 1929). Sufficient extract was prepared at one time to last 8 to 10 days.

EXPERIMENTAL RESULTS

In an effort to simulate normal pregnancy in the experimental animal, a young female rabbit was spayed during estrum. Histological examination of the ovaries showed the presence of large follicles, but the absence of corpora lutea. The examination of the mammary gland revealed only the growth of a duct system (Fig. 6). Following recovery from the operation, 20 rat units of theelin were given daily for three days to insure an estrus condition at the initiation of the experiment. Then the injection of 1 c.c. of corporin daily for 11 days was initiated. The gland removed at the close of the period was similar to the check gland indicating the lack of growth of lobules (Fig. 7).

Similarly a castrate male rabbit with a mammary duct system previously grown as the result of the daily injection of 20 rat units of theelin during the course of 30 days, was given 1 c.c. of corporin daily for 30 days. Glands removed at 10 day intervals exhibited neither additional growth of the ducts nor the pregnancy type of lobule growth (Figs. 8, 9, 10). An additional 10 day period of injection of 36 rat units of theelin and 0.5 c.c. of corporin daily resulted in a marked proliferation of the ducts with end buds and the beginning of lobule growth (Fig. 11).

These experiments indicated that theelin was a necessary supplement to corporin to stimulate the complete lobule growth similar to that which develops during the first half of pregnancy. Accordingly, the experiments were begun using castrate male rabbits whose mammary glands had been previously grown with theelin. Two animals were used in this experiment.

A14 was given 12 rat units of theelin and 1 c.c. of corporin daily for 27 days. Sections of glands taken at 10 day intervals exhibited a very rapid growing condition of the ducts and lobule hyperplasia in the region adjacent to the teat. At the end of 27 days, both the type and extent of development resembled very closely that of a gland after 14 days of pregnancy or pseudo-pregnancy, except for the extreme ends of ducts which still showed indications of actively growing end buds.

A18 was given 20 rat units of theelin and 1 c.c. of corporin daily for 18 days. After 10 days lobule growth was observed surrounding the

base of the teat, while after 18 days the gland exhibited complete lobule growth throughout the entire gland (Fig. 13).

The rabbits used up to this time had the mammary duct system previously grown with theelin. In order to determine the effect of the simultaneous injections of theelin and corporin upon the prepubertal mammary gland, A19, an immature castrated male was used. Injections of 12 rat units of theelin and 0.5 c.c. of corporin were given daily for 27 days. Sections of glands taken at 10 day intervals exhibited a rapidly growing duct system, with lobule hyperplasia in the region surrounding the teat (Figs. 14, 15, 16). The duct system, however, was not as extensive in the first gland. After 20 and 27 days there was little difference in appearance between the glands with the duct system previously developed with theelin and those developed by the above method. These observations indicated that it was not necessary to grow the duct system previous to the theelin and corporin treatment to secure typical pregnant type of growth of the gland.

The next question of interest upon which information was sought was the relation between the level of injection of the two hormones and the most rapid growth of the gland. In the first experiment, the amount of corporin was held constant, while increasing amounts of theelin were administered except in one case where double the amount of corporin was given. The details of the experiment are shown in Table 1.

TABLE 1.—EFFECT OF SIMULTANEOUS INJECTIONS OF OVARIAN HORMONES UPON THE DEVELOPMENT OF THE MAMMARY GLAND

Rabbit Number	Type of Gland Growth at Start	Amount of Theelin Given Daily	Amount Corporin Daily	Results (Type of gland growth)
		r.u.	*c.c.*	
A27 ♂	Ducts stimulated with theelin	1 r.u. 1st day, increasing 1 r.u. daily	0.5	Estrus
A28 ♂	Ducts stimulated with theelin	1 r.u. 1st 10 days 10 r.u. 2nd 10 days 20 r.u. 3rd 10 days	0.5	Estrus
A29 ♂	Ducts stimulated with theelin	1 r.u. 1st day, increasing 1 r.u. daily	1.0	Pregnancy
A30 ♂	Normal	1	0.5	Estrus
A31 ♂	Normal	5	0.5	Estrus
A32 ♂	Normal	15	0.5	Estrus
A33 ♂	Normal	25	0.5	Estrus

The preparation of the corporin used in these experiments was the same as previously except that the solutions were evaporated with a hot air dryer instead of under reduced pressure. Injections were continued for 27 days with check glands being removed on the 11th, 21st, and 28th days.

The results were disappointing for the reason that in only a single animal was the characteristic lobule growth of the gland produced. It appeared evident from these observations that the amount of corporin injected was the limiting factor. The experiment showed also that increasing amounts of theelin could not take the place of a deficiency of corporin.

Due to the poor results obtained in this experiment, indicating a lack of potency of the extracts, a new method of extraction was tried (Fevold and Hisaw by letter). (As pointed out later the probable cause of the low potency of the extracts was due to the oxidation of part of the hormone in using the hot air dryer). This method also outlined a procedure for the separation of relaxin (Hisaw, Fevold and Meyer, 1930) and theelin from the corporin.

Two experiments were run on adult normal male rabbits A20 and A114. As only a small amount of fresh sow and cow corpora lutea were available, separate extracts were made without removing the relaxin or theelin. The extract was stored at 20 degrees Centigrade in 95 per cent alcohol. The alcohol was removed before being injected.

A20 was given 20 rat units of theelin daily for 30 days (Fig. 17). Then 12 rat units of theelin and 1 c.c. of cow corporin were given daily for 30 days. Lobule development was produced equal to that of about 10 days of pregnancy. The end buds at the extremities of the ducts were in a rapidly growing condition as shown by the deeply staining periphera. (Fig. 18).

A114 was a normal male rabbit about 3 or 4 years old which was used for breeding purposes. A check gland exhibited duct growth equal to that present after continued estrum in the female. Twelve rat units of theelin and 0.5 c.c. of sow corporin were given daily for 30 days. Sections of gland taken at 10 day intervals exhibited lobule hyperplasia beginning close to the base of the teat and extending to the extremities of the ducts at 30 days. Six days after the injections were stopped a section of the mammary gland was removed. The ducts and lobules contained a small amount of thick, whitish secretion.

These experiments indicate that the corpora lutea of cattle as well as the sow contain corporin. The method of extraction and probable loss of potency due to oxidation of the hormone made impossible a comparison of the two sources.

With a new supply of sow corpora lutea, it was decided to carry the separation and purification further than previously. Therefore, the method of removing the relaxin as well as the theelin was followed. The removal of theelin was of special interest as it would make possible a more accurate quantitative relationship to be established between thee-

lin and corporin. The final extract was taken up in very small amounts of water so as to provide a more concentrated product than that previously used.

Normal male and immature castrate female rabbits were used in this series. Check glands at the beginning were taken from litter mates. Both the corporin and theelin was varied in the amount injected. Injections were continued for 30 days, with sections of glands being taken at 10 day intervals. The details of the experiment are given in Table 2. As only negative results were obtained with this extract it would appear that the corporin was lost in the process of extraction and purification.

TABLE 2.—EFFECT OF SIMULTANEOUS INJECTIONS OF OVARIAN HORMONES UPON THE DEVELOPMENT OF THE MAMMARY GLAND

Rabbit Number		Type of Gland Growth at Start	Amount of Theelin Given Daily	Relaxin Given Daily	Corporin Given Daily	Results (Type of Gland Growth)
			r.u.	*c.c.*	*c.c.*	
A65	♀	Prepubertal	1		0.3	Estrus
A8	♂	Normal	12		0.3	Estrus
A66	♀	Prepubertal	12		0.3	Estrus
A7	♂	Normal	1 r.u. 1st 10 days 12 r.u. 2nd 10 days 20 r.u. 3rd 10 days		0.3	Estrus
A69	♀	Prepubertal	12		0.1	Estrus
A70	♀	Prepubertal	12		1.0	Estrus
A71	♀	Prepubertal	1 r.u. 1st 10 days 12 r.u. 2nd 10 days 20 r.u. 3rd 10 days		0.3	Estrus
A9	♂	Normal	12	0.5	0.3	Estrus
A10	♂	Normal	12	0.5		Estrus
A11	♂	Normal		0.5	0.3	Estrus

Another series of experiments followed in which the corporin was extracted from cow corpora lutea. The above procedure was followed except that no attempt was made to separate relaxin. Injections were made over a 10 day period. The rabbits used in the previous experiment were continued. In three cases increased lobule growth appeared, although it was not as striking as the growth observed in the earlier experiments. Marked lobule growth was present near the teat, while the greater portion of the gland consisted chiefly of ducts. Two of the three rabbits received 1 c.c. of corporin with 12 rat units of theelin while the other received 1 c.c. of corporin with 25 rat units of theelin. Negative results were obtained when smaller amounts of corporin were injected.

These further experiments confirmed the opinion that our procedure of extraction was producing an extract low in physiological activity. In fact, the results obtained in stimulating lobule growth in comparison to our earlier results, were very disappointing. A probable explanation of the poor results obtained by the technique of extraction employed

in our laboratory was noted in a report of the studies of Fevold, Hisaw, and Leonard (1932) in which they stated that corporin was easily oxidized. It was stated further that corporin was soluble in ether. Considering our procedure of extraction, the first loss of hormone probably resulted from the oxidation resulting from the use of the hot air dryer in evaporating the solution. Then in the separation of the theelin with ether a further loss probably occurred. As a result of this information the method of extraction was modified, resulting in a far more potent corporin extract.

PRESENT METHOD OF EXTRACTION OF CORPORIN

The method of extraction was changed to eliminate as far as possible losses of the hormone. The results to be reported later are believed to indicate that the method now employed is quite successful. The method follows: The frozen tissue is ground very finely in a meat chopper and extracted at room temperature for 72 hours in two volumes of methyl alcohol. The residue is separated from the alcohol by straining through a cloth. The tissue is then re-extracted in one volume of acid alcohol for 2.5 hours in a reflux condenser.

The two extracts are now separately evaporated under reduced pressure to a thick consistency or sludge. (Excessive foaming is observed at this point). The sludge from the two extracts are now combined and extracted three times with benzol. The benzol extractions are evaporated under reduced pressure and the thick oily residue extracted three times with acetone. The acetone precipitate is removed and the solution evaporated under reduced pressure. The residue containing the hormone is preserved in about 10 volumes of absolute alcohol until ready for use. The alcohol is then evaporated prior to its injection. By this method corporin has been stored for 42 days at 20 degrees Centigrade without any noticeable loss of potency.

About 30 c.c. of the crude corporin extract was obtained from each kilogram of fresh tissue. On the average, about 0.25 to 0.3 c.c. gave a rabbit unit (progestational proliferation) when injected over a period of 5 days.

For the test either normal females were castrated while in estrum, or spayed females were brought into estrum with theelin.

RECENT EXPERIMENTS

As a result of the improvement in the method of extracting corporin and the stability of the product, it became possible to extract larger amounts of tissue and assay the extracts in terms of rabbit units previous to its use in stimulating the growth of the mammary gland. With this known physiologically active extract, the experiments to determine the relation between corporin and theelin were repeated.

Using male and immature castrated female rabbits with only prepubertal duct development, increasing amounts of corporin were injected with a constant amount of theelin. After the daily injection for 15 days of 0.1 c.c. of corporin and 20 rat units of theelin, the ducts were found to have developed very markedly. True lobule hyperplasia was not observed, however, a beginning of lobule growth could be detected histologically (Fig. 25). The ends of the ducts were in an active growing condition (Figs. 19, 24).

Following the injection of 0.2 c.c. of corporin and 20 rat units of theelin daily for 15 days true lobule proliferation could be observed close to the base of the teat. Further toward the extremities of the gland, however, only actively growing ducts with end buds were present (Fig. 20). When 0.3 c.c. of corporin (equal to 1 rabbit unit) was given daily with 20 rat units of theelin for 12 days, greatly increased lobule growth was observed. Lobules were absent only at the end of the growing ducts (Fig. 21). This condition is very characteristic of the mammary gland after 10 or 12 days of the first pregnancy. These results would indicate that approximately five times as much corporin was required daily to stimulate the growth of the lobules of the mammary gland as is required to cause "progestational proliferation" of the uterus. (A rabbit unit is the total amount of corporin required to produce a +++ or ++++ progestational proliferation of the rabbit uterus when injected daily for five days).

Rabbits having the ducts previously grown with theelin (20 rat units daily for 30 days) exhibited a complete lobule proliferation at the end of 12 days injection of 0.3 c.c. of corporin and 20 r. u. of theelin daily (Fig. 22, 26).

This last condition would more nearly simulate normal pregnancy. The ducts were previously grown with theelin, which would correspond with the growth produced during continued estrus and followed by the administration of corporin with theelin which occurs during pregnancy. The type and rate of growth so nearly corresponding to that of the first pregnancy was taken to indicate that the hormones were being administered in approximately the proportion normally present.

The administration of corporin alone did not produce mammary gland growth (Fig. 23). Males and immature castrate females were given 0.3 and 0.7 c.c. of corporin alone for 15 days. Glands at the close of injection were the same as the controls at the start. In rabbits with the ducts previously grown with theelin there were no changes. In a few cases where the rabbits were first given theelin in large amounts, 40 r. u. daily for 6 days, followed by the administration of 0.3 c.c. of corporin, there was a beginning of lobule production, but no true lobules present. This was possibly due to the presence of theelin in the circulation of the

animal after injections have ceased and the presence of corporin immediately following would induce mammary development until all the theelin was eliminated. Thus it was not present over a sufficient period of time to produce complete lobule growth.

The above experiments indicate that about 0.3 c.c. or one rabbit unit of corporin and 20 rat units of theelin daily is the minimum amounts of the hormones which will produce experimental hyperplasia of the mammary gland of the rabbit characteristic of normal pregnancy. As compared to the progestational proliferation of the uterus, the lobule development of the mammary gland requires five times the concentration of corporin daily over a period of at least 10 or 12 days to complete the development of the gland.

In addition to the histological evidence that the combination of corporin and theelin stimulates the growth of the lobules of the mammary gland, a series of experiments was conducted in cooperation with W. U. Gardner (unpublished) in which the glands of three male and one female rabbits grown as outlined above were brought into lactation corresponding to that observed at the end of pregnancy by means of an extract of the pituitary of sheep.

These experiments are believed to offer conclusive evidence not only that the growth of the mammary gland may be stimulated experimentally, but that the glands so developed are capable of responding to the lactation promoting hormone of the pituitary.

SUMMARY AND CONCLUSIONS

The injection of about 20 rat units daily of the estrogenic hormone (theelin) in gonadectomized immature rabbits for about 30 days causes the growth of the duct system and slight lobule proliferation comparable to that of the mature virgin rabbit after continuous estrum. The injection of larger amounts of the hormone over a similar period of time did not carry the development beyond this stage. The long continued injection of theelin caused some slight further duct growth but extensive lobule growth was not produced. After 90 days of continuous injection of theelin a regression of the duct system was noted.

The injection of a physiologically active extract of the corpus luteum (corporin) into gonadectomized rabbits either with or without a previously developed duct system was ineffective, in stimulating the growth of the ducts or lobules of the mammary gland.

Upon the simultaneous injection of corporin and theelin for 15 days a hyperplasia of the gland lobules was observed resembling in every respect the growth of the mammary gland during the first 15 days of pregnancy or pseudo-pregnancy. Maximum growth was obtained with daily injections of 0.3 c.c. of corporin (about 1 rabbit unit) and 20

rat units of theelin. This is taken to indicate that the growth of the lobules of the mammary gland requires daily about five times the amount of corporin needed to cause "progestational proliferation" of the uterus.

In addition to the histological evidence that the combination of corporin and theelin stimulates the growth of the gland corresponding to that observed at the middle of pregnancy, it was found possible to stimulate the lactation phase of development normally observed during the second half of pregnancy by means of injections of extracts of the pituitary of sheep.

BIBLIOGRAPHY

Allen, E., and Doisy, E. A. 1923. *Ovarian hormone; Preliminary report on its localization, extraction, and partial purification, and action in test animals.* Jour. Amer. Med. Assn., vol. 81, p. 819.

Allen, W. M. 1930. *Physiology of the corpus luteum. V. The preparation and some chemical properties of progestin, a hormone of the corpus luteum which produces progestational proliferation.* Amer. Jour. Physiol., vol. 92, p. 174.

Bouin P., and Ancel, P. 1909. *Sur la fonction du corps jaune; action du corps jaune vrai sur l'utérus.* Compt. rend. Soc. de Biol., vol. 66, p. 505.

Ancel, P., and Bouin, P. 1911. *Recherches sur les fonctions du corps jaune gestatif. II. Sur le déterminisme du développement de la gland mammaire au cours de la gestation.* J. de Physiol. et de Path. Gén., vol. 13, p. 31.

Corner, G. W., and Allen, W. M. 1929. *Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum.* Am. Jour. Physiol., vol. 88, p. 326.

Corner, G. W. 1930. *The hormonal control of lactation. I. Non-effect of the corpus luteum. II. Positive action of extracts of the hypophysis.* Amer. Jour. Physiol., vol. 95, p. 43.

Fevold, H. L., Hisaw, F. L., and Leonard, S .L. 1932. *Hormones of the corpus luteum. The separation and purification of three active substances.* Jour. Amer. Chem. Soc., vol. 54, p. 254.

Grigorieff, W., 1897. *Die Schwangerschaft bei der Transplantation der Eierstöcke.* Centralbl. f. Gynäk., vol. 21, p. 663.

Halban, J. 1900. *Ueber den Einfluss der Ovarien auf die Entwicklung des Genitales.* Monatschr. f. Geburtsh. u. Gynäk., vol. 12, p. 496.

Hammond, J., and Marshall, F. H. A. 1914. *The functional correlation between the ovaries, uterus, and mammary glands in the rabbit, with observations on the oestrus cycle.* Proc. Roy. Soc. B., vol. 87, p. 422.

Hisaw, F. L., Fevold, H. L., and Meyer, R. K. 1930. *The corpus luteum hormone. II. Methods of extraction.* Physiol. Zool., vol. 3, p. 135.

Knauer, E. 1900. *Die Ovarientransplantation.* Arch. f. Gynäk., vol. 60, p. 322.

Loeb, L., and Hesselberg, C. 1917. *The changes in the pregnant guinea pig, the effect of lutein injections, and the correlation between the cycle of the uterus and ovaries and the cycle of the mammary gland.* J. Exper. Med., vol. 25, p. 305.

Nelson, W. O., and Pfiffner, J. J. 1931. *Studies on the Physiology of lactation. I. The relation of lactation to the ovarian and hypophyseal hormones.* Anat. Rec., vol. 51, p. 51.

Turner, C. W., Frank, A. H., Lomas, C. H., and Nibler, C. W. 1930. *A study of the estrus producing hormone in the urine of cattle during pregnancy.* Mo. Agr. Exp. Sta. Res. Bul. 150.

Turner, C. W., and Frank, A. H. 1930. *The effect of the estrus producing hormone on the growth of the mammary gland.* Mo. Agr. Exp. Sta. Res. Bul. 145.

Turner, C. W., and Frank, A. H. 1931. *The relation between the estrus-producing hormone and a corpus luteum extract on the growth of the mammary gland.* Science, vol. 73, No. 1889, p. 295.

Turner, C. W., Frank, A. H., Gardner, W. U., Schultze, A. B., and Gomez, E. T. 1932. *The effect of theelin and theelol on the growth of the mammary gland.* Anat. Rec. vol 53, p. 227.

PLATES

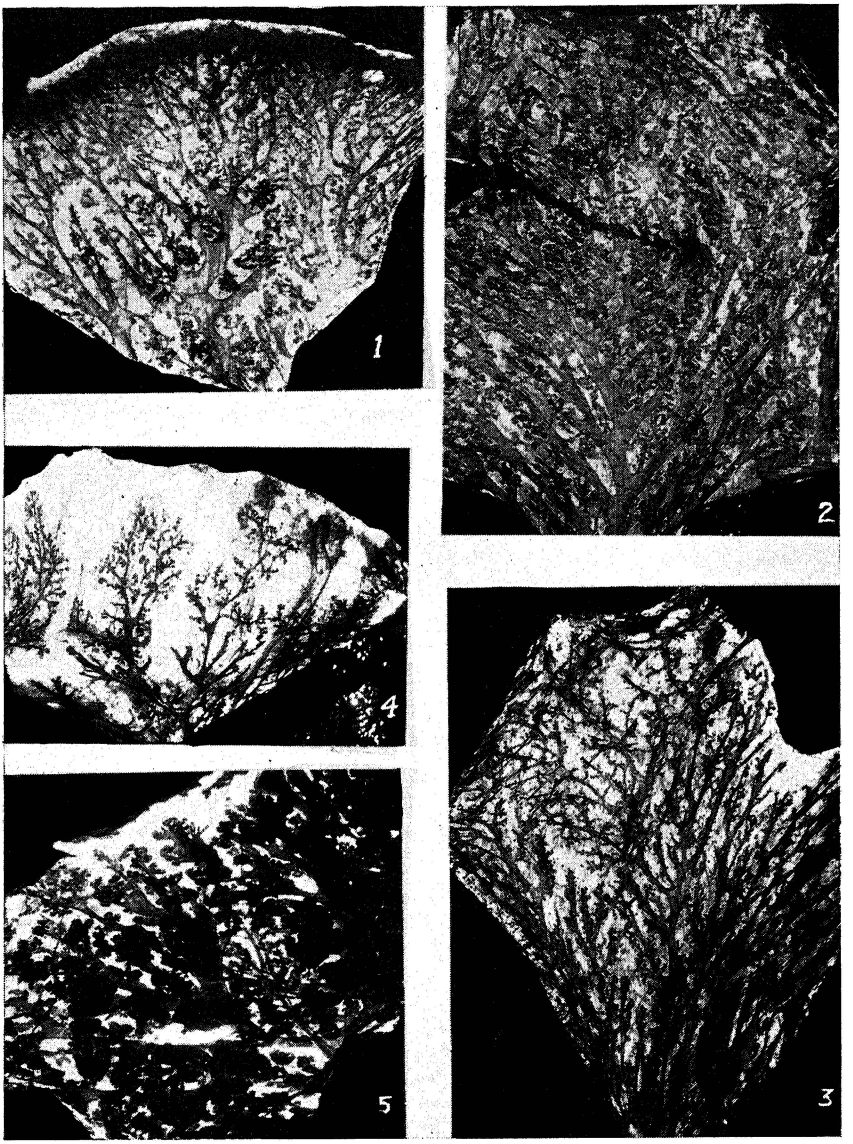


PLATE I

PLATE I

Fig. 1. A section of a mammary gland of R106, a castrate male rabbit after 30 daily injections of 20 rat units of a crude extract of the estrogenic hormone (theelin) recovered from the urine of pregnant cattle. The ducts are generally increased in length and diameter, with extensive branching and a rapidly growing periphery. X2.

Fig. 2. A section of mammary gland of R106 after 60 daily injections of 20 rat units of theelin. There is a slight increase in length of the ducts with an increased number of branches. X2.

Fig. 3. A section of mammary gland of R106 after 90 daily injections of 20 rat units of theelin. The ducts and end buds appear to be in an involuted state. X2.

Fig. 4. A section of mammary gland of R106 after 120 daily injections of 20 rat units of theelin. The ducts and end buds are in a further state of involution. X2.

Fig. 5. A section of mammary gland of R106 after an additional injection period for 10 days of 20 rat units of theelin and 0.3 c.c. of corporin. Lobule hyperplasia is very marked, extending from the teat close to the extremities of the ducts which exhibit proliferated end buds. X2.

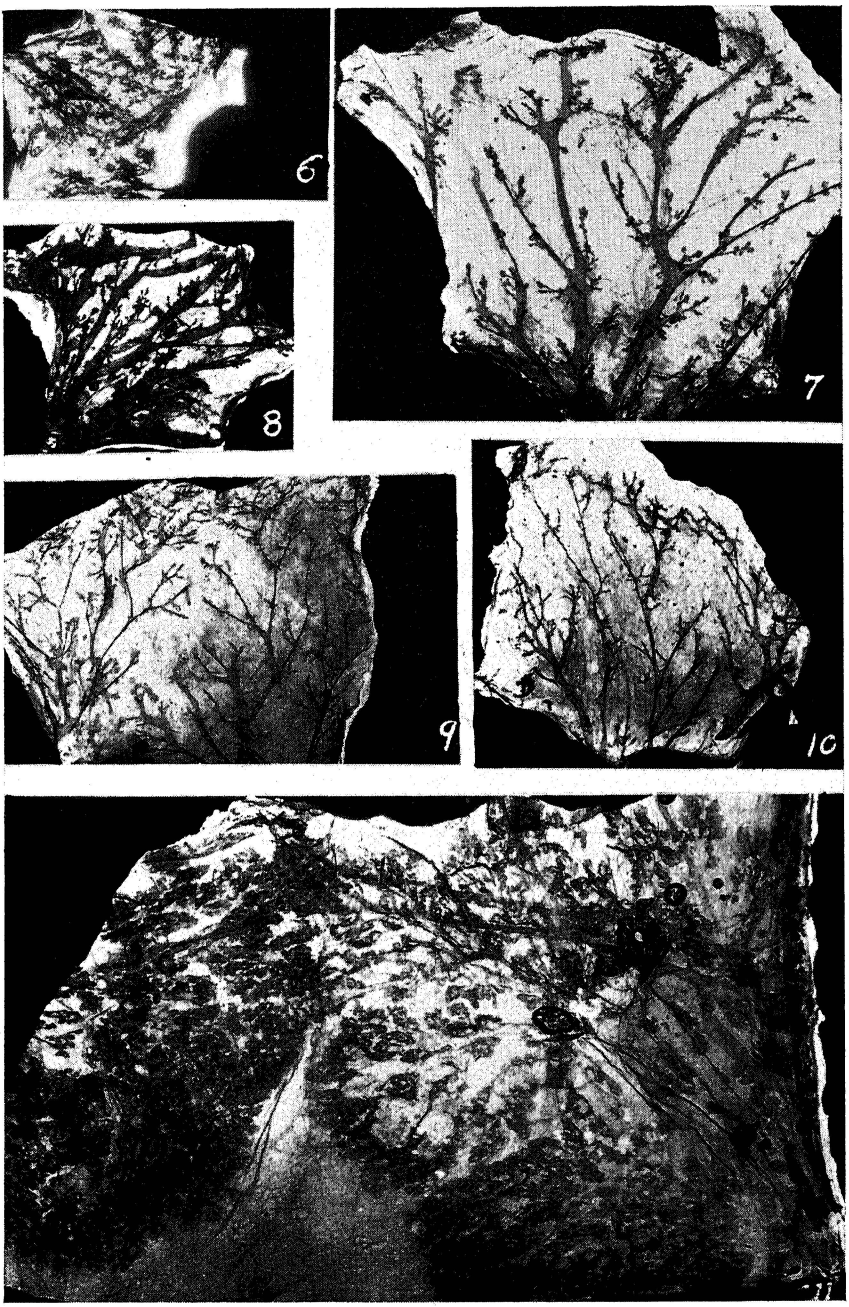


PLATE II

PLATE II

Fig. 6. Control gland of a normal female after continued estrum. (Gland was taken at time of castration). X2.

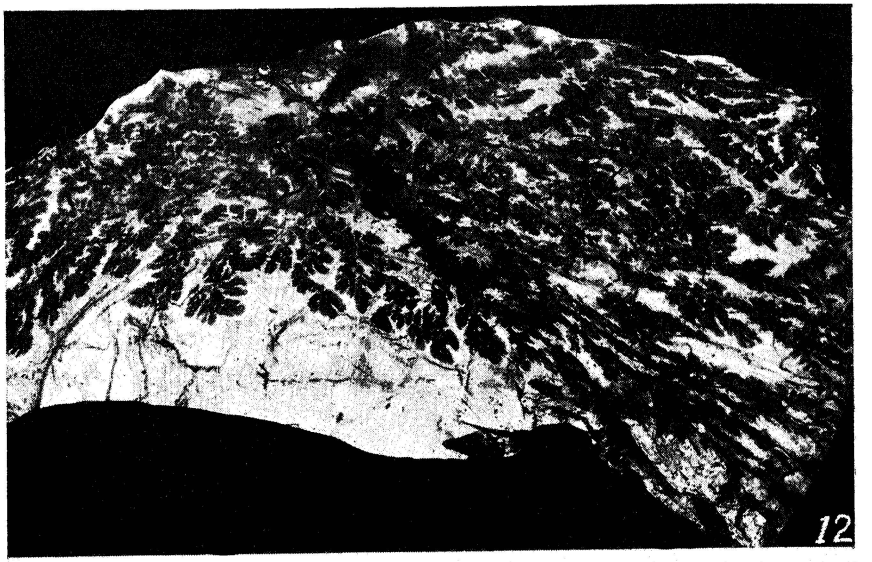
Fig. 7. A section of gland of above female after 11 daily injections of corporin. There is no increase in lobule growth over that of the control. X2.

Fig. 8. A section of gland of a castrate male rabbit after 10 daily injections of 1 c.c. of corporin. (The ducts were previously grown with 30 daily injections of 20 rat units of theelin daily). X2.

Fig. 9. A section of gland of above male rabbit after 20 daily injections of corporin. No change in duct or lobule growth could be noted after the second period, of injections. X2.

Fig. 10. A section of gland of above male after 30 daily injections of corporin. There is no change in the type of growth from that of the 10 and 20 day period. X2.

Fig. 11. A gland of above male after 10 additional daily injections of 0.5 c.c. of corporin and 36 rat units of theelin. The ducts show additional growth with an extensive hyperplasia of lobules. X2.



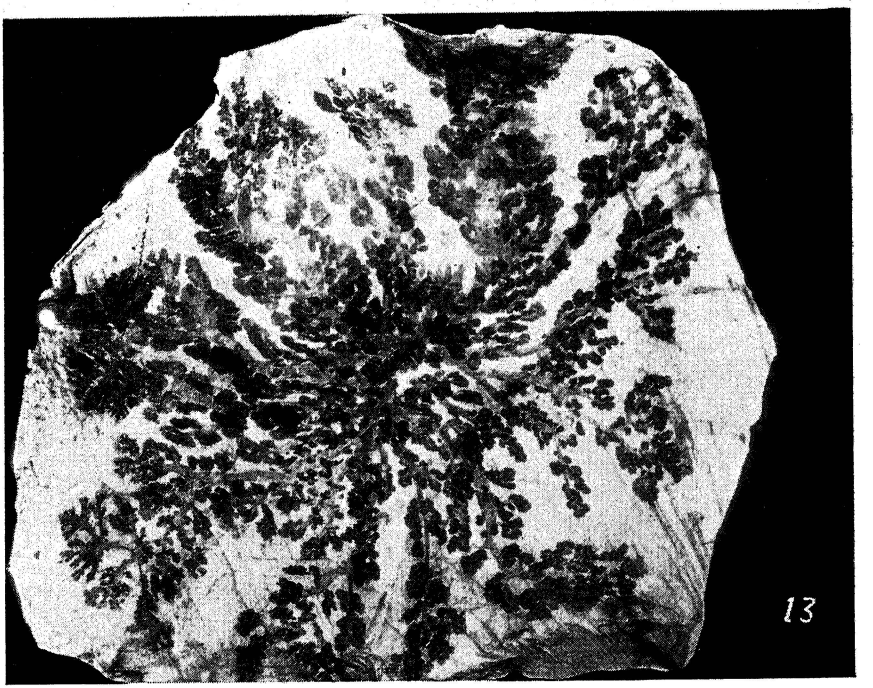


PLATE III

PLATE III

Fig. 12. Mammary gland of a normal pseudo-pregnant rabbit at 20 days. X2.

Fig. 13. Mammary gland of a castrate male rabbit A18 after 18 daily injections of 20 rat units of theelin and 1 c.c. of corporin. Lobule hyperplasia is complete. X2.

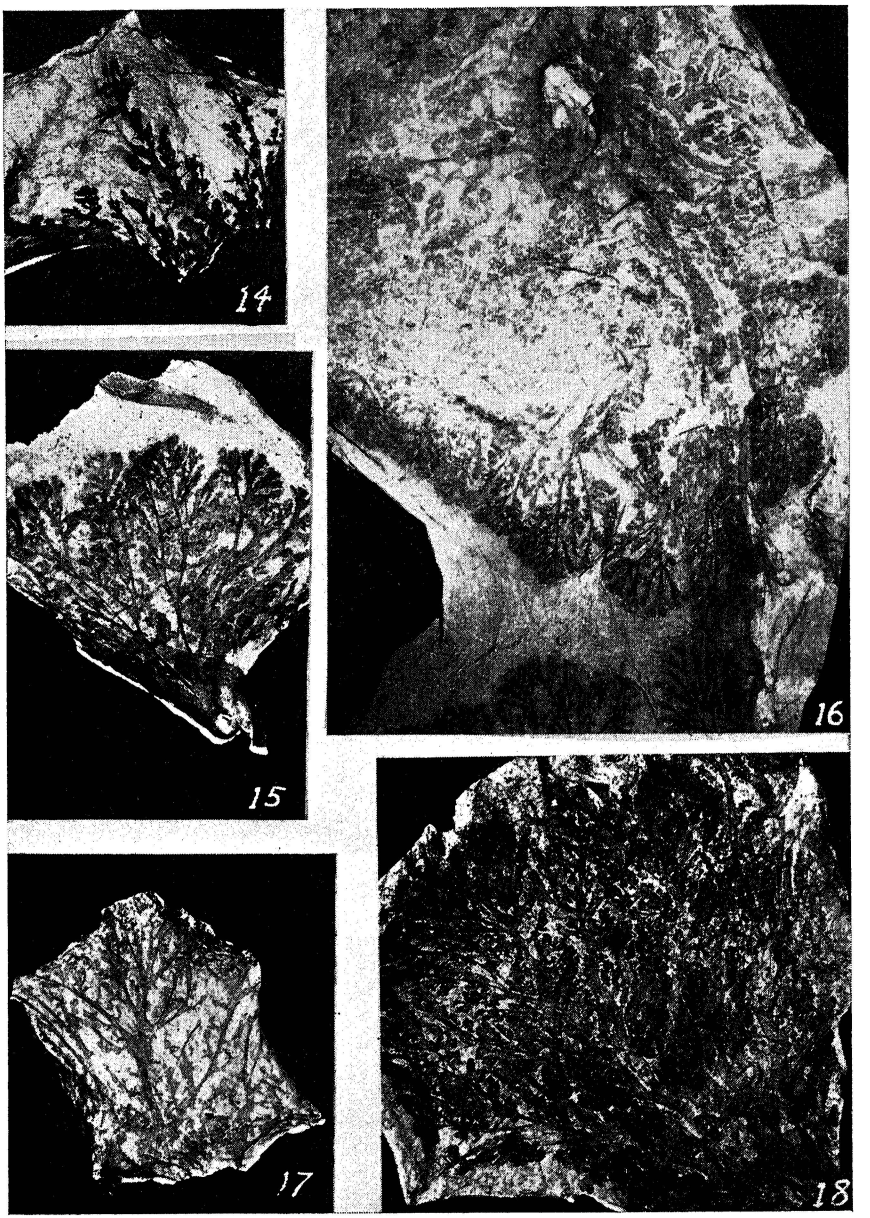


PLATE IV

PLATE IV

Fig. 14. A section of mammary gland of A19, an immature castrate male after 10 daily injections of 12 rat units of theelin and 0.5 c.c. of corporin. Growth of the ducts was very rapid with lobule hyperplasia near the teat. X2.

Fig. 15. A section of gland of A19 after 20 daily injections of 12 rat units of theelin and 0.5 c.c. of corporin. The ducts show an increase in extent of growth. X2.

Fig. 16. A gland of A19 after 30 daily injections of 12 rat units of theelin and 0.5 c.c. of corporin. Lobule hyperplasia extended close to the periphery of the rapidly growing ducts. X2.

Fig. 17. A section of gland of A20, a normal male after 30 daily injections of 20 rat units of theelin. Ducts with very little lobule hyperplasia are present. X2.

Fig. 18. A section of gland of A20 after 30 daily injections of 12 rat units and 1 c.c. of cow corporin. Marked lobule hyperplasia is present near the teat with rapidly growing end buds at the periphery. X2.

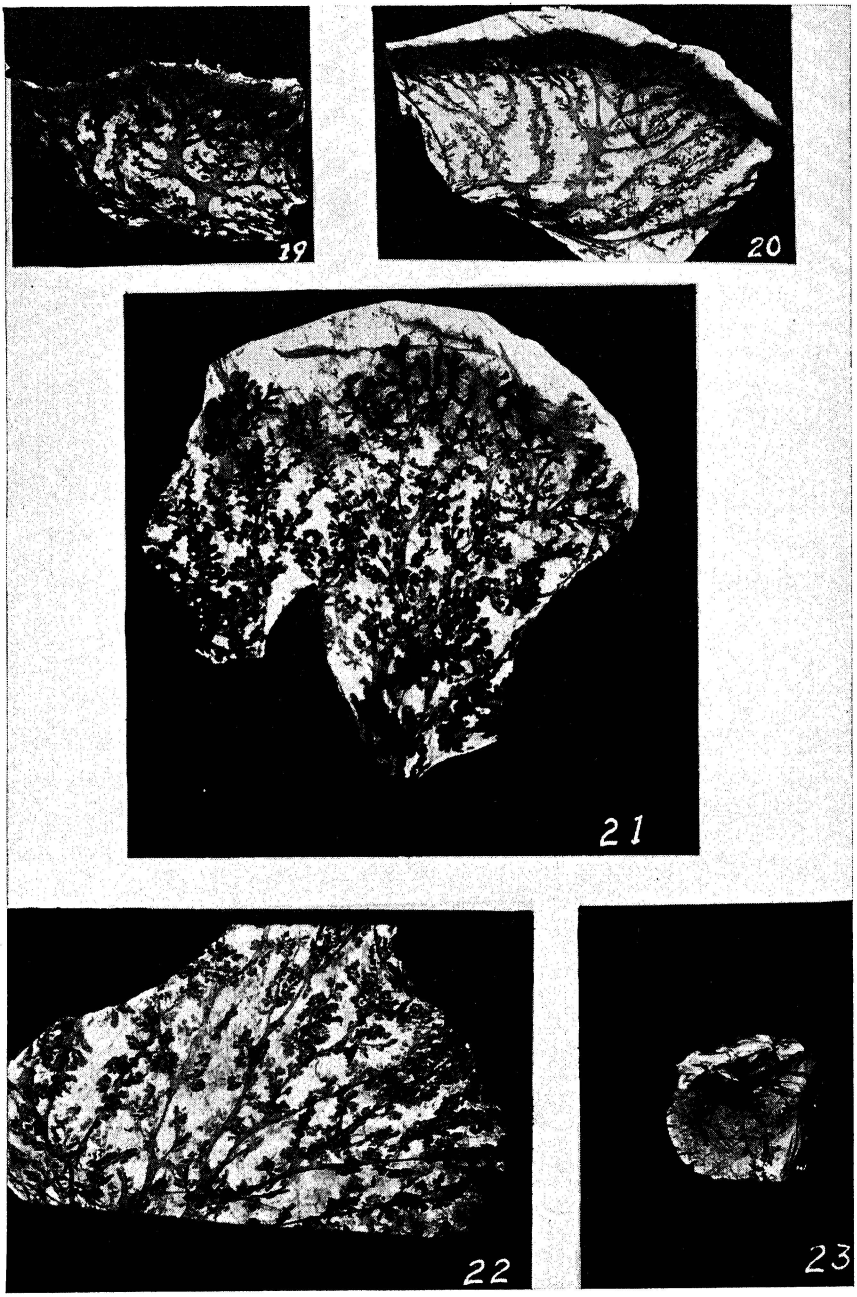


PLATE V

PLATE V

Fig. 19. A section of gland from a castrate immature female rabbit after 15 daily injections of 20 rat units of theelin and 0.1 c.c. of corporin. The ducts are in a rapidly growing condition. True lobule hyperplasia is not present (see Fig. 25). X2.

Fig. 20. A section of gland from a castrate immature female after 15 daily injections of 20 rat units of theelin and 0.2 c.c. of corporin Slight lobule hyperplasia was present near the base of the teat. X2.

Fig. 21. A section of gland from a castrate immature female after 12 daily injections of 20 rat units of theelin and 0.3 c.c. of corporin. Lobule hyperplasia extends close to the extremities of the ducts, which still exhibit a growing condition. X2.

Fig. 22. A section of gland from a castrate male rabbit after 12 daily injections of 20 rat units of theelin and 0.3 c.c. of corporin. Lobule hyperplasia is present throughout the gland equal to that of mid-term pregnancy (see Fig. 26). X2.

Fig. 23. A gland from an immature castrate female after 15 daily injections of 0.3 c.c. of corporin. There was no change in the gland from that of the check. X2.

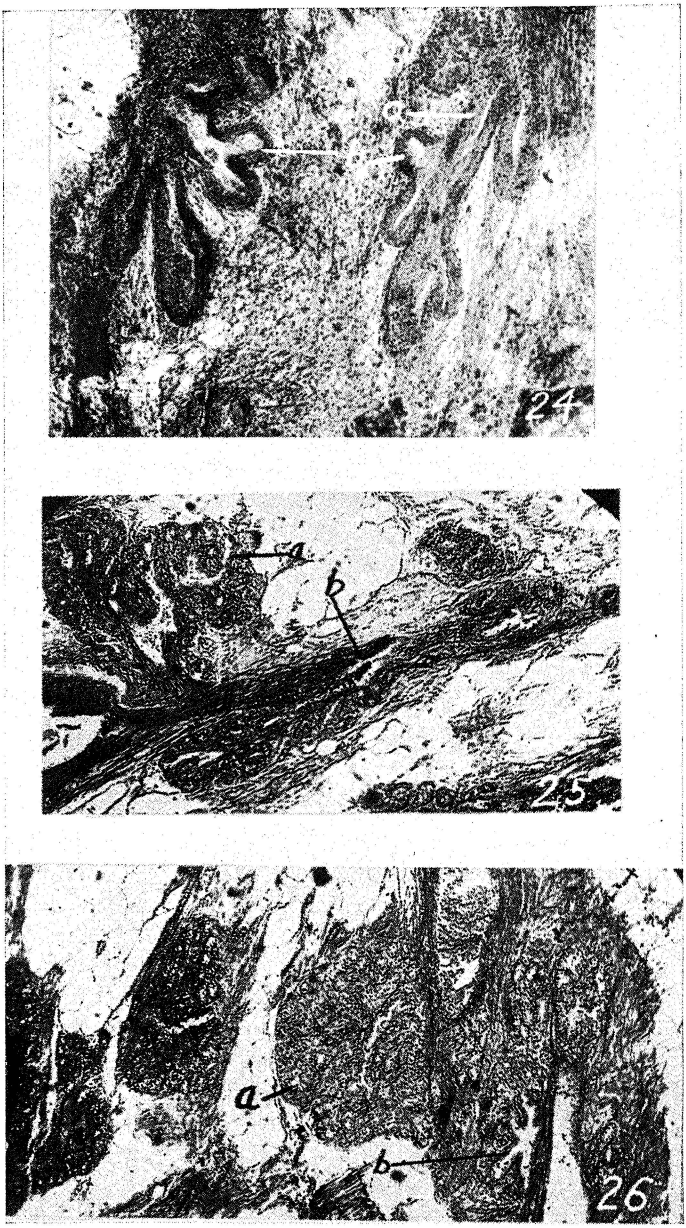


PLATE VI

Fig. 24. Section of the mammary gland of A119 after 30 daily injections of 12 rat units of theelin. The presence of end buds indicates a rapidly growing duct system. X60.

Fig. 25. Section of the mammary gland of A166 after four daily injections of 20 rat units of theelin immediately followed by 0.3 c.c. of corporin for 19 days. A condition of early lobule formation was produced. X60.

Fig. 26. Section of the mammary gland of A165 after 4 daily injections of 20 rat units of theelin immediately followed by 0.3 c.c. of corporin and 20 rat units of theelin for 19 days. Complete lobule growth was present. X60.