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The Function, Assay and Preparation of Galactin, a Lactation Stimulating Hormone of the Anterior Pituitary and An Investigation of the Factors Responsible for the Control ot Normal Lactation

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ABSTRACT

This paper presents the results of further studies on the lactation-stimulating hormone of the pituitary which has been called "galactin." Galactin has been found effective in stimulating or initiating lactation in the rabbit, guinea pig, dog, and sow. The rat, mouse, and monkey have not, thus far, responded in the same manner. The response of the mammary glands of the rabbit to galactin depends on the degree to which the glands are developed and to some other factor, presumably of ovarian origin, which conditions them for response. Galactin was ineffective in stimulating the growth of the mammary glands of young ovariectomized female rabbits, young male rabbits, or of restoring involuted mammary glands of ovariectomized female rabbits. Galactin has been obtained from bovine and sheep pituitaries. The galactin content of the extracts was independent of the gonad-stimulating hormone content. The methods of extraction of the pituitaries and method of assay of the extracts are presented. In the light of the results presented, a discussion of the control of normal lactation is also included.

ACKNOWLEDGMENTS

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INTRODUCTION

The factors responsible for the initiation of mammary growth and secretory activity have been assumed for a number of years to be hormonal in nature. The temporal relationship between reproduction and mammary function lead to the assumption that a common control or connection existed between reproductive and mammary activity. Mironoff (1894), and Goltz and Ewald (1896) observed that the severing of the nerves to the mammary glands or the removal of the posterior portion of the spinal cord did not alter the secretory activity of the mammary gland following parturition. That mammary growth and function is independent of nervous connections between the ovaries or uterus and the mammary glands has been demonstrated frequently by ovarian and mammary transplants (Ribbert 1898, Pfister 1901, Grigorieff 1897, Halban 1900, and Stricker 1929).

During the past few years convincing evidence has been presented showing that mammary growth or hyperplasia is stimulated by ovarian hormones (Turner 1932, Turner and Frank 1932). Evidence has also been accumulated indicating the inadequacy of ovarian hormones in the activation of the secretory cells of the mammary glands with the possible exception of the guinea pig.

Following the initial reports of Stricker and Grüter (1928 and 1929) the work of several investigators has verified their original observation that the pituitary contains a hormone which will experimentally initiate mammary secretory activity. Suitable anterior pituitary extracts have been consistently found to contain a galactopoetic or a galactogogic material when tested under suitable conditions. It is thus strongly indicated that the stimuli for the growth and secretory activity of the mammary glands are independent, the former being under ovarian control, the latter being under the control of the pituitary.

Many factors are known to be capable of modifying mammary activity. It is generally considered that within certain limits, mammary secretion may be modified quantitatively and qualitatively by altering nutritional conditions. Pathological and environmental conditions may likewise alter mammary activity. To the writer's knowledge, however, the pituitary extracts are distinctive in the changes they initiate in the mammary glands. They have the function of a true galactogogue in the experimental activation of the mammary glands. The part played by the pituitary in the control of normal lactation both from the standpoint of initiation and persistency, however, has not been sufficiently investigated.

The investigations reported here have been undertaken as a continuation of the study of the nature and effects of the lactation-stimulating extracts of the pituitary. The investigations undertaken may be grouped under four headings. (1) The nature of the response in different individuals of the same and different species. (2) A method of standardizing experimental animals for a biological assay of the pituitary extracts. (3) The nature of the active material concerning its stability, relationship to other pituitary hormones and methods of extract preparation and purification. (4) A study of the mechanism for the control of normal lactation.

MATERIALS AND TECHNIQUE

Experimental Animals.—Though rabbits have been used most frequently in the work to be reported here, rats, mice, guinea pigs, a dog, and a sow have also been used. In addition, through cooperation with Dr. E. Allen, Dean of the School of Medicine, four monkeys were given galactin injections.

As rabbits may ovulate spontaneously when kept in contact with other rabbits, they have all been kept isolated. Accurate records of induced pseudo-pregnancies and of extract administrations have been kept so that the approximate condition of the animals as related to the development of their mammary glands was known at all times. Observation of either the intact or removed glands, however, has been relied upon for verification of the condition of the mammary glands.

Operative Technique.—The method of removing the mammary glands or sections of mammary gland has been described previously (Turner and Gardner 1931). When small sections of gland were desired, the operation was performed under local anesthesia. A cranial-caudal incision was made just medial to the teat. The skin was cut loose from the underlying gland. With reasonable care in the preservation of the blood vessels to the skin and in leaving as much as possible subcutaneous connective tissue, necrosis seldom occurred. The glands were then cut free from the underlying muscle and removed. Hemostats were sufficient to prevent bleeding unless the large mammary arteries and veins were cut. The skin was then sutured with silk.

When whole glands of mature rabbits were removed the operations were performed under ether anesthesia. The same procedure was used in removing the gland. The large blood vessels were ligated before being cut. The skin was then cut away for an area of 3/8 to 1/2 inch surrounding the incision and the wound sutured with silk. As the rabbits occasionally pull out the stitches, the wounds were protected by covering them with gauze and painting with collodion. With moderate care in operating rapid healing occurs.

Ovariectomies and laparotomies were performed by either ventral or lateral incision under ether anesthesia. As feed was kept before our animals at all times and the viscera thus always well filled, the lateral method was usually used. Incisions were made on both sides for the inspection or removal of both ovaries. After the removal of the ovary bleeding was stopped by either ligature or cautery. The muscle layers and skin were separately sutured. In rats ovariectomies and laparotomies were performed by mid-ventral incision.

Hysterectomies were performed by mid-ventral incision in both rats and rabbits. Bleeding was controlled by ligatures and cautery.

Histological Technique.—The mammary glands were pinned out flat on thin cork plates immediately upon removal and fixed in Bouin's fluid for 12 to 24 hours or more, depending on the thickness of the glands. The glands were then washed in running water and all muscles and as much connective tissue as possible removed under the dissecting microscope. The glands were then stained 12 to 24 hours in Mayer's hemalum, destained in acid alcohol, dehydrated in graded alcohols, cleared in xylol, and mounted in balsam.

Sections of gland for histological study were not trimmed following fixing, but washed, dehydrated, cleared and embedded in paraffin. Sections were cut from 6 to 10μ and stained in Delafield's hematoxylin and eosin, or Delafield's hematoxylin and Mallory's triple connective tissue stain.

Ovaries have likewise been fixed and sectioned when removed.

Method of Extract Injection.—All injections of the pituitary extracts except for the injection into rabbits for the determination of the gonad-stimulating content have been made subcutaneously. Antuitrin-S has been injected intravenously for the purpose of initiating ovulation. Theelin has in all cases, been injected subcutaneously. Unless otherwise stated, injections have been made once daily during the time indicated.

Methods of Extract Preparation and Source of Extracts.—Antuitrin-S, theelin and a sheep pituitary extract have been kindly and generously supplied by Dr. Kamm of Parke, Davis and Company. The greater part of the pituitary extracts used, however, have been prepared in our laboratory by extraction of fresh sheep or bovine pituitaries obtained directly from Armour and Company.

As various methods or modifications have been used in the preparation of galactin-containing extracts, a detailed discussion will be given in a following section (Part III).

Part I. Species Response to Galactin

REVIEW OF LITERATURE

The Normal and Experimental Growth of the Mammary Glands of the Rabbit.—In studying the experimental growth and activation of the mammary glands it is desirable to have the normal development of the glands well in mind. As the normal development of the mammary glands has been recently reviewed elsewhere (Turner and Frank 1930), but a brief summary will be presented here. Ancel and Bouin (1911) and Hammond and Marshall (1914) originally reported on the development of the glands during the later stages of their development.

A slow growth of the duct system of the glands occurs during the time between birth and puberty. During estrus, which persists for a considerable period of time in the rabbit, unless interrupted by pregnancy or pseudo-pregnancy, the duct system undergoes considerable hyperplasia, greatly extending the area of the gland. During pregnancy or pseudo-pregnancy the lobules and alveoli of the glands are developed. The greater part of this development takes place during the first 14 days. In pseudo-pregnant rabbits involution then sets in. Only a slight change is observed in the mammary glands during the latter half of pregnancy until the secretory phase becomes well established shortly before parturition.

Experimentally the growth of the duct system has been induced following injections of estrus-producing hormone obtained from the urine of pregnant cows (Turner and Frank 1930), and by the purified estrogenic preparations theelin and theelol (Turner et al. 1932). The complete growth of the mammary glands is not produced following extensive administration of theelin (theelin will be used to designate any estrogenic preparation).

Simultaneous injections of theelin and an extract of the corpus luteum produce the complete growth of the mammary glands similar to that occurring during pseudo-pregnancy or during the first half of pregnancy (Turner and Frank 1931 and 1932).

The ovarian hormones are thus capable of inducing complete growth of the mammary glands of the rabbit. As will be mentioned in a following section, however, several investigators have reported mammary hyperplasia following the injection of anterior pituitary extracts.

Briefly stated, the growth changes of the mammary glands, as determined from observations of normal animals as well as experimental observations, are stimulated by the ovarian hormones, theelin and corporin or progestin. The former hormone stimulates the development of the duct system of the mammary glands, the latter, however, is required in addition for the stimulation of complete mammary growth, namely, the development of the alveoli. In the writers' opinion, considerable inconsistency of experimental work reported by various investigators may possibly be due to a lack of a proper understanding of the significance of various developmental stages of the mammary gland.

The Effects of Pituitary Extracts on the Mammary Glands.—The initial demonstration of the existence of a gonad-stimulating hormone or hormones in the anterior pituitary by Zondek and Aschheim (1927) and Smith and Engle (1927) lead to the assumption of an indirect pituitary-mammary gland relationship. That is, the mammary glands might be affected by the ovaries, which are stimulated by the gonad-stimulating principles of the pituitary. Parkes (1929) observed complete mammary growth and a slight lactation in a rabbit injected with an anterior pituitary extract for the purpose of maintaining functional corpora lutea in the ovaries for a period equal to normal pregnancy.

As has been mentioned, Stricker and Grüter (1928 and 1929) first report the direct lactation-stimulating effect of anterior pituitary extracts. Female rabbits were ovariectomied on the tenth day of pseudo-pregnancy and lactation was initiated following the administration of the pituitary extracts. A similar though somewhat delayed response was obtained in rabbits whose mammary glands had undergone considerable involution. Lactation was not induced in immature female rabbits. Grüter (1928) mentioned that marked ovarian stimulation was observed in two normal 5- to 6-week-old rabbits following four to five daily injections of anterior pituitary extracts. During the time of injection no changes were observed to occur in the mammary glands. It was therefore concluded that the mammary glands must be at least partially developed under the influence of the corpora lutea before the lactation-stimulating effect of the pituitary could be observed.

Corner (1930) obtained lactation in mature ovariectomized virgin female rabbits following pituitary extract treatment. Two of the rabbits which he used had been isolated at 4 months of age and he concluded were undoubtedly virgins. Control glands removed at the beginning of the period of injection showed but slight development, while glands removed after an extended period of pituitary extract treatment showed a development comparable to the condition observed during late pregnancy. It was thus concluded that mammary hyperplasia as well as secretory activity was initiated by the pituitary extracts. These extracts were found to contain no appreciable amount of theelin or progestin.

Extracts similar to those used by Corner were used also by Asdell (1931). Spayed virgin female rabbits were brought into full mammary development. He also found that the extracts would not produce mammary development in the immature male or female rabbit. It was concluded that some sensitizing substance—presumably other than the corpus luteum—was required before the extract was active in stimulating mammary development and lactation.

Mature, non-lactating ovariectomized female rabbits were brought into full mammary activity by daily injection of pituitary extracts for periods up to ten days (Turner and Gardner 1931). Rabbits having glands considerably involuted responded to the extracts. Observations made on whole mounts of control and experimental glands lead to the opinion that growth of the gland was not produced following the injection of these extracts. Their activity was thought to be concerned only in the activation of the existing secretory epithelium.

Nelson and Pfiffner (1931) observed milk secretion in the rabbit following the administration of pituitary extracts when the injections were made following a previous treatment with the corpus luteum extracts. Normal female rabbits responded similarly. Examination of the ovaries of these treated animals showed large masses of lutein tissue. Young normal and castrate male rabbits whose glands had been developed by ovarian hormone treatments were activated so that milk could be expressed from the teats. Normal and castrated males whose glands had not been previously developed did not respond to the pituitary extract. They concluded that ovarian hormones were essential for the initial development of the mammary glands but that the profound growth of the gland during pregnancy was due to anterior lobe hormone or a substance physiologically similar.

The non-effect of the pituitary extracts on the glands of immature rabbits was reported by Turner, Gardner and Schultze (1932).

In view of the galactopoetic nature of the active material of the pituitary extracts the term "galactin" has been applied to it

(Turner and Gardner 1932). Galactin appeared to exist in the extracts independently of the growth and gonad-stimulating material.

Riddle, Bates and Dykshorn (1932 and 1932a) observed that a sheep pituitary extract capable of initiating crop-gland activity in doves and pigeons was capable of initiating lactation in the rabbit. These extracts were ineffective in producing mammary hyperplasia. Their lactation-stimulating material has been called prolactin.

Lactation has also been initiated in young virgin rabbits by Catchpole and Lyons (1933). They state that these virgin female rabbits possessed mature mammary glands but no milk. Observations made in our laboratory are not in agreement with theirs on this point as completely grown glands showing both duct and lobule proliferation have never been observed in virgin female rabbits. They likewise report obtaining lactation in male rabbits with pituitary extract following a "sensitization" of the mammary glands with corporin.

Though all of the above investigators agree that the pituitary extracts contain an active principle capable of initiating lactation in the rabbit, there is considerable variation of opinion concerning the developmental condition of glands at the time at which they are responsive. Lactation is always initiated following pseudo-pregnancy or in mature non-lactating multiparous rabbits, either in the absence or presence of the ovaries. Though the mammary glands of immature female or male rabbits have been found to be non-responsive, the mammary glands of mature virgin ovariectomized female rabbits or the mammary glands of male or female rabbits "sensitized" by corporin appear to be responsive. From observations made in our laboratory it appears to the writers, however, that in some cases, at least, the growth stages of the glands were inadequately known.

The Changes Induced in the Mammary Glands of the Rat and Mouse Following the Administration of the Pituitary Hormone.—Mammary hyperplasia was observed in adult virgin rats by Evans and Simpson (1929) after a month of daily subcutaneous injections of an alkaline extract of the pituitary rich in the growth-stimulating hormone and comparatively poor in gonad-stimulating hormone. More rapid mammary hyperplasia took place following the administration of gonad-stimulating hormone from bovine or rat pituitary implants or from placenta or pregnancy urine extracts. The mammary glands of immature animals responded rapidly to gonad-stimulating implants or extracts (Evans and Simpson 1929).

Evans and Simpson (1931) reported further observations on the rat. They state that growth of the mammary tree was caused by rat and bovine pituitary implants, aqueous acid extracts of placenta and urine of pregnancy precipitated by alcohol. The latter two products were practically free from folliculin and growth hormone. Males and spayed females failed to respond to the injection of the alkaline pituitary extract and pituitary implants. Normal virgin females made pseudo-pregnant and castrated from 18 hours to 3½ days later did not respond to the alkaline extract.

Turner and Schultze (1931) likewise observed the absence of any effect of pituitary implants or extract on the mammary glands of male and of castrate female rats. The mammary glands of normal immature females developed rapidly when pituitary implants were administered. Lactation resulted when the implants were continued over a longer period or when simultaneous injections of corporin, theelin and galactin were given (Schultze and Turner 1933).

Implants of pituitaries were followed by no changes in the mammary glands of castrate animals though normal animals responded rapidly (Nelson and Pfiffner 1931). Castrate rats given simultaneous treatment with corpus luteum and pituitary extract developed fully mature and functional glands. They concluded that "in the rat some ovarian factor must act synergistically with the anterior lobe hormone in order to induce extensive mammary gland growth."

Selye, Collip and Thomson (1933) found that complete mammary growth could be produced by the treatment of normal rats with an extract of pregnancy urine. Lactation, however, resulted only following the removal of the ovaries. In a second paper the same writers report that hypophysectomy would not prevent the onset of lactation in the rat but would prevent the continuation of lactation directly following parturition or during lactation.

The development of the mammary glands of mice was observed following the injection of gonad-stimulating hormones (Bradbury 1932). The injection of the gonad-stimulating substance of pregnancy urine was not followed by as rapid a development of the glands as was observed following pituitary administration though the ovaries contained abundant lutein tissue. Pituitary extracts were observed to induce the formation of alveoli and lactation following ovariectomy providing the glands were sufficiently developed at the time of the operation. In the mouse hysterectomy as early as the 11th day of pregnancy was followed by lactation.

The Effect of Pituitary Extracts on The Mammary Glands of the Guinea Pig and Other Species.—The mammary glands of mature ovariectomized virgin guinea pigs were observed to undergo secretory activation following pituitary extract injections (Nelson and Pfiffner 1930). The same writers later (Nelson and Pfiffner 1931) reported obtaining lactation in male and female, adult and immature, normal and gonadectomized guinea pigs following the injection of pituitary extracts. In all cases where lactation was obtained, however, the guinea pigs had been previously treated for 10 to 15 days with a luteal extract. Riddle, Bates and Dykshorn (1932) likewise observed lactation in the guinea pig following the treatment with a pituitary extract.

The effect of the lactation-stimulating extracts has also been studied in other animals—sow, dog and goat. In these instances, however, but few animals have been used. In the sow no work has been reported on the initiation of lactation. Grüter and Stricker (1929), however, observed that their pituitary extracts caused an increased secretion of milk in a sow producing an insufficient quantity for the proper nourishment of her litter.

The same writers re-initiated lactation in a dog 10 days after the last milk secretion was observed. One injection resulted in the reappearance of secretory activity. After two more injections lactation was so stimulated that two adopted puppies were nourished for several days. Putman, Benedict and Teel (1929) observed the growth of the mammary glands and a slight secretion of milk in a dog following a long continued injection of a pituitary extract.

In the goat lactation has been initiated in one case (Asdell 1931). A young goat kid was brought into lactation following the injection of an alkaline pituitary extract. The same extract, however, was ineffective in improving the milk yield of a goat whose milk yield was declining. In a further report evidence was obtained indicating that extracts of sheep pituitary tend to prevent for a time at least, the normal decline in the milk yield of goats (Asdell 1932).

Grüter and Stricker (1929) have also injected cows with their lactation-stimulating extracts of the anterior pituitary. In cows which were giving small amounts of milk (0.5 to 1 liter per milking) the milk flow was increased. Stocklausner and Daum (1932) presented evidence from which they concluded that injections of an anterior pituitary extract "vantasan" were followed by increased persistency of milk production.

The Effect of Galactin on the Initiation and Stimulation of Lactation.—*In the Rabbit.*—The observations previously made on the lactation-stimulating effect of galactin have been frequently verified. Over 70 rabbits have responded positively to various extracts prepared in our laboratory. Over 150 rabbits have been injected with various fractions of the pituitary extracts. Some of these extracts, however, have been found to contain no galactin or to contain galactin in sub-minimal amounts. In other instances no response has been obtained due to the unfavorable developmental condition of the mammary glands at the time the injections were made. The variation in response to extracts prepared in various ways and in young and mature rabbits will be reported in sections to follow.

The opinion has been stated previously that galactin acts only on the existing secretory epithelium. This opinion has been substantiated by further observations. As will be reported in more detail in the following section, galactin has been observed to produce no change in the mammary glands of immature female or male rabbits. Likewise, galactin is ineffective in activating the mammary glands of mature females whose glands have undergone extensive involution following ovariectomy.

Some interesting observations have been made on the effect of galactin following theelin injections or theelin and corporin injections. Two male rabbits and two immaturely castrated female rabbits whose glands had been grown under the influence of theelin and corporin have been brought into lactation following the injection of galactin. In one of these rabbits—a castrate male—the mammary glands were not completely developed, that is, there was but a partial development of alveoli. Upon examination of the lactating gland following galactin injections it was found that the ducts were swollen with milk, and that those alveoli present, if there were such, were likewise greatly distended with secretion. A similar observation has been previously reported (Turner and Gardner 1931) though in this case a mature female rabbit whose glands had undergone considerable involution was used.

In a more recent experiment an immaturely ovariectomized rabbit whose mammary glands had greatly involuted since the initial growth had been stimulated under the influence of theelin and corporin was brought into a definite lactation when galactin was injected following theelin treatment. Before theelin was injected the glands were greatly involuted. They consisted of but a "naked" duct system (Fig. 1). Sixteen and two-thirds rat units

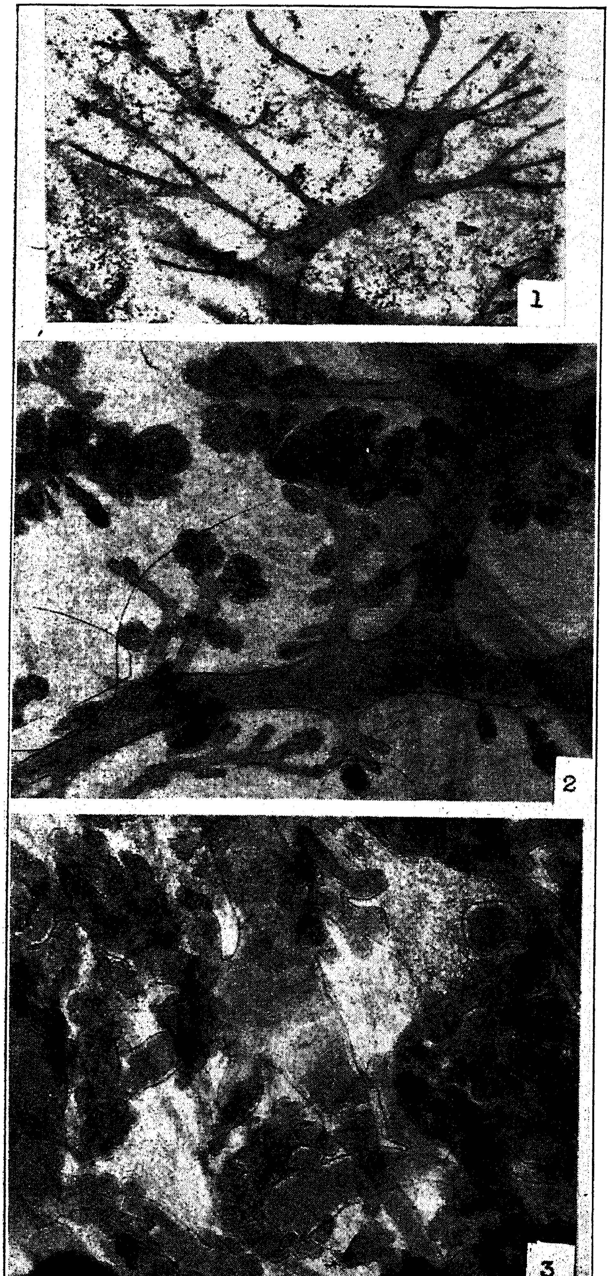

Plate I

Fig. 1.—A section of mammary gland removed from an immaturely ovariectomized rabbit approximately 10 months after mammary development had been previously induced by theelin and corporin. Only the naked duct system remained. Enlarged approximately 12 times.

Fig. 2.—A section of a mammary gland of the above rabbit after 1050 rat units of water soluble theelin had been administered during a period of 29 days. The small lateral ducts have again appeared. A small amount of a watery secretion was present in the larger ducts. Enlarged approximately 12 times.

Fig. 3.—A section of a mammary gland of the above rabbit following a nine day period of injection with galactin. The ducts are greatly distended with milk or a milk-like fluid. Milk could readily be pressed from the teats at this time. The secretion can be seen shrunken away from the enlarged ducts and "end buds." Enlarged approximately 12 times.

of theelin were injected daily for 12 days. This was followed by a 17-day period during which injections of 25 rat units of theelin were made twice daily. Following this extended theelin treatment the duct system was well developed. A large number of small branches had grown out from the "naked" ducts (Fig. 2). Galactin was then injected for nine days. The gland removed following this treatment was filled throughout with milk (Fig. 3). The type of gland, however, was similar to that of the previously removed gland.

This observation is particularly interesting since it has been frequently observed that lactation is not induced by galactin alone in rabbits whose mammary glands are greatly involuted. It likewise indicates that secretory activity of the mammary glands is not necessarily confined to the alveoli alone but that some or all of the ducts may take an active part. Though the gland was considerably hypertrophied and milk could be obtained readily from the nipple, the gland was not normally or completely developed.

Lactation has also been induced in five cases in hysterectomized rabbits following galactin injections. Galactin is thus effective in initiating lactation in the absence of either the ovaries or uterus.

In several cases galactin and theelin have been injected simultaneously. The response is similar to that observed when galactin alone is injected. Thus, the results indicate that there is no direct antagonism between theelin and galactin. These observations will be discussed in more detail in a following section.

In Rats and Mice.—A number of experiments have been conducted using animals of different ages for the purpose of determining the effect of the pituitary extracts used to initiate lactation in the rabbit. The purpose of these experiments was primarily twofold; first, to determine the response of the rat to the lactation-stimulating hormone; and second, to determine the gonad-stimulating hormone content of the extracts. As will be mentioned later, these preliminary investigations were likewise for the purpose of obtaining a smaller animal for use in the assay of the various extracts.

If the extracts contained sufficient gonad-stimulating hormone to produce ovarian changes the development of the mammary glands might be assumed to progress under ovarian control. In this case no change would be expected in the mammary glands of male or ovariectomized animals.

Lactation has not been initiated in the rat or mouse following the injection of the pituitary extract alone. It thus seems evident that the mammary glands of the rat and mouse do not respond in the same manner as do those of the rabbit. As it had been concluded from experiments with rabbits that our pituitary extracts acted only on the existing secretory epithelium, mature female animals were used exclusively for the purpose of testing the extracts for the presence of the lactation-stimulating hormone.

In the mouse it was found that the mammary glands involute very rapidly following weaning. At the tenth day there was no indication of secretion and the lobules had undergone marked involution. Six mice given daily injections of the pituitary extract starting 10 to 14 days after weaning and continuing for a period of 10 days were not brought into lactation. Though 0.5 cc. of the extract were injected daily, the ovaries and uterus presented no consistent changes and no evidence of increased gonad activity was observed.

The results obtained in experiments conducted with the rat have been similar to those obtained with the mouse. In one experiment two mature females were used. One received during seven days a total of three sheep pituitaries as implants. Another received 0.5 cc. of an anterior pituitary extract daily for a period of seven days. Lactation was not observed in either animal. In another experiment two multiparous female rats were injected daily with 0.6 cc. of pituitary extract for a period of 22 days. If the extracts contained appreciable quantities of gonad-stimulating hormone the response might be assumed to be similar to that observed following pituitary implants; that is, development and activity of the mammary glands as observed by Evans and Simpson (1931) and Schultze and Turner (1933). Control glands were removed at the time the injections started, and a second gland was removed at the close of the experiment. In no case was there any evidence of lactation. The final glands removed were involuted and resembled the glands of non-pregnant untreated females. These two rats will again be mentioned in connection with the assay of extracts for gonad-stimulating hormones.

Several observations have been made which agree somewhat with those reported by Evans and Simpson. In one instance two rats 29 days old and one rat 31 days old were injected daily with 0.2 to 0.3 cc. of pituitary extract B12 for a period of 11 to 13 days. Two littermate rats served as controls. Glands removed at the end of the experiment were larger and denser than the mammary glands of the controls. The glands, however, as yet consisted of only a complex system of ducts. This development of the glands, however, appeared to be due to ovarian influences. The ovaries of the three treated rats were slightly larger than the controls. Upon sectioning, however, no corpora lutea were observed in any of the ovaries and the increased size appeared to be due to the increased number of larger follicles.

In another instance four 60-day-old female rats were given 0.5 cc. of the pituitary extract daily for periods of from 11 to 28 days. The vaginas of two of the rats were open at that time and the other two opened the following day. Control glands were removed at the time injections started and a second series of glands were removed at the time they ceased. The glands showed some increase in size but this was confined to a proliferation of the duct system as occurs normally during a series of estrus cycles. In no case was there any evidence of lactation.

It appears evident that lactation is not initiated in the rat or mouse as readily as in the rabbit. The positive results of Nelson and Pfiffner (1931) have not been repeated in our laboratory. Due to the fact that the glands of these two species undergo such rapid involution following the removal or cessation of the stimulus to growth or continued secretory activity, it is probable that the pituitary injections have not been made when the glands were at optimum condition for the response. It may be, however, that, as Nelson and Pfiffner state, the simultaneous injection of the ovarian hormone is necessary.

In endeavoring to obtain rats with fully developed and actively proliferating mammary glands, pregnant females have been hysterectomized or ovariectomized on the 12th and 14th days of pregnancy. In both cases the glands were well developed though the alveoli were small at the time of the operation. Sections of gland removed two days later showed that lactation had been initiated. The lobules were enlarged and alveoli distended. On the fourth day following the operation the glands had markedly regressed. Since lactation appears to result normally in the rat following hysterectomy or ovariectomy at mid-pregnancy or shortly after, at which time the glands are completely developed, the effect of galactin administrations at this time would at best be questionable. Therefore, this method of preparing the mammary glands for response to galactin was dropped.

In conclusion it may be said that the rat and the mouse do not respond in the same manner as the rabbit. This may be due to the rapid and extensive involutionary changes occurring in the rat. On the other hand, it might be that ovarian hormones are necessary as conditioning or supplementary stimuli for the initiation of lactation. The evidence presented here is not sufficient, however, to warrant such a conclusion.

In the Guinea Pig.—Four non-lactating multiparous female guinea pigs have been given galactin. In one case the animal died on the fifth day of treatment. Milk could be pressed from the teat and upon examination the glands were found to be filled with milk. The injections were continued for 11 days in two other guinea pigs. In one case there was no evidence of lactation. In the other milk could be removed from the nipple for several days before the injections ceased. Upon examination of the glands, however, they were found to be relatively poorly developed. The lobules and alveoli were atrophic and the secretion was confined entirely, or nearly so, to the ducts. The mammary glands of the fourth guinea pig were apparently unchanged following the administration of theelin and galactin simultaneously for nine days.

Though the guinea pig has been found to respond to galactin the results obtained have been very inconsistent on the small number of animals used. Others in our laboratory are continuing the study of the effect of galactin in the guinea pig.

In the Sow.—Lactation has been experimentally initiated in one sow by the use of galactin. The injections of the extract were started 15 days after parturition. The young had been removed at birth. The day following parturition a mammary gland was removed. The glands were well developed and filled with milk (Fig. 4). After the glands had involuted for 15 days a second section of gland was removed. The glands at this time were not secreting and were quite involuted (Fig. 5). No milk could be pressed from the teats. After three days of treatment a few drops of milk could be obtained. The amount of milk that could be removed increased up to the sixth day. The gland was filled with milk and in an actively secreting condition (Fig. 6).

In the Dog.—In our laboratory the dog has likewise been found to respond to galactin. In the dog, mammary development takes place following estrus that is quite comparable to that during pregnancy, in other words, the dog exhibits a "complete" pseudo-pregnancy. At the end of the pseudo-pregnancy lactation has been reported to occur. The administration of galactin every other day for a period of 10 days was found to stimulate lactation in a dog whose glands had started to undergo regression following such a "complete" pseudo-pregnant development. A few drops of viscous, milk-like fluid could be obtained from the teats at the time of the second injection. The amount of milk that could be removed increased with the number of injections. Histological study of the sections of gland removed before and after the galactin injections prove that increased secretory activity had been stimulated (Fig. 10 and Fig. 11).

In the Monkey.—Galactin was administered to four mature ovariectomized monkeys for periods of from two to 29 days following various preliminary injections with theelin and corporin. The experiment was conducted in connection with work being undertaken by Dr. E. Allen, Dean of the School of Medicine. Secretory activity was observed in no case. The experiments, however, were not sufficiently extensive to warrant any conclusions as to the general ineffectiveness of the pituitary extract in the monkey.

The Effect of the Pituitary Extract in Initiating the Growth of the Mammary Glands.—In view of the conflicting data reported by the several investigators concerning the nature of the response to the lactation-stimulating hormone in immature animals or in mature animals whose glands were undeveloped, several experiments have been undertaken. In addition some evidence of the nature of the response to the lactation-stimulating extract has been obtained incidental to other experiments and will likewise be mentioned here.

Recently it was found that the growth of the mammary glands of immaturely ovariectomized and male rabbits may be experimentally induced by the use of ovarian hormones (Turner and Frank 1930 and 1932). The estrus-producing hormone (theelin) produces a development of the duct system of the glands. Theelin and an extract of the corpus luteum (corporin or progestin) produce the marked lobule proliferation which occurs normally during pregnancy or pseudo-pregnancy. The question now arose as to whether the pituitary extract could likewise stimulate the growth of the mammary glands. The injection of immaturely ovariectomized female rabbits and a male rabbit should offer a means of testing this question.

In the experiments reported here immature or young ovariectomized female rabbits and a normal male rabbit were injected for variable periods of time with galactin. The mammary glands of the young female rabbits were at variable stages of development at the time the experiments were started. In some cases the glands were very small, being confined to an area extending but slightly beyond the base of the teat. In most of the rabbits, however, the

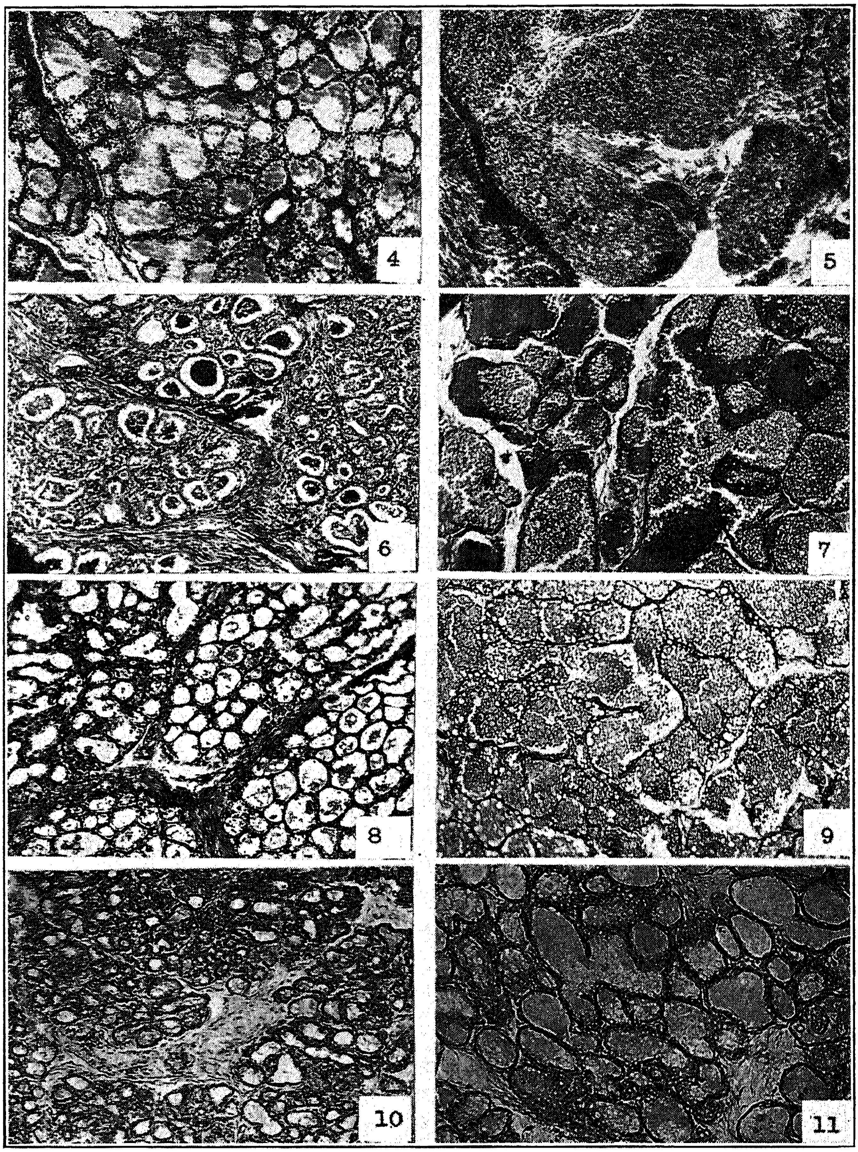

PLATE II.—For Explanation of figures, see opposite page.

Plate II.—See opposite page.

Fig. 4.—A microphotograph of a section of mammary gland removed from a sow on the day of parturition. The alveoli are large and distended with secretion. Sectioned at 8u. Stained in Delafield's hematoxylin and Mallory's triple stain.

Fig. 5.—A microphotograph of a section of mammary gland removed from the same sow after two weeks involution. The lumen of the alveoli had almost entirely disappeared. The gland parenchyma was greatly reduced. Secretion was absent.

Fig. 6.—A microphotograph of a mammary gland removed from the same sow after six daily injections of galactin. The alveoli were again partially distended with milk. The gland parenchyma was greatly increased.

Fig. 7.—A microphotograph of a section of the mammary gland of a rabbit (R240) showing the extent of lactation induced following galactin administration. The ducts and alveoli are greatly distended with milk.

Fig. 8.—A microphotograph of a section of mammary gland removed from R168. This gland was fully grown but there was no macroscopic evidence of lactation. Some secretion, however, can be seen in the alveoli. This gland served as a control to the gland in Fig. 9.

Fig. 9.—A microphotograph of a section of mammary gland removed from R168 after the rabbit had been given six daily injections with galactin. The alveoli were greatly distended with milk.

Fig. 10.—A microphotograph of a histological preparation from a mammary gland removed from a dog prior to the injection of galactin.

Fig. 11.—A microphotograph of a histological section of mammary gland from the same dog following five galactin injections during 10 days.

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gland area was from 1 to 3 centimeters in diameter (Figs 14, 15, and 16). In one instance it was much more extensive (Fig. 12). Though the extent of the mammary development was variable, the type of development was uniform. In all of the rabbits, whether the glands were small or relatively large, the glands consisted of only a branching system of ducts. Though the number of small lateral branches was far greater in the larger glands, there was no indication of alveoli in any case.

The type of development observed in these young rabbits was comparable to that observed following theelin injections. It might thus be assumed that the ovaries of these rabbits had been functional in the production of theelin for variable periods of time prior to the selection of the rabbits for the experiment and before ovariectomy. This was further substantiated by observations made on the ovaries at the time of their removal. Relatively large follicles were observed in all the ovaries. There was no macroscopic evidence for the existence of corpora lutea in any case. This condition of the ovaries as well as the existing developmental condition of the mammary glands indicated conclusively that all of the rabbits were virgins.

The treatment of these rabbits with tested galactin extracts should offer proof of the presence or absence of a mammary gland growth-stimulating effect as well as a test for the lactation-stimulating effect in immature rabbits.

All extracts used were simultaneously tested for their lactation-stimulating hormone content by injecting them into non-lactating multiparous female rabbits and were found to be effective in the amounts used. Extracts prepared in our own laboratory as well as those prepared by Parke, Davis and Company were used. Subcutaneous injections were made once daily. In two instances theelin was injected following the galactin injection. The growth of the mammary glands stimulated following theelin injections served to increase the obviousness of their lack of response following the galactin injections.

Nine rabbits were used. In most cases entire glands were removed before the experiment started. Other mammary glands were removed usually at intervals during the period of treatment or following it. These removed glands were all studied as whole mounts. It is possible that there was some individual variation in the extent of the area covered by the different glands. It was observed that the inguinal glands were usually smaller than those in the thoracic region. Therefore, all of the glands removed in these experiments were removed from the two thoracic pairs.

The protocols of the experiments are as follows:

R84. A young female rabbit. Control glands and ovaries removed 3-21. Ovaries were small, contained no corpora lutea and few large follicles. Glands were immature; only duct development was present. Two cc. of pituitary extract were given daily from 3-21 to 4-1. Gland section removed 4-1. No change was observed in condition of gland. One cc. of theelin was injected daily from 5-23 to 6-3 and from 6-7 to 6-27. Sections of glands were removed 6-3, 17 and 27. Glands had undergone extensive duct growth and some lobule proliferation.

R86. A young female rabbit. Control gland and ovaries removed 3-21. No indication of corpora lutea in ovaries. Glands were more extensive than those of R84 but had only developed ducts. Two cc. of a bovine pituitary extract were injected daily from 5-23 to 6-2. Sections of gland were removed 5-23 and 6-2. The glands had involuted following ovariectomy and were unchanged by the injections. One cc. of theelin was given daily from 6-17 to 27. A gland was removed 6-27. Mammary growth was restored.

R178. Young female rabbit. Born 1-23 (approximately 5 months old at the beginning of the experiment). Ovariectomized 6-17. Ovaries were infantile. Control gland removed 6-17. The glands were in an infantile condition. Two cc. of Parke, Davis and Co. Pituitary Extract No. 095109A were given daily from 6-18 to 6-28 and from 7-10 to 7-20. Glands were inspected and removed 6-28 and 7-20. Mammary glands had appeared to have slowly and continually developed, but the growth was confined entirely to a slight extension of the duct system.

R180. Littermate to R178. Treatment and response identical to above.

R292. A young female rabbit. Ovariectomized 3-19. Glands could be seen by slight elevation of the skin around the teat. Two cc. of pituitary extract B27 (1) were injected daily for eight days beginning 4-1. Entire gland removed 4-9. Glands had not changed. Two cc. of Parke, Davis and Co. Extract No. 095270A were injected daily from 4-11 to 4-19. Mammary glands were examined 4-19. Glands were still small and teats were undeveloped.

R294. A young female rabbit. Ovariectomized 3-20. Mammary glands were inspected at this time and one whole gland removed. The gland was approximately 6 cm. x 3 cm. in area, and consisted entirely of developing ducts. Ovaries contained large follicles but no corpora lutea. Further treatment was identical to R292 except that 3 cc. of the extract were injected daily. No mammary proliferation was observed.

R148. A normal young male rabbit. No control gland was removed but the teat was small and glands appeared undeveloped. Two cc. of B1 were injected daily from 10-4 to 10-11 at which time the rabbit died. Mammary glands were examined and found to be undeveloped.

R140. A young female rabbit ovariectomized 10-1. Teat was small and glands appeared undeveloped. Two cc. of B1 were injected daily from 10-4 to 10-17. A gland was removed 10-17. Gland was not over 1 cm. in diameter and consisted of only a duct system.

R156. A young female rabbit ovariectomized 10-3. The teat was small and glands appeared but slightly developed. Two cc. of B1 and 400 rat units of estrus-producing hormone were injected daily from 10-4 to 10-17. Mammary glands were inspected and a section of mammary gland removed 10-17. The glands were found to be as well developed as a gland after a corresponding period of theelin injection.

Galactin was administered to five young female rabbits starting within three days following ovariectomy and to one on the 11th day, one on the 12th day and one on the 63rd day following ovariectomy. One rabbit (R156) was injected simultaneously with galactin and theelin. In no case was there any evidence of the stimulation of secretory activity. In but two of the rabbits which received galactin alone (R178 and R180) was there any evidence of mammary gland growth (compare Figs. 14 and 15). Both of these rabbits were injected for two ten-day periods with a pituitary extract No. 095109A supplied by Parke, Davis and Company. Treatment was started the day of ovariectomy. In both of these rabbits the glands removed at the end of the experiment were approximately twice as large as the control glands previously removed. The type of development, however, had not changed. The final glands removed resembled those observed after a brief period of stimulation with theelin.

The mammary glands of the young male rabbit likewise gave no indication of response to galactin. Not only did galactin fail to induce growth in six of the females studied but in some cases there was evidence that regression of the glands had taken place

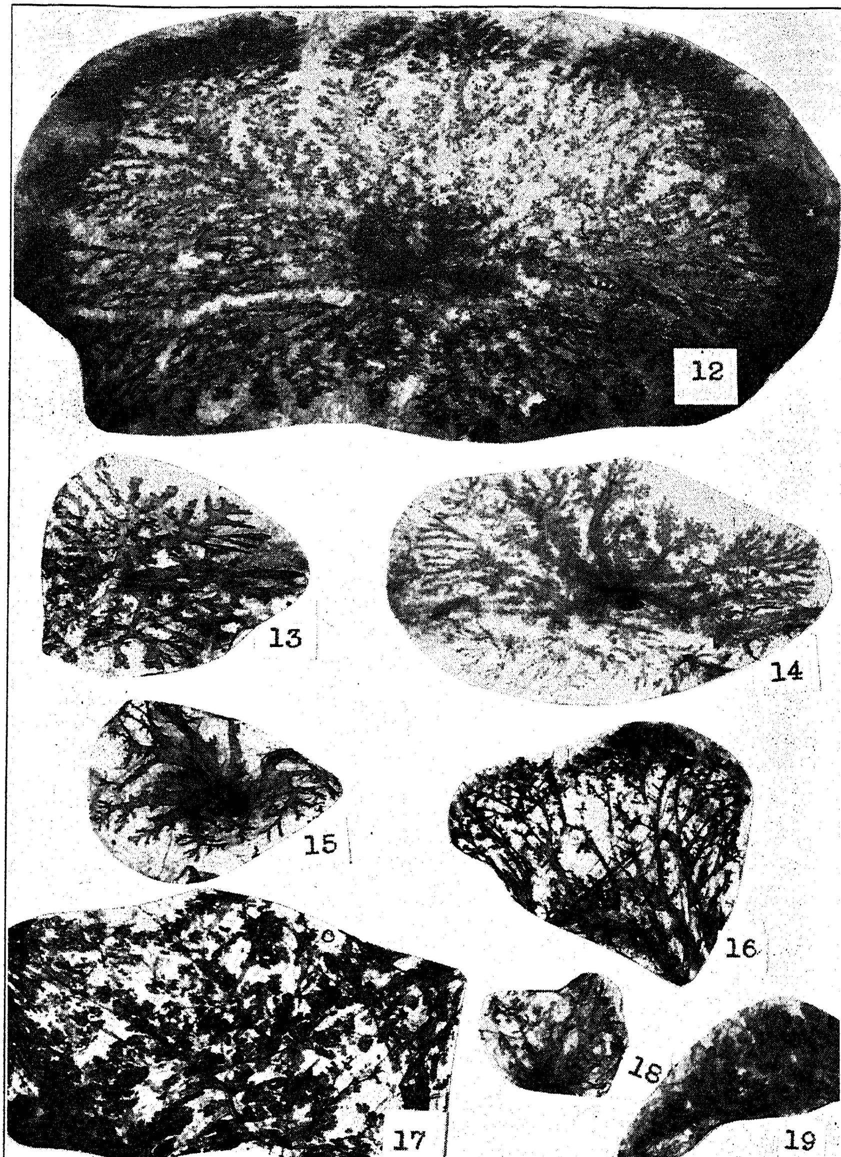

PLATE III. For explanation see opposite page.

Plate III.—See opposite page

Fig. 12.—A control mammary gland removed from R294 prior to the injection of galactin (two extracts were used—one was prepared in our own laboratory and one supplied by Parke, Davis and Co.). The glands possessed a well developed duct system. The distal ends, however, were still undergoing proliferation. The remaining glands were not stimulated to growth or secretory activity. Enlarged 2 times.

Fig. 13.—A gland removed from R292 following a period of treatment with galactin and prior to treatment with a second lactation-stimulating extract. This gland was unaffected by the pituitary extracts. Enlarged 2 times.

Fig. 14.—Mammary gland removed from R178 on 7-20-32. The gland was removed from an ovariectomized immature female rabbit after two ten-day periods of daily injection with Parke, Davis and Co. Pituitary Extract No. 095109A. Compare with control gland, Fig. 15. Enlarged 1⅔ times.

Fig. 15. Control gland removed from R178 on 6-17-32. Enlarged 1⅔ times.

Fig. 16.—Control gland of R84. The glands of this individual were extensively developed. This gland exemplifies the type of development experimentally produced by estrogenic hormones. Enlarged 1⅔ times.

Fig. 17.—A section of the mammary gland of R84 after 30 days of estrus-producing hormone injection. This gland compares closely with Fig. 28 in general structure.

Fig. 18.—An entire mammary gland removed from R140 after 14 daily injections of an extract of sheep pituitaries. The gland was very small and appeared to have been unaffected by the pituitary extract. Enlarged 1⅔ times.

Fig. 19.—A section of a mammary gland of R156 removed after the daily injection of 400 rat units of estrus-producing hormone and pituitary extract B1. The gland appears to be in an actively growing state. Enlarged 1⅔ times.

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during the period of treatment. The regression of the mammary glands was observed at the end of a ten-day period of injection which began on the day of ovariectomy in one rabbit (R84). The mammary glands of two rabbits which had received both extracts prepared in our laboratory and Parke, Davis and Company Extract No. 095270A had likewise undergone involution. The enlarged ends of the proliferating ducts which could be readily distinguished through the skin at the time of ovariectomy had greatly regressed. The small mammary gland of R140 (Fig. 18) also had assumed the appearance of an inactive gland. The mammary glands of the rabbit (R86) which had involuted for a period of two months following ovariectomy were unchanged following galactin treatment. The mammary glands of the rabbit which had been injected with both theelin and galactin at the same time had undergone a development comparable to that observed following theelin treatment alone (Fig. 19).

With the two exceptions mentioned above, there was no indication that the lactation-stimulating extracts will stimulate the growth of the mammary glands. In addition there was no indication that these extracts were capable of initiating lactation in any of the immature glands studied. In no case was the degree of mammary hyperplasia reported by other investigators obtained (Corner 1930, Asdell 1931 and Catchpole and Lyons 1933). The apparent growth obtained in the two exceptional cases was very slight, considerably less than that induced after an equal period of theelin injection. Even in these instances the mammary glands did not appear to be in a state of active growth.

Theelin, on the other hand, was found capable both of restoring the growing condition in glands following a two-month period of castrate involution and of initiating further development of the mammary glands (Fig. 17). The effect of theelin thus contrasts strikingly with that of galactin.

Five normal immature female rabbits were injected with different lactation-stimulating extracts. As these extracts contained some gonad-stimulating hormone as well as galactin a precocious sexual development was induced which in turn stimulated a precocious development of the mammary glands. In none of these rabbits, however, did the mammary glands develop at a more rapid rate than that observed following the first ovulation and only when the growth of the glands was almost complete did evidence of secretory activity appear.

Extensive atrophy of the mammary glands of the rabbit occurs during anestrus and following ovariectomy. Involutionary changes may be so great after an extended period following castration that formerly completely developed mammary glands are reduced almost to the prepubertal type of development (Fig. 23), though the area covered by the duct system may remain very nearly normal. Galactin has likewise been found incapable of stimulating renewed development of these glands. As previously mentioned, even those involuted mammary glands that have been brought into secretory activity after being "conditioned" by theelin have shown no indication of further growth following galactin administration.

In addition to the observations made following ovariectomy of immature rabbits those made on the involuted mammary glands likewise indicate the ineffectiveness of galactin in the stimulation of mammary gland growth or hyperplasia. Further, the rate of development of the mammary glands following precociously induced sexual maturity was not increased by the lactation-stimulating extracts which likewise contain gonad-stimulating hormone.

Part II. A Method of Assay of Galactin

For purposes of comparing various galactin-containing extracts, a series of test animals responding as uniformly as possible and to a minimum of extract are desirable. As preliminary experiments have shown that the rat and mouse do not respond readily to galactin these animals were not used in our tests though they would have been highly desirable from the standpoint of their size. The guinea pig was found to respond to galactin but the results were not consistent and as will be mentioned later, it is much more difficult to maintain the mammary glands of the guinea pig in a condition at which they will respond quickly to galactin.

Ovariectomized and normal female rabbits have been injected with various extracts of pituitary hormone. The response of the mammary glands was similar whether the ovaries were removed or present. Ovariectomized rabbits were used entirely in our earlier experiments (Turner and Gardner 1931). However, it was soon observed that the response of the mammary glands was not uniform in all cases. The lack of uniformity was observed both in normal and in ovariectomized rabbits even when equal amounts of the same extracts were used.

A quantitative measure of the intensity of the initiated secretory activity was thus desired. It was observed that in most cases evidence of secretory activity, as determined by pressing milk from the nipple, appeared on the second or third day following the administration of the extracts in the amounts injected. From the fourth to the sixth day the glands of animals responding strongly became greatly hypertrophied. The glands of other animals exhibited varying degrees of response and in some cases had apparently undergone no changes. The variations in response have been comparatively evaluated.

Five values have been assigned. One value, minus one (-), is used to designate the absence of response. The other four values are positive and for brevity are designated +, ++, +++, and ++++. As these values are determined entirely by observation of the glands after a given period of injection, it is obvious that it would be impossible to definitely distinguish them; that is, they grade into one another. The following description of the glands will give some idea as to the significance of the ratings. Plus one (+), all the ducts are filled with milk, lobules are flesh colored and not enlarged. Plus two (++), all ducts and most of the lobules are filled with milk though not greatly thickened (Fig. 20). Plus three (+++), entire gland is filled with milk. Plus four (++++), mammary glands are greatly extended with milk throughout (Fig. 21).

The following table will give some of the variations in the response observed in different animals to the same extracts. The degree of hypertrophy of the mammary glands has been rated according to the above standard.

TABLE 1.—THE RESPONSE OF THE MAMMARY GLANDS OF THE RABBIT AT VARIOUS DEVELOPMENTAL STAGES TO GALACTIN

Rabbit No.	Daily Dose of Extract Used	Condition of Control Gland	Time Ovariectomized	Days Injected	Condition of Final Glands
140	B12—2 cc.	15 days pseudo-pregnancy	1 day	4	++++
165	B12—2 cc.	Glands grown by theelin and corporin		10	++++
172	B12—2 cc.	Slightly involuted pseudo-pregnancy growth		4	+++
173	B12—2 cc.	Slightly involuted pseudo-pregnancy growth		7	++++
146	B12—3 cc.	Severely involuted gland	5½ mo.	6	—
106	B13—2 cc.	Glands grown with theelin and corporin		5	+++
126	B13—2 cc.	Glands grown with theelin and corporin		7	+++
176	B13—2 cc.	Involuted glands of mature female		5	—
145	B13—2 cc.	Glands greatly involuted	6 mo.	6	—
196	B14b—1 cc.	Markedly involuted pseudo-pregnant glands	3 days	8	++++
175	B14b—.5 cc.	Involuted mature gland		8	++
201	B17—2.5 cc.	Involuted mature		6	—
198	B17—1.5 cc.	Full pseudo-pregnancy growth		3	+++
215	B20c.p.—1 cc.	14 days involution since induced lactation		8	—
249	B20c.p.—1 cc.	13 days pseudo-pregnancy growth		8	++++
218	B20b—2 cc.	16 days pseudo-pregnancy growth		5	++++
274	B20—2 cc.	19 days pseudo-pregnancy		8	++++
185	A5G1—2 cc.	16 days pseudo-pregnancy		9	++++
263	A5G1—2 cc.	12 days pseudo-pregnancy		9	++++
207	B51—2 cc.	16 days pseudo-pregnancy		8	++++
212	B21—2 cc.	Slightly involuted pseudo-pregnancy		8	—
147	B21—2 cc.	Hysterectomized-involuted pseudo-pregnancy		8	+
210	B21 (2)—2 cc.	16 days pseudo-pregnancy		7	++++
213	B21 (2)—2 cc.	14 days since +++ induced lactation			—

In this series are included four ovariectomized animals. One of these had been ovariectomized one day before the injection of the extract was started and at the 15th day of pseudo-pregnancy. In this case the response was rated plus four (++++) on the fourth day of treatment. Another female had been immaturely ovariectomized and the mammary glands had been grown by the application of the ovarian hormones, theelin and corporin. Two of the rabbits had been ovariectomized 5½ to 6 months previous to the injection of the pituitary extract. Immediately following ovariectomy lactation had been induced by pituitary extract injection and from that time on the rabbits had been undisturbed. Marked involution of the mammary glands had occurred (Fig. 23) during this period. Only the duct system was retained. In these individuals the pituitary extracts were ineffective in initiatng lactation. Sections of gland removed at the end of the period of injection were identical with those sections removed before the injections were started.

Rabbits at various stages of pseudo-pregnancy have been used. Ancel and Bouin (1911) observed that during pseudo-pregnancy complete mammary growth occurs. This development reaches its maximum at about the 14th day after ovulation. The rabbits used in this experiment have been injected beginning on the 12th to the 17th day following ovulation, which has been induced either by sterile coitus or intravenous injection of gonad-stimulating extracts. In all cases a plus four (++++) response was obtained.

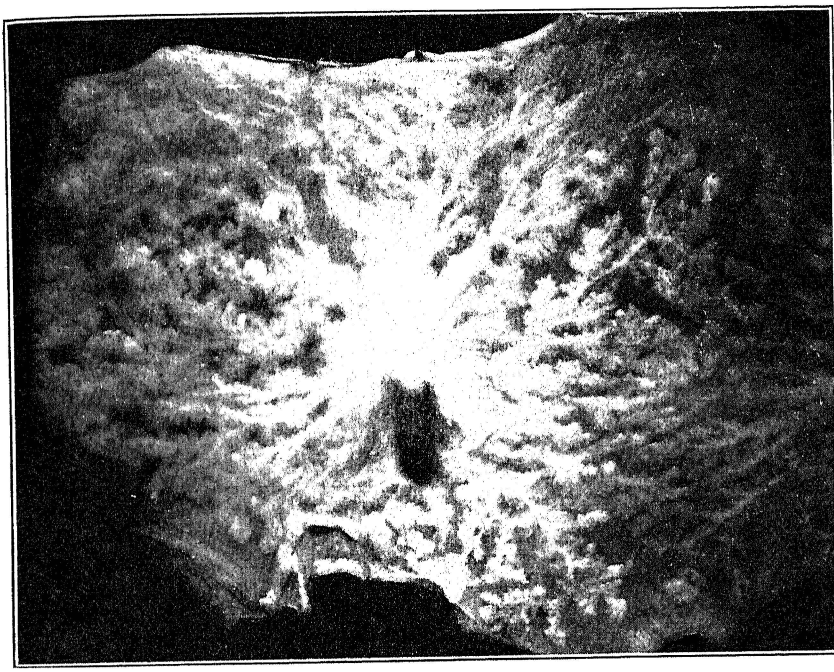

PLATE IV

Fig. 20.—A photograph of a mammary gland removed from a rabbit in which a plus two (++) response had been initiated by galactin injection. All of the ducts were filled with milk and most of the lobules were slightly distended.

Two rabbits were injected 14 days after lactation had been induced previously. Though extracts of known activity were used lactation did not result in either case. The other rabbits in which lactation was not induced possessed mammary glands involuted to various degrees (Figs. 24, 25 and 26).

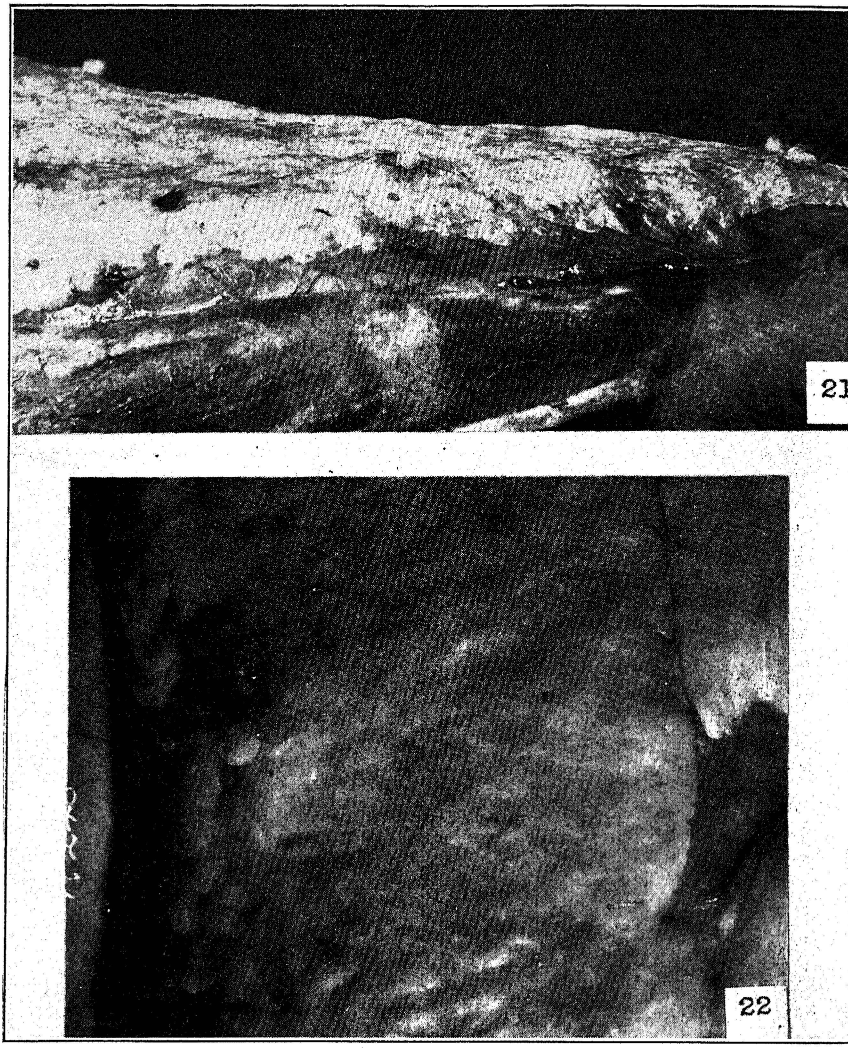

PLATE V

Fig. 21.—A side view of the intact mammary glands of R240 following a seven-day period of galactin administration (B 26 p 2—acid soluble portion of the alkaline pituitary extract.) The mammary glands are greatly thickened with milk throughout. This response is rated plus four (++++).

Fig. 22.—A surface view of the mammary glands of R240. The cross at the right marks the place where a section of gland had been removed to serve as a control. Compare with side view (Fig. 21).

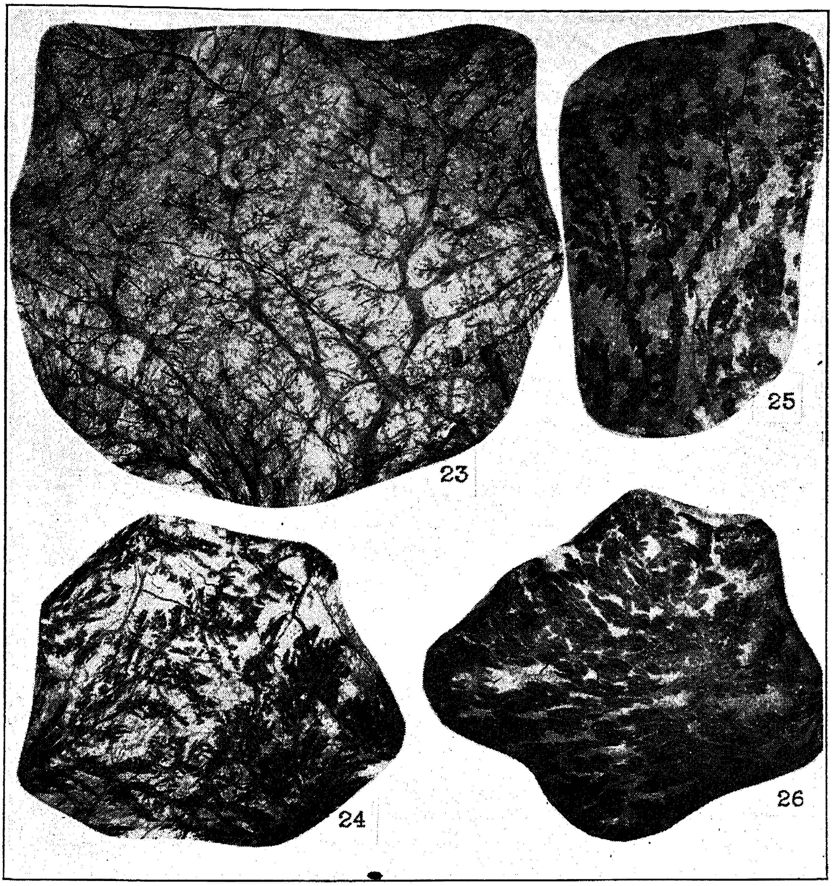

PLATE VI

Fig. 23.—A section of mammary gland of R145 removed approximately six months after ovariectomy. Involution had progressed so far that it is doubtful if any alvolar epithelial cells remained. Such glands without previous theelin "conditioning" were not responsive to galactin.

Fig. 24.—A section of mammary gland removed from R176. The feathery appearance of the lobules is characteristic of involution. This gland was not responsive to an extract of known potency. Enlarged 2 1/3 times.

Fig. 25.—A section of mammary gland removed from R201. This gland did not respond to a galactin extract of known potency. The gland has involuted to a considerable degree. Enlarged 2 1/3 times.

Fig. 26.—A section of a mammary gland of R212. Though this gland was but slightly involuted, it did not respond to galactin. Enlarged 2 1/3 times.

The animals listed above include but a part of those in which lactation has been induced. In most cases extracts have been injected into rabbits from the 12th to the 14th day of pseudo-pregnancy and only in a few cases have duplicates been used.

It appears evident from the above results that the fully developed mammary gland responds most readily to galactin. Involuted or involuting glands respond less readily or not at all, at least to the amount of galactin injected. In some cases, lactation has been initiated in glands showing considerable involution. As has been mentioned, glands possessing only a duct system have been activated by galactin following theelin treatment.

The mammary glands of male rabbits that have been developed by the ovarian hormones likewise undergo activation under the influence of galactin. However, this would be expected as conditions were assumed to be identical to those of castrate female rabbits possessing fully developed glands.

As pseudo-pregnant rabbits were found to be most responsive to the extracts they are being used at present for the assay of galactin. Pseudo-pregnancy is readily brought about in the rabbit. Ancel and Bouin (1911) first observed this condition following sterile coitus performed, for example, by mating a female to a vasectomized male. Bellerby (1929) and Friedman (1929) observed that ovulation in the rabbit was likewise produced by gonad-stimulating hormones. Relatively small amounts injected intravenously caused ovulation in young and mature female rabbits. We observed that the mammary hyperplasia resulting from pseudo-pregnancy initiated either by coitus or hormone injection was identical. Asdell and Salisbury (1933) have recently made similar observations.

Both methods of initiating pseudo-pregnancy have been used. During the fall and winter months very few of our female rabbits will accept the male, and gonad-stimulating hormone injections must be relied upon. Antuitrin-S, a gonad-stimulating hormone prepared from pregnancy urine, has largely been used. In a few cases pregnancy urine itself has been used. Laparotomies were at first performed after the injection of the hormone for the purpose of verifying ovulation. However, as an excess of gonad-stimulating hormone was usually injected (50 to 100 rat units), ovulation was found to have resulted in most of the rabbits in which fresh extracts were used. As Antuitrin-S tends to lose its potency at the rate of about 25 units per month, increased amounts of old extracts should be used (Kamm 1933).

The mammary glands of all rabbits used were examined previous to galactin treatment. In most cases where ovulation was observed the mammary gland showed the typical pseudo-pregnant development. Observations were usually made on from the 12th to the 14th days. The appearance of the mammary glands at this time offers as good a criterion for pseudo-pregnancy as observations on the ovary. The picture was particularly contrasting when rabbits with immature or involuted glands were used. The nature of the development of the glands may be determined by observations through the shaved skin in some rabbits; in others it is necessary to observe glands through an incision in the skin. In numerous cases sections of gland have been removed in a manner previously described. In this manner our observations have been verified almost without exception, by the stained and cleared whole mounts of the glands or by histological sections.

The galactin extracts were administered subcutaneously, once daily usually for a period of eight days. The injections were started on the day of the inspection of the glands. Another inspection of the glands was made on the ninth day following and the degree of response determined. In some cases where the response was very marked at an earlier date, the period of injection was shortened. From these observations it is clear that for consistent response to a comparatively small amount of galactin the mammary glands must be well developed and preferably completing development. It might well be assumed that some change produced in the glands, other than the growth of the glands, is performed by the functional corpora lutea of pseudo-pregnancy or by some other ovarian secretion. This point will be discussed later.

The degree of mammary development in the rabbit during pseudo-pregnancy is somewhat variable. This factor has been observed by various investigators. Apparently the degree to which secretory activity appears has been the most frequent criterion by which mammary development has been observed following pseudo-pregnancy. Smith and Smith (1932) state that lactation frequently followed pseudo-pregnancy. Though many observations have been made on pseudo-pregnant rabbits in our laboratory no pronounced lactation has ever been observed. To determine the extent of mammary development following pseudo-pregnancy, a separate series of rabbits has been used. Pseudo-pregnancy was induced in 20 rabbits. Three other animals in the series failed to ovulate following mating or Antuitrin-S injection. The results are recorded in Table 2.

TABLE 2.—THE DEVELOPMENT OF THE MAMMARY GLANDS DURING PSEUDO-PREGNANCY

Rabbit No.	Treatment	Days since pseudo-pregnancy started	Condition of Glands
282	Mated to vasectomized male	21	No milk in gland. Glands developed.
208	Mated to vasectomized male	21	No milk in gland. Glands developed.
264	Mated to vasectomized male	21	Small amount of milk at distal end of ducts.
184	Mated to vasectomized male	20	Glands undeveloped. No milk in ducts. Milk in a few scattered lobules.
212	Mated to vasectomized male	19	No milk in gland. Glands developed.
295	1 cc. Antuitrin-S	18	No milk in gland. Glands developed.
221	1 cc. Antuitrin-S	18	Glands undeveloped. Milky fluid in large ducts.
288	1 cc. Antuitrin-S	18	No milk in gland. Glands fully developed.
230	1 cc. Antuitrin-S	19	No milk in gland. Glands fully developed.
275	1 cc. Antuitrin-S	19	Watery fluid in ducts. Glands appeared undeveloped
286	1 cc. Antuitrin-S	18	Glands well developed. Small amount of milk
217	1 cc. Antuitrin-S	18	Glands well developed. Small amount of milk
270	1 cc. Antuitrin-S	14	Glands well developed. Small amount of milk
240	1 cc. Antuitrin-S	14	Glands well developed. Small amount of milk
271	1 cc. Antuitrin-S	14	Glands well developed. Small amount of milk
185	1 cc. Antuitrin-S	14	Glands well developed. No milk
252	1 cc. Antuitrin-S	20	Glands well developed. No milk
251	1 cc. Antuitrin-S	20	Glands well developed. Almost lactation +
184	1 cc. Antuitrin-S	17	Glands well developed. No milk
223	1 cc. Antuitrin-S	20	Glands well developed. Small amount of milk
274	1 cc. Antuitrin-S	20	Glands well developed. Small amount of milk
256	1 cc. Antuitrin-S	21	Glands well developed. Small amount of milk
235	1 cc. Antuitrin-S	21	Glands well developed. Small amount of milk

In nine cases there was no evidence of milk in the glands of the rabbits in which ovulation had occurred. Milk, in small though variable amounts, appeared in the glands of 11 of the rabbits. In most instances the secretion was found in the larger ducts near the base of the teat. Milk was observed in the ducts of none of the rabbits in which pseudo-pregnancy had not been produced. A watery fluid was present, however, in small amounts in the gland ducts of two of these rabbits. In the third rabbit a few scattered lobules were white and distended with milk. This latter condition has been observed several times in rabbits. Occasionally glands that are greatly involuted will have a few scattered individual lobules distended with secretion.

The above observations verify those previously made on a large number of pseudo-pregnant rabbits. If, however, the criterion of lactation were taken as the obtaining of a few drops of milk from the nipples, it is probable that lactation might be frequently observed following pseudo-pregnancy, as small quantities of milk may be removed when it is present in the ducts. Macroscopic examination of these glands, however, does not reveal lactation. Rabbits at various stages of pseudo-pregnancy, thus, may be satisfactorily used for the assay of galactin.

Though sufficient evidence is not yet available to definitely define galactin on the animal unit basis, some evidence has accumulated indicating that variations in the quantity of extract injected induce corresponding variations in response. Likewise, extracts potent when injected in a given amount, produce no effect when given in greatly reduced amounts. The response of the mammary glands to galactin may be compared to the response of the uterus to corporin or progestin in that varying amounts of the corpus luteum hormone produce varying degrees of progestational proliferation in the uterus (Corner and Allen 1929). Allen (1930) observed that the progestational proliferation could be produced by the injection of progestin following theelin. The mammary glands on the other hand are activated by galactin following ovarian stimulation which induces growth changes in the glands, and perhaps also other changes which will be discussed later.

The only lactation induced approximating that observed following parturition in the rabbit has been induced in fully developed glands. Therefore, animals with fully developed glands should be used for the assay of galactin. The rabbit is an animal remarkably adapted for this assay in that the growth of the mammary glands may be so readily induced by the stimulating effect of pseudo-pregnancy.

It is therefore proposed that galactin be expressed in terms of rabbit units. A rabbit unit of galactin is defined as that minimum amount of extract which injected during a period of seven days at daily intervals is required to induce a plus three (+++) or plus four (++++) response in rabbits previously pseudo-pregnant for a period of from 12 to 16 days.

Riddle, Bates and Dykshorn (1932 and 1933) have used pigeons and doves for the assay of their lactation-stimulating extract, prolactin. The response of the crop glands of these birds is initiated by the same substance found to stimulate lactation in the rabbit and guinea pig. In the pigeon and dove they have likewise apparently found a quantitative variation in the response obtained as they have graded their results on the extent of crop-gland proliferation as determined by the weight of the glands.

Part III. Preparation and Nature of Galactin

REVIEW OF LITERATURE

Relatively few methods have been used for the extraction of the lactation-stimulating hormone from pituitaries and in all cases the extracts have not been highly purified. Grüter and Stricker (1928-29) state that the lactation-stimulating extracts they used were prepared by saline extraction of bovine pituitaries. Evans and Simpson (1929 and 1931) used rat pituitary implants and alkaline extracts of bovine pituitaries and observed mammary hyperplasia and lactation in the rat though they attributed both reactions to the gonad-stimulating effect and observed no direct lactation-stimulating effect. Corner (1930), Asdell (1931), and Nelson and Pfiffner (1931) used an extract prepared from sheep pituitaries by Parke, Davis and Company. Turner and Gardner (1931) prepared a lactation-stimulating extract from sheep pituitaries using an alkaline method of extraction.

Recently Riddle, Bates and Dykshorn (1932) have briefly described a method used in the preparation of a galactopoetic hormone, prolactin. The active material was isoelectrically precipitated from the acid soluble material of dehydrated and defatted sheep pituitaries.

With the exception of Stricker and Grüter it is interesting to note that all investigators who have indicated the existence of a galactopoetic hormone of pituitary origin have used extracts of sheep pituitaries. Corner, Asdell, Turner and Gardner, and Riddle et al. have concluded from their observations that the lactation-stimulating hormone was distinct from the other pituitary hormones—growth hormone and gonad-stimulating hormones—though none of the evidence at the present time, with the possible exception of that presented by Riddle et al., is at all conclusive.

METHODS OF EXTRACTION

The extract first used in our laboratory was prepared by digesting the ground pituitaries in water suspension at a pH of approximately 9 for a period of from 24-36 hours at ice box temperatures. The material was then reduced almost to neutrality, centrifuged, and the clear reddish fluid preserved with dilute tricresol at ice box temperatures.

Various modifications of the original procedure of extraction have been tried and tests have been made to determine the stability and nature of the active material.

After working out a method of assaying galactin for the purpose of attaining as uniform a response as possible, various fractions of the extracts have been tested for their galactopoetic activity. Similarly, the stability of galactin to heat, age, and various reagents has been studied.

It was observed that a small amount of white precipitate settled out of the clear, light red material obtained by centrifuging the neutralized tissue. The addition of tricresol appeared to hasten the formation of the precipitate. These precipitate-containing extracts have been recentrifuged after the formation of the precipitate. The supernatant liquid and the precipitate have been tested for galactin. Both portions have given positive tests. The precipitate, however, appears to contain a larger amount of the active material.

In attempting to concentrate the clear liquid portion retained after the removal of the precipate mentioned above more acid has been added. Upon the addition of acid a flocculent white precipitate forms which goes into solution upon the further addition of acid. The addition of NaOH resulted in the reprecipitation of the material. This precipitated material has been repeatedly found to contain galactin. The response has always been negative when the clear yellowish solution left after the removal of the precipitate has been injected.

The above observations have led us to believe that the active material is soluble or is associated with a substance or group of substances which are soluble in both acid and alkaline solutions but which are precipitated through a range of hydrogen ion concentrations extending from somewhat below to very slightly above neutrality.

As a result of the above observations the original method for the precipitation of galactin has been modified. It had been observed that the addition of alkali to the original tissue did not apparently alter the activity of the extract produced (in the range of 8.4 to 9.6). Likewise, the addition of acid in excess of neutrality prior to the first centrifugation gave weak or inactive extracts. The active material was apparently largely contained in the precipitated material which was at first discarded. At the present time the alkaline extract is centrifuged before neutralization. A viscous, turbid fluid is separated from the ground tissue by the first centrifugation. Distilled water is added to the tissue and the mixture recentrifuged. The fluid obtained is added to the fluid portion obtained from the first extraction. Acid is slowly added to the mixture until it is nearly but not quite neutral. The mixture is again centrifuged. A considerable amount of a white precipitate is obtained. This precipitate is washed two to three times with small amounts of water and the washings added to the clear solution obtained from the first centrifugation.

The precipitate obtained here contains largely inactive material. Though the number of trials has not been sufficiently numerous to make a definite statement, the evidence at hand indicates that the precipitate is more active the closer the original neutralization is to neutrality and conversely, the material first precipitated upon the addition of acid is inactive. Complete removal of the precipitate, however, cannot be accomplished by centrifuging until the mixture is practically neutral.

The solution obtained after centrifuging the slightly alkaline mixture is further acidified until a definite acid reaction to litmus is reached. More white precipitate is obtained. This is removed by centrifuging. This precipitate is usually highly active. For injection it is suspended in approximately two volumes of water and injected subcutaneously. The clear, reddish solution is likewise highly active. This solution is comparable to that obtained by the previous method and has been previously mentioned. The addition of more acid results in the further precipitation of materials which when removed leave the solution inactive.

The materials precipitated following the addition of acid to the alkaline extracts are partially soluble in increased amounts of acid. The materials of the alkaline extracts soluble at a pH of from 2.5 to 3.0 and reprecipitated by the addition of alkali have been found to be active in initiating lactation when subcutaneously injected in water or saline suspension.

In the following outline the various steps in the preparation and concentration of galactin are briefly listed. The potencies of the various fractions that have been tested are also given. The hydrogen ion concentrations are but approximate as they have been determined by a series of indicator papers.

1. Grind or macerate pituitaries.

2. Add two volumes of distilled water.

3. Add 10 per cent NaOH with constant stirring until a pH of 8.4 to 9.6 is reached.

4. Allow the extract to stand at ice box temperatures for 24 to 36 hours.

5. Centrifuge—a semi-gelatinous mass of tissue and a viscous, turbid fluid are obtained.

6. Add small amounts of distilled water to the tissue and recentrifuge several times.

7. Add the fluid removed following (6) to the original fluid obtained.

8. Add 10 per cent HCl to the fluid portion to reduce pH to 7.4 to 7.6.

9. Centrifuge—a considerable volume of precipitated material is removed. Wash this precipitate with successive additions of distilled water and add washings to the fluid portions first obtained. (This precipitate is toxic when neutralized and injected. Up to the present time it has been found ineffective in stimulating lactation.)

10. Add acid to the fluid portion obtained from (9) until a pH of 7 is reached.

11. Centrifuge—wash the precipitate and add the washings to the clear fluid obtained. (Both the solution and the precipitate contain the active material).

12. Add more acid to reduce the pH to approximately 5.5. A flocculent white precipitate forms. (This precipitate contains a large amount of active material. The solution remaining is inactive). Tricresol is added at the rate of 0.4 per cent by volume to all suspensions and solutions kept for injection.

THE RELATION OF GALACTIN TO THE GONAD-STIMULATING HORMONES OR HORMONE

The question as to whether the lactation-stimulating hormone is distinct from the other hormones contained in the pituitary, that is, the growth and gonadotropic hormones, is of considerable interest. No definite steps have been taken to eliminate these hormones in our preparations. In some of our preparations they appear to be present in considerable amounts; in others they appear in very small amounts, or are absent. Rats, mice, and rabbits have been used to test the extracts for gonad-stimulating properties. The presence of gonad-stimulating hormones causes precocious sexual maturity when the preparations are given to infantile rats and mice in suitable amounts as first pointed out by Smith and Engle (1927) and Zondek and Aschheim (1927). Such a condition is determined by the enlargement of the ovaries and uterus and the opening of the vaginal orifice. Bellerby (1929) and Friedman (1929) observed that ovulation was induced in the mature female rabbit by injections of gonad-stimulating extracts. Intravenous injections of these extracts were particularly active. Typical pseudo-pregnancy follows ovulation thus induced (Hill and Parkes 1930, Friedman 1932, and Asdell and Salisbury 1933).

In view of the variations observed in the gonad-stimulating hormone content of the various pituitary extracts tested and also the various methods used in assaying the extracts for gonad-stimulating hormones, several experiments will be described. At the same time the lactation-stimulating hormone content of the extracts will be given.

Mature female rats have been injected with the lactation-stimulating extracts B12 and B13 (See Table 1). That these extracts contained galactin was demonstrated by the initiation of lactation in several mature female rabbits and ovariectomized female and male rabbits whose glands were grown under the influence of ovarian hormones. Daily smears taken during a period of 22 days failed to demonstrate any alteration of the estrus cycle of these female rats when 0.5 cc. of the pituitary extract were injected daily. The ovaries of three mature female rabbits receiving the extracts were inspected at the end of the period of injection. In one case in which a plus four (++++) lactation response was observed, the ovaries appeared entirely normal. In two other cases the ovaries showed numerous large blood follicles or hemorrhagic follicles.

The significance of the blood follicles is not definitely known. Hammond and Marshall (1925) observed them in varying numbers in normal animals and suggested that they were especially numerous during sustained sexual activity. Jares (1932) observed hemorrhagic follicles to occur as a result of the administration of anterior pituitary substance. Both their number and size were dependent upon the frequency and magnitude of the forced stimulations. Their occurrence in large numbers might thus be taken as indicating the presence of gonad-stimulating hormones in the extracts.

Four 60-day-old female rats were given daily injections of 0.5 cc. of the pituitary extract for periods varying from 11 to 28 days. Two of the rats were sexually mature at the time the experiment started as indicated by the open vaginal orifice. The vaginas of the other two opened the following day. Typical estrus smears were obtained three and four times respectively in two of the rats injected for 21 days. Four complete estrus cycles were observed in one rat injected for 28 days and one estrus period in the rat injected for 11 days.

One extract was also administered to five female rats 38 days of age at the beginning of the experiment. Injections were continued for 25 days. The vaginal orifice of one rat opened at 57 days of age. The vaginas of the others remained closed until the end of the experiment. The ovaries of but one rat were well developed. In the other four animals the infantile condition persisted. Mammary glands removed at the end of the experiment likewise indicated the absence of ovarian stimulation.

Rabbits have been given intravenous injections of some of the extracts and varying results have been obtained. Only those extracts free from precipitated material or from which the precipitated material had settled could, however, be tested in this manner. In Table 3 are grouped a number of the results obtained from different extracts.

TABLE 3.—THE GONAD-STIMULATING HORMONE CONTENT OF GALACTIN EXTRACTS
(Determined by the Effect of Intravenous Injections Upon the Induction of Ovulation in the Rabbit)

Rabbit No.	Galactin Extract No.	Amount Injected	Days Ovaries Examined After Injection	Result
262	B20a	cc. 2	3	Left ovary—6 corpora and 3 blood follicles. Glands undeveloped at 10th day.
261	B20b	2	3	Left ovary—no corpora. Mammary glands undeveloped at 10 days.
263	B20	2	3	Left ovary—several blood follicles and corpora. Gland removed at 10th day showed no development.
240	B21	2		Mammary glands examined—pseudo-pregnancy was not indicated.
228	B22	2	3	Left ovary examined—several new corpora and many blood follicles.
237	B23G.3	2	2	Left ovary—contained five corpora.
231	B26	3	4	Left ovary—results questionable.

The ovaries of several animals have been examined at the end of the period of treatment. In this case the extracts have been injected subcutaneously for varying periods. The results are given in Table 4.

As seen from Tables 3 and 4, variable results have been obtained for the gonad-stimulating effects of the lactation-stimulating extracts. It is interesting to note in two cases reported in Table 3 that corpora lutea were observed to be present from laparotomies performed on the third day following the injection, while examination of the mammary glands on the tenth day revealed an involuted condition. If corpora lutea had formed they were certainly not functional and the original observation is questioned.

TABLE 4.—THE GONAD-STIMULATING HORMONE CONTENT OF GALACTIN EXTRACTS
(Determined by Ovarian Changes Following Subcutaneous Injections)

Rabbit No.	Galactin Extract No.	Amount Injected	Lactation Response	Condition of Ovaries
213	B19 al. s.	1 cc. daily for 8 days	+++	Contained many large follicles showing partial luteinization and internal hemorrhage
215	B19 (2)	2 cc. daily for 8 days	+++	Same as No. 213.
206	B19	2 cc. daily for 8 days	—	Same as No. 213.
216	B20	2 cc. daily for 8 days	++++	Ovaries showed large cystic follicles some of which showed slight hemorrhage.
264	B23G3	2 cc. daily for 4 days	+	Ovaries on examination appeared to contain several large corpora lutea.
249	A5	1 cc. for 2 days	+	Ovaries appeared normal.
240	B26 ppt. 2 acidsoluble fraction	1 cc. daily for 8 days	++++	Ovaries normal.

Large follicles congested with blood from internal hemorrhages are frequently observed. Sometimes they appear in very large numbers. At times a lactation of plus four (++++) has been obtained when the ovaries appeared entirely normal.

The presence of gonad-stimulating hormones in two of the lactation-stimulating extracts has also been determined indirectly by observations of the precocious development of the mammary glands induced in immature female rabbits. As previously mentioned, these extracts have been found inactive in the stimulation of mammary growth in ovariectomized animals. Therefore, the changes induced in the glands of normal animals were attributed to direct stimulating effect of the ovaries which were stimulated in turn by the extracts.

Five young female rabbits were injected with potent lactation-stimulating extracts for variable periods of time. The extracts used were the same ones used with R84, R86, R140 and R156 described in Part I of this paper (pages 24 and 25).

In all cases mammary gland growth was observed following the injection of the extracts. All control glands removed were small and consisted of a branching duct system. Three of the young females were injected daily for a period of 28 to 30 days. Sections of mammary glands removed after 20 injections were developed to variable degrees (Figs. 27, 28, 29 and 30). In no case,

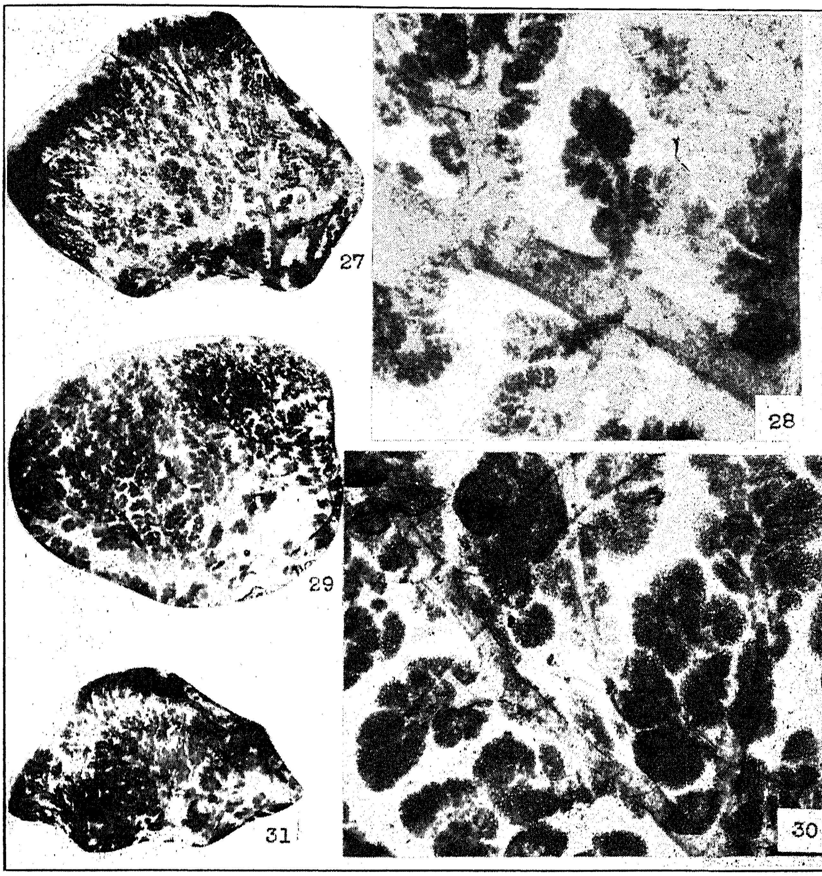

PLATE VII

Fig. 27.—A photograph of a section of mammary gland removed from a normal young female rabbit (R82) after 2 cc. of the pituitary extract had been given daily for 20 days. The gland was developed to a stage at which the anlage of the lobules were present. The proliferating ends of the larger ducts may be observed. The square marked in the lower right corner is the area shown under greater magnification in Fig. 28. Enlarged 1½ times.

Fig. 28.—An enlarged section of the mammary gland shown in Fig. 27. This gland has developed to a stage at which the lobule anlage are present. Under the dissecting microscope gross mounts of the developing mammary glands may be studied at even greater magnification. Enlarged 11 times.

Fig. 29.—Section of mammary gland removed from R88. With the exception of the distal portions, the gland has complete lobule development. The individual alveoli of this mount may be distinguished upon sufficient magnification. Enlarged 1½ times.

Fig. 30.—A section of mammary gland removed from R88. The lobules are well developed and individual alveoli may be distinguished. The gland is developed to a condition resembling normal pseudo-pregnant development. The same gland is shown at lower magnification in Fig. 29. Enlarged 11 times.

Fig. 31.—A section of mammary gland of R88 removed after 26 daily injections of the pituitary hormone. The ducts and lobules were distended with a milk-like fluid. Enlarged 1½ times.

however, did the degree of development exceed that found at the 14th day of pseudo-pregnancy. A continuation of the period of injection for another period of six to ten days resulted in lactation (Fig. 31).

Two other young female rabbits were given daily injections of galactin for 13 days. In these cases also, mammary development was observed. The rate of development was likewise slower than that observed during pseudo-pregnancy.

Though the ovaries of none of the animals were examined, the growth of the mammary glands was attributed to the effects of the ovarian hormones theelin and corporin. The relatively slower rate of development observed, as compared to that following pseudo-pregnancy, is attributed to the assumption that ovulation was not induced directly following the start of the experiment. Evidence for this assumption was obtained from examination of the glands after the first ten-day period of treatment. At this time the glands appeared to be just starting active growth.

Though the results are not uniform they are considered to be sufficiently so to warrant the conclusion that there is no relation between the galactin content of the extracts and their gonad-stimulating properties and hence that the lactation-stimulating and gonad-stimulating hormones are not identical.

The same conclusion might be drawn from observing the effect of gonad-stimulating hormones on the mammary glands. The only strictly gonad-stimulating extract used has been Antuitrin-S. Antuitrin-S in amounts of from 50 to 100 rat units has been injected daily for periods up to 10 days. In no case has lactation been initiated.

That the lactation-stimulating effect of the pituitary is not due to the growth-stimulating hormone has been assumed as Bugbee, Simond and Grimes (1930) state that tricresol is destructive to the growth-stimulating material. All of our extracts were preserved in 0.4 per cent tricresol.

Riddle and Brausher (1931) have demonstrated that Antuitrin-S would not stimulate crop-gland secretion in doves and pigeons. The same substance stimulating crop-gland activity was found capable of initiating lactation in rabbits and guinea pigs (Riddle, Bates and Dykshorn 1932 and 1932a). Thus, they have likewise demonstrated that the crop-gland stimulating substance was distinct from the gonad-stimulating substances.

Extracts have been prepared from the uterus of pregnant sows and from sow's placenta, using the same method of extraction as used to obtain galactin. Rabbit muscle tissue has been treated likewise. In no case have the extracts of these tissues been found to initiate mammary secretory activity.

Both sheep and cattle pituitaries have been found to contain the lactation-stimulating hormone. On one occasion the posterior lobes of cattle pituitaries were removed from the anterior before the extraction. In this case the response was the same as that observed when whole pituitaries were used. Whole sheep pituitaries have always been extracted as it is difficult to separate the posterior and anterior lobes.

STABILITY OF GALACTIN

At the present time there is some disagreement in the literature on the stability of the lactation-stimulating extract. Corner (1930) stated that the extracts which he used appeared to lose their mammary-stimulating potency after a few weeks. The same extracts, however, maintained their gonad-stimulating properties over a much longer period. The instability of the mammary-stimulating substance of similar extracts was also experienced by Asdell (1931). On the other hand, the galactopoetic material prepared by Riddle et al. (1932) was claimed to be relatively stable.

Several of the galactin-containing extracts prepared in our laboratory have been assayed after variable periods of time. The results of some of these tests are found in the following table.

As may be seen, the active material of the extracts appears to be relatively stable. Extracts have been kept for over six months and have apparently lost no lactation-stimulating potency. Several extracts have been kept for as long as three to four months. Though there are some inconsistencies in the results, most of the extracts have been found to be relatively stable over this period.

The effect of the condition of storage of galactin has not been determined. All the extracts used here have been kept in rubber stoppered serum bottles at a temperature of 36 to 40 degrees Fahrenheit. Four-tenths per cent of tricresol was used as a preservative. The extracts were removed from the refrigerator only when material was removed for injection.

It may be concluded that galactin when kept under the conditions described, is a relatively stable material. It is possible, however, that differences in the method of preparation or storage of the extracts may affect considerably the stability of the active material.

Though galactin is stable over a considerable period of time when kept at ice box temperatures, it has been found to be rapidly inactivated upon heating when in a slightly alkaline soluble form. Heating these extracts for five minutes at 70 degrees Centigrade has been found to completely inactivate them. The precepitated material has not been studied concerning its heat stability. Riddle et al. (1932), however, have reported that their active material was relatively stable to heat.

Galactin in the soluble form has been dialyzed for periods of 24 hours. It has been found that the active material will not pass through a collodion membrane. Attempts have also been made to dehydrate the precipitated galactin-containing material with acetone. The extracts have been largely inactivated by this treatment.

TABLE 5.—THE STABILITY OF GALACTIN OVER VARIABLE PERIODS OF TIME

Rabbit No.	Galactin Extract No.	Amount of Extract Injected	Condition of Control Glands	Days of Injection	Date of Injection	Age of Extract	Response
		cc.				*months*	
273	B20	2.5	19 days pseudo-pregnant	8	1-8-33	3.50	—
215	B20a	2.0	16 days pseudo-pregnant	5	10-1-32		++++
274	B20b	2.5	19 days pseudo-pregnant	8	1-18-33	3.50	++++
218	B20b	2.0	16 days pseudo-pregnant	5	10-1-32		++++
275	B21	2.5	19 days pseudo-pregnant	8	1-18-33	3.25	++
210	B21	2.0	16 days pseudo-pregnant	7	10-10-32		++++
260	B19	2.5	19 days pseudo-pregnant	8	1-18-33	4.00	+++
215	B19	2.0	slightly involuted multiparous	8	9-8-32		+++
206	B19	2.0	greatly involuted multiparous	8	9-8-32		—
251	B18	2.0	20 days pseudo-pregnant	9	1-20-33	6.25	+++
199	B18	2.0	Involuted multiparous	5	7-14-32		+
193	B18	2.0	Involuted multiparous	3	7-22-32		++
185	A5G1	2.0	20 days pseudo-pregnant	9	1-20-33	1.25	++++
263	A5G1	2.0	12 days pseudo-pregnant	8	12-8-32		++++

Part IV. Mechanism for the Control of Normal Lactation

Though the normal growth of the mammary glands of the rabbit may be entirely experimentally produced in immaturely castrated female or male rabbits by treatment with ovarian hormones and normal lactation may be initiated following the administration of the pituitary hormone, galactin, little is known of the mechanism of the control of normal lactation. The growth of the duct system of the mammary glands may be experimentally produced by theelin (Turner and Frank 1930). Normally this growth takes place to a slight extent during the prepubertal period. During the more or less continuous estrus established following sexual maturity, the duct system of the virgin rabbit undergoes a more rapid development. It thus is logical to assume that the ovarian hormone, theelin, presumably of follicular origin, is responsible for the initial normal growth (the extension of the duct system) of the mammary glands of the normal rabbit.

The growth of the lobules and alveoli from the smaller ducts of the theelin developed mammary tree has been experimentally produced by the combined injection of theelin and corporin (Turner and Frank 1932). In the normal virgin rabbit such development is induced following ovulation which results in either pregnancy or pseudo-pregnancy. Corner (1930) and Turner and Frank (1931 and 1932) observed that corporin alone was ineffective in the production of lobule growth. Thus it is assumed that, in the normal rabbit, the early pregnancy growth and perhaps the complete pregnancy growth of the mammary glands is under ovarian control (follicles and corpora lutea).

It is not stated that there is a definite point of separation between the stages of full duct growth and alveoli growth. In young rabbits during pseudo-pregnancy duct proliferation and lobule growth have been observed simultaneously. In some cases a few structures, probably alveoli, can be observed following theelin alone. However, even when massive or long continued theelin treatment has been given there is no evidence of any extensive lobule development.

The effect of the ovarian hormones on the mammary glands has been investigated in several species—rat, mouse, guinea pig, dog, and monkey. The amount of investigational work has not been as abundant in any of these species as it has in the rabbit.

Though some variations in the rate and degree of response of the mammary glands to the ovarian hormones exist among the species sufficiently investigated, the response appears to be similar in essential points. Theelin will stimulate mammary growth to a certain degree. Theelin and corporin acting synergistically will stimulate complete or continued mammary growth.

Another hormone may be effective in producing mammary hyperplasia. From the work of Corner (1930), Asdell (1931) and Catchpole and Lyons (1933), the anterior pituitary may be functional in stimulating the growth of the mammary glands, particularly the later stages. Bradbury (1932) observed that the later stages of mammary development were induced in the mouse by pituitary extracts. Though it has been attempted several times in our laboratory, growth of the mammary glands of the rabbit has never been stimulated, at least to any marked degree, by injections of pituitary extracts containing the lactation-stimulating hormone.

Though the mechanism of control of mammary gland growth in normal individuals appears to be relatively satisfactorily explained, particularly during the earlier stages, very little is known concerning the mechanism for the control of normal lactation. As has been demonstrated the pituitary extract, galactin, has galactopoetic powers. Is the galactin, however, the stimulating agent in the initiation of mammary secretory activity under normal conditions? It has been established, for example, that the hormones of the posterior lobe will strengthen uterine contraction at parturition. It has been found, however, that parturition will occur normally in rats in which the posterior pituitary has been completely removed (Smith 1932). Though abalation experiments do not necessarily prove that some active material is not necessary for the response (in this case, uterine contractions), they prove that the posterior pituitary which has been considered as the source of the active material (oxytocin) is not required.

Recently investigations have been conducted in which lactation has been studied following the removal of the anterior lobe of the pituitary. Hypophysectomies have been performed on the rat by several investigators following the original reports of Smith (1927 and 1930). Smith and White (1931) and Firor (1933) have been successful in removing the pituitaries of rabbits and Allan and Wiles (1932) have performed hypophysectomies in the cat. Selye, Collip and Thomson (1933) observed that the hypophysectomy of pregnant rats did not prevent the onset of lactation following parturition, but lactation was found to persist for only a very short time in these animals. Hypophysectomy performed during lactation resulted in the immediate cessation of secretory activity. No observations on the effect of hypophysectomy on lactation in the rabbit have been reported. Allan and Wiles (1932) observed that their hypophysectomized cats never suckled their young.

These observations appear to add evidence that the pituitary may be functional in the control of normal lactation. This control, however, could act either directly or indirectly, through the thyroid or adrenal glands. Though there is little, if any, evidence at the present time for the former assumption, the investigations of Carr (1931 and 1931a) and of Brownell, Lockwood and Hartman (1933) have shown that in the rat the adrenal may play an active part in the stimulation of lactation.

Assuming that the anterior pituitary supplies the stimulus to mammary secretory activity, the question of the mechanism regulating or conditioning the stimulus arises. That is, is galactin only released or produced by the pituitary under certain conditions, or are the mammary glands receptive to galactin only under definite conditions?

Experiments have been planned in which the attempt has been made to investigate this mechanism. Attempts have been made to experimentally duplicate, in part, those conditions assumed to exist during normal pregnancy. During pregnancy in some species, at least, large quantities of theelin are produced as this hormone has been found in large amounts in the blood of these animals and in urine excreted by these animals (human—Aschheim 1927, Frank 1929, and others; horse—Zondek 1930; and cows—Hisaw and Meyer 1929, Turner et al. 1930). Theelin has been found to have some effect on the gonad-stimulating capacity of the pituitary (Meyer, Leonard, Hisaw and Martin 1930). Moore and Price (1932) demonstrated a reciprocal pituitary-gonad relationship and this relationship has been assumed to be responsible for the regulation of the periodic sexual activity in most mammals. Perhaps theelin in some way might regulate the lactation-stimulating hormone production or release by the pituitary. If such an assumption were true, lactation might be expected following an extended period of theelin administration. In the rabbit, lactation, at least to any considerable amount, has never been observed following theelin administration (Turner and Frank (1930). In the guinea pig, however, lactation has been reported to occur following theelin administrations (Steinach et al. 1928 and Laqueur et al. 1928). Nelson (1932) and Nelson and Smelser (1933) observed female guinea pigs following ovariectomy and cryptorchid or gonadectomized male guinea pigs bearing ovarian grafts and verified these results. They developed the hypothesis of a reciprocal hypophysis-ovary relationship for the control of normal mammary function and activity. This hypothesis may be summed up as follows: Theelin inhibits the lactation-stimulating hormone secretion of the anterior pituitary when it is present in large amounts, lactation resulting normally only when the theelin supply is removed.

From the evidence cited on the guinea pig such a hypothesis might appear very attractive. However, it does not work out in the rabbit (Turner and Gardner 1931), rat, or mouse (Turner et al. 1931). Likewise, the milk production of the heifer gradually increases during the latter part of pregnancy (Turner 1931 and Hammond 1933) in spite of a gradually increasing theelin elimination in the urine during this time.

At this time it might be well to mention that several investigators have either observed an inhibitory effect of theelin on lactation or have attributed a decline in lactation to the effect of theelin. Smith and Smith (1932) concluded that theelin inhibited lactation in the rabbit. Enzmann and Pincus (1933) made observations in the mouse which they have interpreted similarly. Frei and Grüter (1930) observed that ovariectomy of cows suffering with nymphomania (follicular cysts in the ovaries) caused increased and prolonged lactation. These observations might be considered to agree with the hypothesis proposed by Nelson.

Though the theory of a reciprocal pituitary-ovary relationship may appear attractive in the regulation of lactation, there is also an equally great probability that the ovary may "condition" the glands to a point at which they are responsive to galactin.

The mammary glands function periodically. They undergo slight cellular hypertrophy and atrophy with each estrus cycle in some species. In all multiparous females the mammary gland normally has passed through stages of great functional activity and stages of inactivity and perhaps extensive atrophy. In this respect the mammary gland resembles the uterus. They are glands of periodic development and function.

It has been observed that the rabbit mammary gland just completing development is most responsive to galactin. Likewise, comparatively undeveloped mammary glands have been brought into secretory activity when galactin has been injected following somewhat extended periods of theelin and theelin and corporin injections. It therefore might be concluded that the ovarian hormones in addition to stimulating the growth of the mammary glands have a direct conditioning effect upon the secretory cells which causes them to be increasingly responsive to galactin. In this manner the mammary glands might be compared to the uterus where corporin or progestin is capable of inducing the progestational proliferation of the mucosa only after the conditioning influence of theelin or of corporin and theelin acting synergistically.

Experiments have been conducted with rabbits in which presumably functional corpora lutea have been maintained in the ovaries for periods equal to or greater than the duration of normal pregnancy. Four normal mature female rabbits, three normal young female rabbits, and two mature hysterectomized rabbits have been intravenously injected with Antuitrin-S at seven, ten or fifteen day intervals. Ovulation was observed to result following this treatment and the mammary glands were examined at intervals during the progress of the experiment. In this manner ovarian conditions were maintained similar to those presumably existing during pregnancy and for a time equal to or exceeding normal pregnancy. The mammary glands of all the animals underwent the typical pseudo-pregnant development during the first part of the experiment, that is, the duct system and lobules were apparently completely developed. In one of the hysterectomized rabbits and in two of the normal rabbits there was evidence of the secretory activity, but the response was not rated even as high as plus one (+). Though the observations extended over a period of from 32 to 35 days, there was no evidence of significant secretory stimulation of the mammary glands of any of the rabbits.

As has been previously mentioned, Antuitrin-S has been found ineffective in stimulating lactation in both normal and ovariectomized rabbits. Had lactation resulted it would have been attributed to the fact that conditions in the ovary similar to those existing during normal pregnancy had been maintained for a period equal to or slightly exceeding normal pregnancy. This would have offered evidence of a reciprocal hypophysis-ovary mechanism for the regulations of lactation. The pregnancy-like ovarian conditions were non-functional in the activation of the pituitary in the secretion or liberation of galactin. However, these observations indicate that some extra-ovarian and extra-uterine factor must be involved.

In one of the rabbits a hysterectomy was performed on the 35th day of the experiment. Lactation did not follow. The uterus had no inhibiting effect on lactation. Galactin was injected in seven of the animals at the close of the experiment and lactation was induced in all cases.

Similar observations have been made by Nelson. Asdell, Hammond, and Salisbury (1933) observed that a continuous series of pseudo-pregnancies induced by sterile coitus likewise did not produce a development of the mammary glands beyond the pseudo-pregnancy stage. Nelson (1932a) further observed that the presence of the maternal placenta as represented by experimentally induced deciduomata in the presence of active corpora lutea did not induce complete mammary proliferation or lactation. Likewise, the surgical removal of this tissue was not followed by lactation.

The lactation-stimulating mechanism might be looked upon from another angle, that is, the factors causing the persistency of mammary secretory activity. Are the factors responsible for the original initiation of secretory activity the same as those that are responsible for the continuation of secretory activity during the period of active lactation, or does lactation once initiated, continue under the stimulation of suckling or the removal of the products of secretion?

Two rabbits have been injected continuously with galactin for periods of 20 days. A lactation response of plus three (+++) and plus four (++++) was observed on the eighth day following the beginning of galactin injections. One control rabbit was likewise treated but the injections were stopped on the seventh day. At the 20th day there was very little difference between the glands of the control animal and of those animals which had received continuous galactin injections. A small amount of milk was present in the larger gland ducts of the rabbits in both groups. The glands of the injected animals appeared slightly thicker, however. On the 20th day the control animal was injected with galactin. Lactation could not be re-induced at this time. This observation is similar to the results previously obtained in rabbits which did not respond to galactin 14 days after a previously induced lactation (see Table 1). These results might be contrasted to those obtained on the sow and dog as previously mentioned. However, in two instances galactin reinitiated lactation in two rabbits when treatment was begun on the 19th day following parturition, the young being removed at birth.

The above observations indicate that galactin will not maintain the mammary glands in a state of active secretion over a considerable period of time if the products of secretion are not removed. Likewise, after a galactin-induced lactation the mammary glands are not responsive to further galactin injections for a period of two weeks, at least. The lactation occurring following normal parturition if nursing is not allowed, does not render the glands inactive to later galactin injections. One cannot conclude that such experiments indicate that galactin is not necessary for the persistency of lactation in the normal animal.

The observation that lactation in the rat is immediately stopped following hypophysectomy (Selye, Collip and Thomson) offers the only proof that the pituitary is necessary for the maintenance of lactation. Replacement therapy using galactin should indicate whether the mammary glands are directly or indirectly affected.

Though our present knowledge of the mechanism for the control of normal lactation is gravely incomplete, recent investigations have thoroughly established, for the first time, the existence of a galactopoetic substance.

Summary and Conclusions

1. The lactation-stimulating substance of the anterior pituitary, galactin, initiated or stimulated lactation in the rabbit, guinea pig, dog, and sow. The rat, mouse, and monkey did not respond to galactin in the same manner as did the other species mentioned.

2. The response of the mammary glands of the rabbit varied with the developmental condition of the glands at the time the injections were undertaken. It is probable that some change induced, other then the growth of the glands, is effective in conditioning the glands for response to galactin. The conditioning factor or factors are probably of ovarian origin.

3. Involuted and immature mammary glands did not respond to galactin in the amounts injected.

4. The involuted or immature mammary glands of rabbits, partially but not completely developed by the ovarian hormones, theelin and corporin, or theelin alone, have been found to respond to galactin.

5. The lactation-stimulating extracts were incapable of stimulating the growth of the mammary glands of young virgin female rabbits or male rabbits, of redeveloping involuted mammary glands, or of altering the effect of theelin in the stimulation of the initial development of the glands.

6. A method for the preparation of active lactation-stimulating extracts was described. The active material was soluble in both dilute alkali and acids and insoluble in slightly acid to neutral solutions. Galactin has been obtained from both cattle and sheep pituitaries.

7. Galactin has retained its activity under the storage conditions used for periods up to six months. In the soluble form it was readily inactivated by heat.

8. Galactin was found to be present in extracts containing appreciable amounts of gonad-stimulating hormones and in extracts containing no gonad-stimulating hormone as determined by the methods used. It was concluded that galactin and the gonad-stimulating substances are not identical. As tricresol has been reported to destroy the growth hormone, galactin is considered distinct from this substance.

9. The gonad-stimulating hormone, Antuitrin-S, is ineffective in initiating lactation in the rabbit.

10. A method was presented for biologically assaying galactin-containing extracts using pseudo-pregnant rabbits. A unit of galactin is defined as that minimum amount of extract which injected during a period of seven days at daily intervals is required to induce a plus three (+++) or plus four (++++) response in rabbits previously pseudo-pregnant for a period of from 12 to 16 days.

11. Active corpora lutea maintained in the ovaries of rabbits by repeated ovulation induced by intravenous injections of gonad-stimulation hormone for periods of 32 to 35 days did not induce lactation. The glands developed to the pseudo-pregnant condition and appeared to remain in that condition.

12. Galactin initiated lactation in hysterectomized rabbits and in rabbits simultaneously injected with theelin.

13. Galactin was ineffective in maintaining the mammary glands in a continuous stage of secretory activity when the milk was not removed from the glands. Likewise, galactin was found to be ineffective in the reinitiation of lactation in rabbits following a previous galactin-induced lactation.

14. The mechanism for the control of normal lactation was discussed.

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