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The Lactogenic and Thyrotropic Hormone Content of the Anterior Lobe of the Pituitary Gland

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ABSTRACT

This bulletin presents the results of a study of the lactogenic and thyrotropic hormone content of the anterior lobe of the pituitary gland. Five hundred pituitaries were collected from cattle in a local slaughter house and these assayed for their lactogenic and thyrotropic principles. Weight determinations were made of the pituitaries and the average weights are recorded.

An assay method was developed for the determination of the minute quantities of the lactogenic hormone present in pituitary glands from laboratory animals. An extensive study was made of the rat pituitary gland. As the male rat matured there was an increase in the lactogen content per pituitary gland and this was due entirely to increased pituitary size. In the female there was an increase in lactogen concentration in the pituitary gland with the onset of sexual maturity. During pregnancy there was no increase in the lactogen content whereas following parturition the quantity of the hormone was doubled. Suckling, either with or without the removal of milk, decreased the lactogen content of the pituitary gland. Castration did not alter the lactogen content of the pituitary gland, but ovariectomy decreased the content. The abdominal anchorage of the testes did not alter the lactogenic hormone content of the pituitary gland. Estrogen injections into ovariectomized rats, normal and castrated male rats, and normal male guinea pigs increased the lactogen content per pituitary gland while progestin injections into ovariectomized rats had no influence upon the pituitary lactogen content. Attempts were made to inhibit lactation with ovarian hormone injections.

The lactogen content of bovine fetal pituitaries was the lowest found in any of the cattle pituitary assays. Pituitaries from heifers contained more lactogen than glands from bulls. As the heifers matured there was an increase in the hormone concentration in the pituitary gland, however, as the bulls matured the hormone concentration remained unchanged. Pituitary glands from bulls contained more lactogen than glands from steers. Pituitary glands from dairy cows contained larger quantities of the lactogenic hormone than glands from beef cows.

The thyrotropic content of pituitary glands was determined in the growing and in the mature animal. Pituitaries from bulls contained more of the thyrotropic hormone than glands from heifers and heifer pituitaries contained more than glands from steers. Pituitaries from dairy cows contained more of the thyrotropic principle than glands from beef cows.

The Lactogenic and Thyrotropic Hormone Content of the Anterior Lobe of the Pituitary Gland

R. P. REECE* AND C. W. TURNER

INTRODUCTION

Scientific investigation of the pituitary gland began with Rathke's (1838) description of some phases of its development. The findings during the following 50 years were largely anatomical. However, in the last 20 years much attention has been given to pituitary physiology, cytology, and pathology.

Ott and Scott (1910) presented the first evidence of a pituitary-mammary interrelationship. An extract of the posterior lobe was injected intravenously into a lactating goat and as a result they were immediately able to withdraw an increased amount of milk. It now seems certain that posterior lobe extracts are not true galactogogues but cause a contraction of the smooth musculature of the mammary gland, thus giving a more complete emptying of the gland. Moreover, Smith (1932) and Houssay (1935a, b) have shown that the posterior lobe is not essential for lactation.

Even though the posterior lobe plays no part in milk secretion it has been demonstrated by numerous investigators that the anterior lobe contains a principle, the lactogenic hormone, which is capable of initiating lactation. With the development of the hypophysectomy technique it was established soon that the lactogenic hormone is essential for the induction and maintenance of lactation.

The establishment of the above facts causes one to wonder if one of the differences in the ability of cows to secrete milk may not be due to differences in the amount of lactogen they are secreting. Moreover, experimental work has demonstrated that the addition of desiccated thyroid tissue to the diet of cows or injecting them with thyroxine will cause an increase in milk flow and a very marked increase in milk fat yield. In addition to these

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facts research work has shown that the anterior lobe of the pituitary gland produces a principle, the thyrotropic hormone, that stimulates the thyroid gland to increased activity. It was thought that the primary reason for normal thyroid glands to vary in their production of thyroxine was due to differences in the amount of thyrotropic hormone being secreted and excreted by pituitary glands. Consequently, it seemed probable that the pituitary glands of cows would vary in their ability to secrete the thyrotropic hormone.

Since the study of Eckles and Reed (1910) little progress has been made in determining the differences between profitable and unprofitable dairy cows. It is interesting to note that these investigators concluded that the main difference between profitable and unprofitable dairy cows was not to be found in the coefficient of digestion or in the amount of food required for maintenance, a superior cow being one with a large capacity for using food above the maintenance requirement and one that uses this available food for milk production.

As an attacking point on the above mentioned thoughts it seemed logical to study the lactogen and thyrotropic content of the gland secreting the hormone. The interpretation that could be placed upon the results obtained was not known, for there was little evidence that the hormone content of an endocrine gland necessarily indicated the rate of hormone excretion. After the cattle pituitary collecting had been under way for about six months it became evident that a great number of pituitaries were not going to be available from heavily lactating animals. Therefore, attention was turned toward determining the lactogen content of pituitary glands from laboratory animals. The assay results obtained on pituitaries from small animals have proved useful in aiding one to interpret the assay results of cattle pituitaries. All of the observations, with one exception on laboratory animals, indicate that a pituitary gland with a high lactogen content is one that is actively excreting the hormone into the blood stream.

The results obtained in this study are encouraging and it is believed that this type of work offers the possibility of determining the fundamental reasons why some cows are good producers and why others are low producers. Furthermore, there is every reason to believe that suitable tests can be evolved which will enable one to evaluate dairy cattle on their endocrine system, and if this is accomplished, can not these tests be employed to locate animals with superior productive abilities?

MATERIALS AND TECHNIQUE

Collection of Cattle Pituitary Glands

Pituitary glands were collected in a local slaughter house. The individual collecting the glands was always present before the animals were slaughtered. This made possible the recording of such information as estimated age, estimated weight, sex, breed, and the stage of the reproductive cycle of the animals.

Immediately following decapitation the top of the skull was cleaved open and the meninges laid back. As the brain was pulled in a caudal and dorsal direction the optic chiasma and the infundibulum were severed. The diaphragma sellae was severed from its attachment to the sphenoid bone and as it was lifted up the pituitary gland was freed from the rete mirabile. The pituitary glands were then placed in numbered vials. Whenever pregnant animals were encountered the fetuses were taken from the uterine horns, their pituitaries removed and crown-rump measurements made. Using Hammond's (1927) figures on the crown-rump length of fetuses of known age, estimates were made of the length of time the animals had been pregnant. The glands were then brought to the laboratory.

Laboratory Work Upon Cattle Pituitary Glands

The thin membrane around the pituitary gland was carefully removed, the posterior lobe separated from the anterior lobe, and weighings made of each lobe to an accuracy of 0.1 mg. The anterior lobes were kept in a frozen state until a number of a kind was secured.

In making the acetone dried powder the glands were removed from frozen storage, sliced into thin pieces and placed in centrifuge tubes which contained about ten times their volume of acetone. The anterior lobe tissue remained in the acetone 10 to 15 minutes and then the tubes were centrifuged. The acetone was decanted and the above procedure repeated three times. The tissue was placed in a desiccator overnight. Employing a porcelain mortar and pestle the dehydrated tissue was ground until it would pass through an 80 mesh sieve. The acetone dried powder was then weighed, placed in a dry vial and kept in a desiccator until it was assayed.

Collection of Pituitary Glands From Laboratory Animals

In studying the factors altering the lactogen content of the pituitary gland, animals were usually paired upon the basis of body weight, one of the pair serving as a control, the other as the experimental animal. Control animals were sacrificed at the same time as the experimental animals.

Animals were completely anesthetized and their spinal cords cut in the neck region. The skull top was removed and the brain lifted out. The pituitary glands were then carefully removed and weighed to an accuracy of 0.1 mg.

The pituitary glands were either assayed immediately or kept in a frozen condition until they were assayed.

Source of Hormones

In attempts to experimentally alter the lactogen content of the pituitary gland, several hormones have been employed. The theelin and progestin were kindly and gratuitously supplied by Dr. Oliver Kamm of Parke, Davis and Company. Dr. E. Schwenk of the Schering Corporation kindly and gratuitously supplied the progynon-B. Thyroxine and oestroform-B were secured from the British Drug House, Ltd.

Methods of Injections

All of the injections into experimental animals have been made subcutaneously. Unless stated to the contrary, daily injections have been made.

Histological Technique

Occasionally it has been of interest to determine the condition of the mammary glands of animals in which the lactogen content of the pituitary glands was being determined. All tissue for histological study was fixed in Bouin's fluid where it remained for 12 hours or more. The tissue was then prepared for histological examination by the standard technique.

Assay Methods

Lactogen assay.—In all lactogen determinations the common pigeon has been used as the test subject. As a test subject the pigeon has several advantages and one or two disadvantages. In its favor are the facts that large numbers are available, it is relatively cheap and requires no preliminary preparation and it offers the possibility of ruling out test subject variability. The pigeon loses some attractiveness as an assay subject because it is not a mammal and because it must be sacrificed at the close of the test period. All points considered, the pigeon is the best test subject available for lactogen assay work.

Riddle and Braucher (1931) were the first to present evidence that an anterior pituitary extract would cause glandular enlargement and functioning of the crop glands of immature and other non-breeding male and female doves and pigeons. Sectioning of nerves to the crop gland did not interfere with this growth and function. Riddle, Bates and Dykshorn (1932a, b, 1933) reported that the anterior lobe principle which caused lactation in mammals also induced proliferation and crop-milk formation in the crop glands of pigeons and doves. In the last noted paper these investigators presented a quantitative assay method of the lactogenic hormone, based on the weight of the stimulated crop glands.

Lyons and Catchpole (1933) called attention to the fact that one need not depend upon a weight increase in the crop glands over and above controls. For a positive response they suggested the observation of beginning growth changes, inasmuch as these changes could be observed in crop glands that weighed less than the average normal.

McShan and Turner (1936) published an assay method based upon minimum crop gland proliferation of 50 ± 11 per cent of 20 common pigeons weighing 300 ± 40 grams.

These above mentioned assay methods have proved useful in the further purification of the lactogenic hormone, but they have been of no aid in detecting lactogen in minute quantities.

Lyons and Page (1935) reported the detection of lactogen in the urines of eight lactating women. Their urine extract was injected intradermally over the crop glands of squabs. They claimed that this method of local administration of lactogen made possible the detection of the hormone in a single microgram dose. The birds were sacrificed at the end of 48 hours.

McQueen-Williams (1935a) demonstrated that one normal rat pituitary contained sufficient lactogen to elicit a good response when implanted subcutaneously over the crop gland of a one month old squab.

Attempts were then made to see if the work of McQueen-Williams (1935a) could be confirmed. Pituitary glands from male rats were implanted subcutaneously over the crop glands of mature pigeons and the birds sacrificed 96 hours later. Indications of a beginning response were secured, but the glands were difficult to rate. Pituitary glands were then macerated, suspended in distilled water and this suspension injected intradermally over the crop glands of pigeons for four days, the birds being sacrificed on the fifth day. This procedure produced responses in the crop glands that were more readable.

The mature pigeon has always been used for assay purposes and there are advantages in so doing. First, because the mature pigeon's crop gland is more sensitive to the lactogenic hormone than is the squab's crop gland, and secondly, because there are more loose folds of skin over the crop gland of the mature pigeon, thus making it easier to localize the injections.

In those cases when a comparison was desired between any two groups of acetone dried powders, one began by making one or more preliminary trials. The acetone dried powder was suspended in 0.2 cc. of distilled water, for each pigeon that was to be injected. Five-hundredths of this suspension was injected intradermally daily for four days over the crop gland of a pigeon.

It is important that the injections be made intradermally, otherwise the sensitivity of the test is lost. Close observation of the needle after it enters the skin will permit one to decide if it is intradermally or subcutaneously placed. If the injection has been made intradermally, a blister-like formation will appear on the skin over the crop gland. Otherwise, the outward appearance of the skin does not change.

Suspensions were made up containing varying amounts of the acetone dried powder, as it was desirable to determine the amount of powder required to proliferate an area of the crop gland about the size of a nickel. The pigeons were sacrificed on the fifth day, their crop glands removed and a visual rating made of the degree of proliferation. When an area of the crop gland about the size of a nickel was proliferated it was called one bird unit. In making these ratings the spread and thickness of the proliferation were taken into consideration. When minimal amounts of the hormone were injected only that part of the crop gland which was immediately underneath the site of injection became proliferated. By injecting the two acetone dried powders, which were being compared for their lactogenic potency, into the same pigeon, one powder over the right crop gland and the other over the left crop gland, one was then certain that any difference in the degree of proliferation of the glands was due to a difference in the lactogen content of the powders and not due to bird variability. All crop glands were rated by two individuals, with one not knowing their identity.

Preliminary trials indicated the amount of hormone required to give the desired proliferation of the pigeon's crop gland. This amount of hormone was then injected into ten birds. After the crop glands were rated, the ratings were totaled and this figure used to express the number of bird units in the total amount of hormone injected. No correction was made for ratings greater or less than one since work upon this point showed that doubling the amount of hormone injected also doubled the degree of proliferation. For example, ten birds were injected with the same acetone dried powder over the right and left crop glands. Each bird was injected with a total of 0.5 mg. over the right crop gland and 1.0 mg. over the left crop gland. The ratings of these crop glands showed that the 5 gm. of powder injected on the right side contained 7.75 bird units while the 10 mg. of powder injected on the left side contained 17 bird units. If the degree of proliferation is not held within a rather limited range this observed relationship may not hold.

It has usually been of interest to express the hormone content of pituitary glands in the number of bird units per milligram of acetone dried powder, total bird units per pituitary gland and bird units per unit weight of the whole pituitary and per unit weight of the anterior lobe. The last expression gives one a measure of the concentration of the lactogenic hormone within the gland.

This assay method, which is based upon the intradermal mode of injection, is the only known assay method whereby the hormone content of two groups of powder can be determined in the same assay animal.

Moreover, this method has made possible the detection of such minute quantities of lactogen as are found in the pituitaries of laboratory animals. The pituitaries of these animals were macerated with an agate mortar and pestle and suspended in distilled water. Occasionally it was necessary to store the pituitary glands until they could be assayed. During this storage period the glands were kept in a frozen condition. Upon removal from storage it was possible to grind the glands into a very fine powder. The injection and crop gland rating procedures used in assaying the glands from laboratory animals were the same as those described above.

The bird unit then may be defined as that amount of hormone which will cause the proliferation of an area of the crop gland about the size of a nickel when injected intradermally over the crop gland of a mature pigeon for four days, the bird being sacrificed upon the fifth day.

Thyrotropic assay.—As early as 1888 Rogowitch reported that thyroidectomy in the rabbit was followed by definite changes in the anterior lobe cells. Only more recently has the importance of the anterior pituitary gland as a regulator of thyroid activity been proved in mammals. Loeb and Bassett (1929) and Aron (1929) produced hyperplasia of guinea pig thyroids by means of anterior pituitary extracts.

Of the common laboratory animals the thyroid glands of the immature guinea pig are the most responsive to the thyrotropic principle. Due to this fact, the guinea pig is the test animal usually employed in assaying materials for their thyrotropic content.

Junkmann and Schoeller (1932) defined a unit as that amount of hormone required to produce a definite histological reaction in one out of two guinea pigs.

Anderson and Collip (1933) suggested an assay method using hypophysectomized rats. Their thyrotropic unit was the smallest amount of the hormone that would produce a 20 per cent increase in the oxygen consumption within 96 hours, when given twice daily to an hypophysectomized rat.

Rowlands and Parkes (1934) employed guinea pigs weighing between 150 and 300 grams. Their thyrotropic unit was that amount of hormone which would cause the thyroids of immature guinea pigs to attain a weight of 60 mg. when administered daily for five days.

Stimmel, McCullagh, and Picha (1936) proposed an assay method based upon a decrease of the iodine content of the thyroid glands. Their unit was the smallest amount of hormone that would elicit a 50 per cent decrease in the percentage of iodine in the thyroid glands of normal guinea pigs weighing between 225 and 280 grams, when injected intraperitoneally for three successive days.

The thyrotropic unit used in the assays to be reported has been that amount of hormone that would elicit a 50 per cent increase in the weight of the thyroid glands of a group of four male guinea pigs weighing between 140 and 170 grams when injected subcutaneously daily for four days and the animals sacrificed on the fifth day. This dosage of hormone has been used because a 50 per cent increase in the weight of the thyroid glands gave a weight that was greater than the weight of any of the thyroid glands from control guinea pigs. Moreover, keeping the response to a significant minimum gave a more sensitive test. The thyroid glands taken from a group of control guinea pigs weighed, on the average, 16.5 mg. The acetone dried powder made from cattle pituitaries was injected into groups of four pigs each until the average weight of the thyroids of one group of animals approximated 24.8 mg. The assay results were expressed in the number of guinea pig units per milligram of acetone dried powder, units per pituitary gland, and units per gram of fresh whole pituitary gland and per gram of fresh anterior lobe tissue.

REVIEW OF LITERATURE

The Lactogenic Hormone

In 1928 Grueter presented the first evidence that the anterior lobe played some part in the control of lactation. Since this pioneer investigation numerous attempts have been made to induce lactation in various species. This work has demonstrated abundantly that the anterior lobe of the pituitary gland contained a principle which would initiate lactation in various animals.

Attempts to Augment Milk Secretion by Pituitary Injection.— Of all the research work carried out with pituitary extracts, those attempts to increase the rate of milk flow have been of most concern in this study.

In the sow. Grueter and Stricker (1929) stimulated abundant milk secretion in sows that produced little or no milk after farrowing by the injection of an anterior lobe extract.

In the ewe. Kabak and Kisilstein (1934) secured a significant increase of milk secretion in a group of ewes well along in their lactation period. Ewes that had lambed in April were separated from their lambs the middle of July. The normal lactation curve of each animal was then determined. The ewes were then injected subcutaneously daily for 15 days with 5 cc. of an alkaline extract of the anterior lobe (equivalent to 1 gram of the initial substance). The increase of milk secretion was secured only during the injection period.

Kabak and Margulis (1935) reported that the injection of an alkaline pituitary extract caused a 25 per cent increase of the milk yield. The increase continued for three five-day periods, diminishing somewhat later on.

In the goat. Grueter (1931) carried out the following experiments in an attempt to obtain some knowledge on the relationship between the anterior lobe of the pituitary gland and the thyroid gland. Six goats shortly after parturition and in full milk flow were used. Complete bilateral thyroidectomy was performed on two goats. These two goats were treated with anterior lobe extracts while two additional goats served as controls. A fifth goat received only one injection and a sixth goat remained untreated. An increase in milk flow was detectable after anterior pituitary injection, the increase being most outstanding after the first injection. The effect of these injections upon the mammary gland was found to be only transitory. After thyroidectomy the milk flow decreased to half of that before operation and the rate of secretion was then increased one-sixth to one-half after anterior pituitary treatment. Similar results were reported by von Fellenberg and Grueter (1932).

Evidence was presented by Asdell (1932) which seemed to indicate that an extract of the anterior lobe was effective in decreasing for a short period, the rate of decline in milk yield of goats. Asdell et al. (1936) summarized rather extensive attempts to alter the lactation curve with lactogen injections. These studies indicated that lactogen would not augment lactation when injected into goats which had just passed the peak of lactation. Lactation was increased in several low producing goats, but there was no tendency for greater persistency.

In the cow. By injecting an extract of the anterior lobe, Grueter and Stricker (1929) succeeded in increasing milk production in cows in normal lactation, ovariectomized cows in normal lactation, and in cows that produced small quantities of milk after calving.

Stocklausner and Daum (1932) injected "vantasan," a commercial preparation of the anterior lobe of the pituitary gland, which probably contained all the anterior lobe hormones, into 20 non-pregnant cows far advanced in their lactation periods. The injections were made weekly for four weeks and twice a week until a total of nine injections per cow had been made. These injections decreased the rate of decline of milk flow. The injected cows produced 0.44 kg. more milk per day than did the non-injected cows.

Evans (1934) injected a fairly pure preparation of lactogen into low producing cows. He was able to increase the milk production of these cows from 25 to 50 per cent above the milk flow maintained immediately before the injections were begun. However, these high levels of production were not maintained after the injections were discontinued.

Asimoff, Skarginskaya, and Ptchelina (1934) found that single injections of an alkaline extract of the anterior pituitary into lactating cows in doses of 30 to 40 cc. within a period of one to three days induced an increase in the daily milk yield of one to three liters. The increase lasted for two to four days. Repeated single daily injections were always followed by an increase in the daily milk yield. Small injections, 10 to 15 cc., did not exert any effect on the milk yield. The extract was only effective in lactating cows, dry cows not responding. The injection of the extract into pregnant cows did not produce abortion. Later Asimoff et al. (1935) reported that the repeated injection of an alkaline extract of the anterior pituitary into cows on pasture during the third to the fifth months after calving produced an increase in milk yield without a subsequent depression of lactation. The rate of decline of lactation was also decreased.

Margulis, Skvortzov, and Poliakov (1935) observed that the daily injection of an alkaline extract of the anterior lobe of the pituitary gland did not cause any change in the milk yield of lactating cows. During the injection period the injected cows gained four times as much in body weight as did the non-injected cows.

Watermann, Freud, and de Jong (1936) treated a cow during a 33 day period with oleum olivarum containing 20,000 i. u. of oestradiol-benzoate rubbed over the udder twice a day. During the treatment milk production was reduced from 5 liters to 0.5 of a liter. The oil alone had no effect upon the control animal. Both animals were then intramuscularly injected with 5500 units of lactogen for a 30 day period. The cow previously treated with estrogen increased to 50 per cent of her former production. In the control cow lactation was maintained at a constant rate for three months.

Folley and Young (1937) reported that the injection of lactogen caused an increase in milk yield of 30 per cent above the original daily yield. The injected lactogen preparation was found to be negligibly gonadotropic and thyrotropic, but possessed high glycotropic activity. The cow (Dairy Shorthorn) received 15 daily subcutaneous injections of one gram of lactogen in 50 ml. of water at a pH of 8. The increased production took some time to subside after the last injection. The percentage of milk fat remained unchanged during the injection period, though it appeared to increase slightly subsequently. The percentage of milk solids-not-fat was considerably increased during the time of injection, and for some time afterward. In addition, there was a definite increase in milk lactose concentration coupled with a decrease in chloride content.

Asimoff and Krouze (1937) made an extensive study of the effect of injecting anterior pituitary preparations into lactating dairy cows. Six hundred cows were used in the experiment, 510 serving as experimental cows and 90 being used for controls. Fifty cubic centimeters of a freshly prepared anterior lobe extract, which probably contained all of the pituitary hormones, were injected subcutaneously. Each cow in group four gave, on the average, 14 liters of additional milk per one injection, groups two, three, and five averaged 9.8 liters, 10.0 liters, and 10.9 liters of additional milk per one injection, respectively. It was observed that individual cows responded differently to the injections of the preparation, some cows not even responding, and the duration of the increase of the milk yield varied with different cows. The percentage of milk fat was slightly increased in several cases, however, this rise did not last long. The injections were found to be most effective during the first half of lactation.

In woman. Kurzrok et al. (1934) reported on the influence of lactogen injections in post-partum women. Twenty-nine cases were chosen in which milk secretion had failed to become adequately established by the sixth day or later post-partum. Seventy-five to 400 units of lactogen were injected intramuscularly. The increase in milk production in 25 cases ranged between 50 and 400 grams. In the remaining four cases there was either a loss or a gain of less than 50 grams in milk production.

While the above mentioned experiments indicate that anterior pituitary extracts augmented lactation, only when the lactogen is relatively free of other pituitary hormones can one attribute the effects to the lactogenic hormone.

Hypophysectomy and Its Effects upon Lactation.—It is sufficient to state here that the hypophysectomy of animals late in pregnancy results either in milk secretion not being initiated or in a transient milk secretion following delivery. The removal of the pituitary gland of lactating animals causes the immediate cessation of lactation. Therefore, the pituitary gland is essential for the induction and maintenance of normal lactation.

Replacement Therapy in Hypophysectomized Animals.—Although the injection of lactogen alone into pituitary-less animals will not induce or maintain lactation it is one of the anterior lobe principles essential for these processes. For a more extensive review of the literature the reader is referred to the recent bulletin by Gomez and Turner (1937).

The Thyrotropic Hormone

Early work has demonstrated that the pituitary gland secretes a principle which stimulates the thyroid gland. This principle causes an hyperplasia of the thyroid epithelium and a discharge of additional quantities of thyroxine into the blood. Because of this pituitary-thyroid interrelationship it is of interest to note the influence of thyroxine upon milk and fat secretion.

Grimmer (1918) and Grimmer and Paul (1930) noted that thyroidectomy of the lactating goat resulted in lowered milk production. Grueter in 1931 reported that the removal of the thyroid from goats soon after parturition caused a lowering of milk production to about one-half of what it was before the operation. Graham (1934a) observed that thyroidectomy of the dairy cow caused a marked lowering of the milk fat secretion distinct from that produced after a control operation in which the thyroid was not involved. The diminution in milk secretion following the removal of the thyroid could not be readily distinguished from that accompanying control operations.

Dragstedt, Sudan, and Phillips (1924) thyro-parathyroidectomized several bitches. These bitches became pregnant and lactated following parturition when tetany was controlled. A similar operation was performed on a group of rats by Nelson and Tobin (1936). These rats were between the 13th and 15th days of pregnancy. Animals surviving the operation dropped normal litters and in every instance raised their young. Houssay (1935c) was able to induce lactation in two thyroidectomized bitches by the administration of the lactogenic hormone. These observations adequately demonstrate that lactation continued in the absence of the thyroid gland, nevertheless the level of milk secretion was markedly reduced.

Graham (1934a) fed small amounts of thyroid to thyroidectomized and unoperated normal cows when they were in the declining phase of lactation. This caused a rapid rise in milk and milk fat production from which the gradual fall continued. The removal of desiccated thyroid glands from the diet of thyroidectomized cows caused a distinct decline in mammary secretion. The feeding of excessive quantities of thyroid tissue to a thyroid-less animal caused a decrease in milk and milk fat production. Graham (1934b) then showed that this stimulating factor in thyroid tissue was thyroxine. Normal cows past the peak of their lactation period were injected with synthetic thyroxine. The animals all responded to these injections by an increase in milk fat production, the increase in milk secretion being more variable. Jack and Bechdel (1935) injected four cows intravenously with thyroxine. These four cows represented three different stages in the lactation cycle. One cow was injected at the peak of her lactation period. She showed a lower milk yield following two partial injections and a slight increase following the third injection. Two cows injected during the declining phase of lactation responded to thyroxine injections. Milk production was increased 11.2 per cent in the one cow and 6.5 per cent in the other. Injections into a cow that had been in lactation six and one-half months were not particularly effective. Folley and White (1936) injected daily four Dairy Shorthorn cows which were in the declining phase of lactation with 10 mg. of thyroxine each. These injections were made subcutaneously and continued for 15 consecutive days. The mean percentage increase in total milk yield over the basal value taken for the last seven days of the injection period was 28. Fat production after eight days was 50 per cent above the basal value. The increase in fat percentage began when the milk yield attained its steady value. The mean increase in pulse rate was 22 beats per minute. In connection with this latter observation it is interesting to note that in 1928 Fuller made a study of some of the physical and physiological activities of dairy cows. Approximately 1700 pulse rate observations were made. Observations of seven high producing cows kept in box stalls and on a higher plane of nutrition had appreciably higher pulse rates as compared with cows in the milking herd.

FACTORS AFFECTING PITUITARY SIZE

Age and Sex

Pituitary size in itself is not indicative of physiological activity and it is of only rather general interest. Increase in the size of a gland may be due to hypertrophy or hyperplasia and in order to employ weight as an index of physiological activity it would be necessary to determine the factor(s) responsible for the increase in size. Nevertheless, only after the determination of pituitary weight in a large number of cases will it be possible to determine what constitutes an abnormal pituitary gland.

The work of Hatai (1913a) shows that the pituitary size of the albino rat in the male and female remains nearly identical until the animals attain a weight of 50 grams. Following puberty the pituitary of the female grows more rapidly than does the gland of the male, so that by the time body maturity is reached the female pituitary gland weighed 75 to 80 per cent more than the male pituitary gland. All of the records show a heavier pituitary gland for the female. The pituitary gland of the Norway rat is somewhat smaller than that of the albino rat, this difference being especially noticeable in the female. The pituitary weight of the female hybrid Norway-albino rat comes about midway between that of the albino and Norway strains.

Pituitary glands from male and female guinea pigs at about the same body weight compared quite favorably.

Livingston's (1916) figures on the rabbit indicate no difference in pituitary size due to sex. Allanson (1932) found a linear relation between the weight of the pituitary gland and the cleaned body weight in 48 female rabbits and 31 male rabbits, indicating that the rate of growth of the pituitary showed no marked difference after puberty. However, when Allason's (1932) figures are averaged the pituitary weight of the female is heavier than that of the male. Rasmussen (1921) reported that a sex difference was revealed by the volumetric analysis of the lobes of the pituitary gland of the woodchuck. The male glands averaged 13 per cent larger than glands from the female.

The extensive work of Wittek (1913) with cattle shows the increase in pituitary size with increase in age and body weight. Until the age of four is reached there appears to be no difference in the weight of glands from bulls and heifers. In older animals the pituitary glands from bulls were somewhat heavier. Swett et al. (1937) reported on the pituitary weights of four breeds of dairy cattle. According to the number of milligrams of pituitary tissue

TABLE 1.—PITUITARY SIZE.

Sex	No. of Animals	Age	Body weight	Pituitary weight mg.	Authority
			Rat—Albino		
F	11		9-70 gm.	2.0	Hatai (1913a)
F	20		71-120 gm.	6.7	Hatai (1913a)
F	28		121-170 gm.	9.8	Hatai (1913a)
F	21		171-up gm.	13.2	Hatai (1913a)
F	27	269 days	141 gm.	9.2	Hatai (1913b)
F	30	158 days	180 gm.	9.4	Hatai (1915)
F	9	208 days	178 gm.	12.0	Hatai (1915)
F	9	221 days	162 gm.	12.9	Hatai (1915)
F	9		129 gm.	8.2	Herring (1920)
F	20		185 gm.	10.7	Stein (1931)
F	22	108 days	184 gm.	10.5	Stein (1933a)
F	10		212 gm.	13.1	Stein (1934)
F	53	120 days	179 gm.	10.9[1]	Andersen's (1933) figs. ave.
F	41		162 gm.	6.1	Lipschutz & Villagran (1936)
M	21		5-70 gm.	1.8	Hatai (1913a)
M	17		71-120 gm.	4.3	Hatai (1913a)
M	19		121-170 gm.	5.6	Hatai (1913a)
M	8		171-220 gm.	7.2	Hatai (1913a)
M	13		221-up gm.	10.3	Hatai (1913a)
M	12	268 days	124 gm.	5.0	Hatai (1913b)
M	27	157 days	249 gm.	9.0	Hatai (1915)
M	6	197 days	239 gm.	8.4	Hatai (1915)
M	7	273 days	274 gm.	10.0	Hatai (1915)
M	8		251 gm.	7.4	Stein (1933b)
M	--		120 gm.	3.0[1]	Loeb and Friedman (1933)
M	72		201 gm.	5.0	Lipschutz & Villagran (1936)
			Rat—Norway—Norway x Albino*		
F	17		227 gm.	8.4	Hatai (1914)
F	84		234 gm.	11.6	Evans & Simpson (1929a)*
M	31		252 gm.	8.0	Hatai (1914)
M	92		325 gm.	8.8	Evans & Simpson (1929a)*
			Guinea Pig		
F	8		422 gm.	13.0	Marrassini & Luciani (1911)
M	12		433 gm.	12.5	Marrassini & Luciani (1911)
M	--		400-500 gm.	7.5[1]	Loeb and Friedman (1933)
			Rabbit		
F	5		1561 gm.	19.0	Marrassini & Luciani (1911)
F	27		2211 gm.	26.2	Livingston (1916)
F	6		44.5[2] gm.	3.4	Allanson (1932)
F	19		702[2] gm.	18.1	Allanson (1932)
F	39		2110[2] gm.	36.9[3]	Allanson (1932)
M	13		1814 gm.	23.5	Marrassini & Luciani (1911)
M	32		2143 gm.	25.0	Livingston (1916)
M	15		740[2] gm.	19.8	Allanson (1932)
M	16		1930[2] gm.	28.8	Allanson (1932)
M	--		1300-1800 gm.	12.0[1]	Loeb & Friedman (1933)
			Rabbit—Flemish Giant		
M	32	1-100 days	460[2] gm.	8.6	Robb (1929)
M	15	100-200 days	2346[2] gm.	23.8	Robb (1929)
M	10	201-up days	3471[2] gm.	25.0	Robb (1929)
			Rabbit—Polish		
M	14	1-100 days	365[2] gm.	7.5	Robb (1929)
M	15	101-200 days	877[2] gm.	12.9	Robb (1929)
M	16	201-up days	1122[2] gm.	14.8	Robb (1929)
			Rabbit—Flemish-Polish		
M	13	159-313 days	2151[2] gm.	22.4	Robb (1929)
			Hog		
--	--			125.0[1]	Loeb & Friedman (1933)
			Sheep		
--	--			350.0[1]	Loeb & Friedman (1933)
			Cattle		
--	--	3 years (ave.)		1115.0[1]	Loeb & Friedman (1933)
--	4	28 months	530 kg.	2676.0	Marrassini & Luciani (1911)
--	6	3.3 years	658 kg.	2868.0	Marrassini & Luciani (1911)
--	5	5.0 years	521 kg.	3128.0	Marrassini & Luciani (1911)
			Cattle—Bull		
M	22	1.0 year	201 kg.	1910.0	Wittek (1913)[4]
M	27	2.0 years	267 kg.	2170.0	Wittek (1913)[4]
M	72	3.0 years	296 kg.	2460.0	Wittek (1913)[4]
M	58	4.0 years	337 kg.	2870.0	Wittek (1913)[4]
M	43	5.0 years	388 kg.	3130.0	Wittek (1913)[4]
M	6	6.0 years	367 kg.	3180.0	Wittek (1913)[4]
M	6	7.0 years	365 kg.	2300.0	Wittek (1913)[4]
M	7	8.0 years	373 kg.	3700.0	Wittek (1913)[4]
M	5	9.0 years	380 kg.	3150.0	Wittek (1913)[4]

TABLE 1.—CONTINUED.

Sex	No. of Animals	Age	Body weight	Pituitary weight mg.	Authority
			Cattle—Nulliparae		
F	8	1.0 year	157 kg.	1610.0	Wittek (1913)[4]
F	10	2.0 years	213 kg.	2240.0	Wittek (1913)[4]
F	12	3.0 years	247 kg.	2450.0	Wittek (1913)[4]
F	18	4.0 years	286 kg.	2900.0	Wittek (1913)[4]
F	7	5.0 years	285 kg.	2860.0	Wittek (1913)[4]
			Cattle—Multiparae (open)		
F	9	3.0 years	231 kg.	2310.0	Wittek (1913)[4]
F	10	4.0 years	246 kg.	2600.0	Wittek (1913)[4]
F	29	5.0 years	266 kg.	2870.0	Wittek (1913)[4]
F	19	6.0 years	255 kg.	2590.0	Wittek (1913)[4]
F	22	7.0 years	267 kg.	2870.0	Wittek (1913)[4]
F	28	8.0 years	235 kg.	3020.0	Wittek (1913)[4]
F	14	9.0 years	268 kg.	3310.0	Wittek (1913)[4]
F	8	10.0 years	244 kg.	3260.0	Wittek (1913)[4]
F	9	Above 10 years	180 kg.	2280.0	Wittek (1913)[4]
			Cattle—Ayrshires		
F	41	7.0 years	1083 lbs.	2890.0	Swett et al. (1937)
			Cattle—Guernseys		
F	61	8.0 years	991 lbs.	3120.0	Swett et al. (1937)
			Cattle—Holsteins		
F	185	8.0 years	1265 lbs.	3590.0	Swett et al. (1937)
			Cattle—Jerseys		
F	210	8.0 years	910 lbs.	2820.0	Swett et al. (1937)
			Cattle—Grades and Misc.		
F	66	7.0 years	1115 lbs.	3360.0	Swett et al. (1937)
			Ox (Normal)		
--	54			1600.0	Saito (1923)
			Mare (Open)		
F	372		336 kg.	1840.0	Saito (1923)
			Man—Fetus		
--	1	11 mm (c-r length)		0.041	Covell (1927)
--	5	35.6 mm (c-r length)		0.523	Covell (1927)
--	2	62.5 mm (c-r length)		2.094	Covell (1927)
--	3	87.6 mm (c-r length)		3.653	Covell (1927)
			Man—Newborn and Adult		
F	4			880.0	Saito (1923)
--	2			30.0	Saito (1923)
			Man—Adult		
M	17	20-30 years		528.0	Rasmussen (1928) Average
M	19	30-40 years		566.0	Rasmussen (1928) of all
M	28	40-50 years		543.0	Rasmussen (1928) (M)—
M	25	50-60 years		485.0	Rasmussen (1928) 526 mg.
M	23	60-76 years		514.0	Rasmussen (1928)
F	60	16-49 years		618.0	Rasmussen (1934)
F	33	50-84 years		620.0	Rasmussen (1934)
			Average of all	618.3 mg. (F.)	

[1]Anterior lobe weight.
[2]Cleaned body weight.
[3]Includes group of rabbits where influence of copulation on pituitaty size was being studied.
[4]All of Wittek's figures on body weight are based on weights of dressed carcass.

per unit of body weight these four dairy cattle breeds ranked in the following descending order: Jerseys, Guernseys, Holsteins, and Ayrshires. Blickenstaff (1934) reported that there was a definite hypertrophy of the pituitary glands in sterile cows.

Rasmussen's (1928, 1934) observations indicated that pituitary glands from women were definitely larger than in men.

The available literature on pituitary size is summarized in Table 1.

The cattle from which the glands were taken in this study included most of the dairy and beef breeds, a majority of the young stock coming from the beef breeds.

TABLE 2.—THE WEIGHT OF THE BOVINE PITUITARY.

Group	No of glands	Mean in grams	Maximum in grams	Minimum in grams
Fetal	51	0.0431	0.2704	0.0005
56 to 140 days	32	0.0228	0.0727	0.0005
141 to 283 days	19	0.0733	0.2704	0.0060
Calf				
Up to and including 3 months	22	0.6485	0.9175	0.3200
Four months to and including 10 months	234	0.9025	1.5568	0.3412
Heifers—None included that was sexually mature	139	0.8898	1.5568	0.5020
Steers	67	0.9609	1.5090	0.5951
Bulls	28	0.8254	1.2694	0.3412
Eleven months to and including 23 months	163	1.1590	3.3375	0.5055
Heifers	114	1.1380	3.3375	0.5055
Open—None included that was sexually immature	76	1.1168	1.8982	0.5055
Pregnant	38	1.1806	3.3375	0.7177
56-140 days	28	1.1928	3.3375	0.7177
141-283 days	10	1.1463	1.3583	0.8295
Steers	42	1.2104	1.8491	0.7076
Bulls	7	1.1926	1.6227	0.9383
Cows—2 years and over	72	1.7867	2.7786	0.7431
Dairy	50	1.7521	2.7786	0.7431
Lactating and open	19	1.9457	2.6218	1.4076
Lactating and pregnant	7	1.6232	2.5349	0.7431
Dry and open	15	1.7271	2.7786	1.1076
Dry and pregnant	9	1.4852	2.4962	1.1000
Beef	22	1.8655	2.5389	0.8876
Lactating and open	3	2.3524	2.5389	2.0261
Lactating and pregnant	3	2.0223	2.1423	1.9087
Dry and open	7	1.5486	1.9926	0.8876
Dry and pregnant	9	1.8972	2.5375	1.3373

As the animals mature there is an increase in the size of the pituitary. There is no constant difference in the weight of the pituitary glands from heifers and bulls, and glands from beef cows weighed, on the average, 100 mg. more than glands from dairy cows. The variation within any one group is quite large as is indicated by the maximum and minimum size of the glands.

A summary of the weights of the total pituitary and anterior pituitary is given in Tables 2 and 3, respectively. Inasmuch as a greater number of pituitary glands was collected from Hereford cattle than from any other breed, a resume of the weights of these glands is given in Tables 4 and 5. Table 4 gives total pituitary weights and Table 5 the anterior pituitary weights.

TABLE 3.—THE WEIGHT OF THE BOVINE ANTERIOR PITUITARY.

Group	No. of glands	Mean in grams	Maximum in grams	Minimum in grams
Fetal	22	0.0478	0.1324	0.0064
56-140 days	7	0.0206	0.0452	0.0064
141-283 days	15	0.0605	0.1324	0.0185
Calf				
Up to and including 3 months	22	0.4962	0.7514	0.2351
Four months to and including 10 months	234	0.6973	1.5090	0.3348
Heifers—None included that was sexually mature	139	0.6885	1.2792	0.3348
Steers	67	0.7392	1.5090	0.5951
Bulls	28	0.6425	0.9992	0.3412
Eleven months to and including 23 months	163	0.9258	2.9449	0.3703
Heifers	114	0.9050	2.9449	0.3703
Open—None included that was sexually immature	76	0.8882	1.5038	0.3703
Pregnant	38	0.9387	2.9449	0.5618
56-140 days	28	0.9535	2.9449	0.5618
141-283 days	10	0.8974	1.1335	0.6063
Steers	42	0.9787	1.6187	0.5542
Bulls	7	0.9458	1.3127	0.7090
Cows—2 years and over	72	1.4962	2.2880	0.6190
Dairy	50	1.4621	2.2880	0.6190
Lactating and open	19	1.6546	2.2880	1.1101
Lactating and pregnant	7	1.3422	2.2296	0.6190
Dry and open	15	1.4320	2.5465	0.8703
Dry and pregnant	9	1.1992	2.1722	0.8247
Beef	22	1.5739	2.2799	0.7006
Lactating and open	3	1.9923	2.2799	1.7836
Lactating and pregnant	3	1.7489	1.7829	1.7230
Dry and open	7	1.3261	1.7176	0.7006
Dry and pregnant	9	1.5687	2.2213	1.1351

TABLE 4.—THE WEIGHT OF THE HEREFORD PITUITARY.

Group	No. of glands	Mean in grams	Maximum in grams	Minimum in grams
Fetal	13	0.0320	0.1656	0.0005
56-141 days	10	0.0143	0.0521	0.0005
141-283 days	3	0.0915	0.1656	0.0503
Calves up to and including 3 months	6	0.7468	0.8995	0.6030
Four months to and including 10 months	102	0.9351	1.5568	0.4866
Heifers—none included that was sexually mature	68	0.9213	1.5568	0.4866
Steers	27	0.9737	1.5090	0.5951
Bulls	7	0.9200	1.0520	0.7722
Eleven months to and including 23 months	53	1.1482	1.8982	0.5055
Heifers	35	1.1364	1.8982	0.5055
Open—none included that was sexually immature	21	1.1350	1.8982	0.5055
Pregnant	14	1.1384	1.3975	0.7434
56-140 days	12	1.1295	1.3975	0.7434
141-283 days	2	1.1916	1.2248	1.1585
Steers	16	1.1842	1.8491	0.8273
Bulls	2	1.0665	1.1948	0.9383
Cows—2 years old and over	13	1.8748	2.5389	0.8876
Lactating and open	3	2.3524	2.5389	2.0261
Lactating and pregnant	2	2.0791	2.1423	2.0160
Dry and open	5	1.5428	1.9926	0.8876
Dry and pregnant	3	1.8144	2.2118	1.4532

TABLE 5.—THE WEIGHT OF THE HEREFORD ANTERIOR PITUITARY.

Group	No. of glands	Mean in grams	Maximum in grams	Minimum in grams
Fetal	5	0.0515	0.1324	0.0005
56-141 days	2	0.0195	0.0235	0.0146
141-283 days	3	0.0732	0.1324	0.0424
Calves up to and including 3 months	6	0.5475	0.7131	0.4266
Four months to and including 10 months	102	0.7285	1.2792	0.3785
Heifers—none included that was sexually mature	68	0.7206	1.2792	0.3785
Steers	27	0.7503	1.0326	0.4590
Bulls	7	0.7212	0.7708	0.6079
Eleven months to and including 23 months	53	0.9164	1.6187	0.3703
Heifers	35	0.9034	1.3520	0.3703
Open—none included that was sexually immature	21	0.9068	1.3520	0.3703
Pregnant	14	0.8984	1.1321	0.5618
56-140 days	12	0.8834	1.1321	0.5618
141-283 days	2	0.9881	1.0065	0.9698
Steers	16	0.9566	1.6187	0.6402
Bulls	2	0.8236	0.9383	0.7090
Cows—2 years old and over	13	1.5856	2.2799	0.7006
Lactating and open	3	1.9923	2.2799	1.7836
Lactating and pregnant	2	1.7530	1.7829	1.7230
Dry and open	5	1.3206	1.6603	0.7006
Dry and pregnant	3	1.5090	1.9388	1.1351

Castration

For a number of years man has been interested in the influence of castration on pituitary size as is indicated by the early work of Fichera (1905).

In the male albino rat castration causes a marked increase in the weight of the pituitary gland, it making no difference whether the rats are castrated before or after reaching sexual maturity. These same changes hold true for the female rat, only in a much smaller degree.

Fichera (1905) obtained an increase in pituitary weight by spaying female guinea pigs, but Marrassini and Luciani (1911) reported no increase in pituitary size in castrated guinea pigs, either male or female. Even earlier than the latter paper, Marrassini (1910) reported that six male and six female guinea pigs had pituitary glands proportional to their body weight. Sometimes the glands from castrated animals were heavier, sometimes equal and sometimes lighter than those of the control animals.

Cimorini (1908) concluded that the castration of young dogs and rabbits produced an hypertrophy of the pituitary gland. The most extensive evidence in the rabbit indicates a slight increase in pituitary size in castrated and spayed animals.

Wittek's (1913) weights on bull and steer pituitary glands indicate a slight increase in pituitary size due to castration. The

TABLE 6.—INFLUENCE OF CASTRATION ON PITUITARY SIZE.

Sex	No. of animals	Age when castrated	Age when sacrificed	Body wt. when sacrificed	Pituitary weight (mg.)	Authority
			Rat—Albino			
F	27		269 days	141 gm.	9.2	Hatai (1913b)
CF	27	25- 30 days	269 days	159 gm.	10.5	Hatai (1913b)
F	41		Adult	162 gm.	6.1[1]	Lipschultz & Villagran (1936)
CF	35		Adult	165 gm.	6.7[1]	Lipschultz & Villagran (1936)
M	12		268 days	124 gm.	5.0	Hatai (1913b)
CM	25	25- 30 days	268 days	122 gm.	8.1	Hatai (1913b)
M	27		157 days	249 gm.	9.0	Hatai (1915)
CM	26	16- 22 days	157 days	226 gm.	12.3	Hatai (1915)
M	6		197 days	239 gm.	8.4	Hatai (1915)
CM	5	79- 93 days	197 days	227 gm.	11.4	Hatai (1915)
M	7		273 days	274 gm.	10.0	Hatai (1915)
CM	8	208-232 days	273 days	264 gm.	13.0	Hatai (1915)
M	8		81-103 days	251 gm.	7.4	Stein (1933b)
CM	8	21- 23 days	81-103 days	202 gm.	10.9	Stein (1933b)
M	72		Adult	201 gm.	5.0[1]	Lipschultz & Villagran (1936)
CM	43		Adult	192 gm.	8.2[1]	Lipschultz & Villagran (1936)
			Guinea Pig			
F	2				14.8	Fichera (1905)
CF	1	(Sacrificed 10 days after operation)			15.0	Fichera (1905)
CF	1	(Sacrificed 20 days after operation)			18.0	Fichera (1905)
CF	1	(Sacrificed 30 days after operation)			22.0	Fichera (1905)
F	8			422 gm.	13.0	Marrassini & Luciani (1911)
CF	8			430 gm.	12.9	Marrassini & Luciani (1911)
M	6			433 gm.	13.0	Marrassini & Luciani (1911)
CM	6			480 gm.	14.7	Marrassini & Luciani (1911)
			Rabbit			
F	2				17.0	Fichera (1905)
CF	1	(Sacrificed 10 days after operation)			20.0	Fichera (1905)
CF	1	(Sacrificed 20 days after operation)			22.5	Fichera (1905)
CF	1	(Sacrificed 30 days after operation)			31.0	Fichera (1905)
F	6			1561 gm.	19.0	Marrassini & Luciani (1911)
CF	6			1823 gm.	18.3	Marrassini & Luciani (1911)
F	11			2268 gm.	28.0	Livingston (1916)
CF	12			2496 gm.	29.0	Livingston (1916)
F	11			2249 gm.	25.9	Livingston (1916)
CF	9			2059 gm.	28.1	Livingston (1916)
F	5	Litter mates		2000 gm.	23.0	Livingston (1916)
CF	6	Litter mates		1878 gm.	25.2	Livingston (1916)
M	7			1726 gm.	24.1	Marrassini & Luciani (1911)
CM	7			1694 gm.	16.0	Marrassini & Luciani (1911)
M	8			2573 gm.	23.0	Livingston (1916)
CM	10			2795 gm.	28.0	Livingston (1916)
M	16			2028 gm.	25.5	Livingston (1916)
CM	21			2049 gm.	28.7	Livingston (1916)
M	8	Litter mates		1944 gm.	25.9	Livingston (1916)
CM	10	Litter mates		2182 gm.	27.0	Livingston (1916)
			Sheep			
--	5		6.0 years	48.4 kg.	827.0	Marrassini & Luciani (1911)
C	3		1.0 years	43.8 kg.	674.0	Marrassini & Luciani (1911)
			Cattle			
M	5				2350.0	Fichera (1905)
CM	5				4460.0	Fichera (1905)
M	5		5.6 years	595.0 kg.	3448.0	Marrassini & Luciani (1911)
CM	4		1.5 years	325.0 kg.	2074.0	Marrassini & Luciani (1911)
CM	8		1.0 years	198.0 kg.	2040.0	Wittek (1913)
CM	11		2.0 years	234.0 kg.	2430.0	Wittek (1913)
CM	18		3.0 years	254.0 kg.	2520.0	Wittek (1913)
CM	64		4.0 years	289.0 kg.	2770.0	Wittek (1913)
CM	112		5.0 years	335.0 kg.	3070.0	Wittek (1913)
CM	24		6.0 years	335.0 kg.	3230.0	Wittek (1913)
CM	18		7.0 years	342.0 kg.	3370.0	Wittek (1913)
CM	18		8.0 years	364.0 kg.	4320.0	Wittek (1913)
CM	11		9.0 years	421.0 kg.	4290.0	Wittek (1913)
			Buffalo			
M	5				1800.0	Fichera (1905)
CM	5				3450.0	Fichera (1905)
			Geldings			
CM	14			333.0 kg.	1760.0	Saito (1923)
			Fowl			
M	50				13.3	Fichera (1905)
CM	50				26.7	Fichera (1905)

[1]Anterior lobe weight. CF = Ovariectomized females. CM = Castrate males.

increase in size becomes more apparent when comparisons are made per unit weight of the dressed carcass.

Probably the most outstanding change in pituitary weight due to castration was that observed by Fichera (1905) in the cock. Based upon 50 observations in the cock and 50 in the capon he found that castration doubled the pituitary weight.

Kolde (1912) reported an increase in pituitary size in woman following castration.

Observations on the influence of castration on pituitary size are summarized in Table 6. For the weights of pituitary glands from bulls see Table 1.

Of the pituitary glands collected in this study, those from 67 steers between the ages of 4 and 10 months, on the average weighed 135 mg. more than the average weight of those from 28 bulls. The average weight of pituitary glands from 42 steers, varying in age from 11 to 23 months, was nearly identical with the average weight of glands from seven bulls of about the same age. Within the Hereford breed the pituitary glands from steers were slightly heavier than those from bulls (refer to Tables 2, 3, 4 and 5).

Pregnancy

Herring (1920) working with nine litter-mate pairs of rats found that the average pituitary weight of the normals was 8.2 mg., while that of pregnant animals was 6.2 mg., a 24 per cent difference. In every case the pituitary body of the pregnant rats was smaller than that of its control. The change appeared to be in the glandular lobe. Stein (1931) also working with litter-mates, reported very little difference in weights of pituitaries from normal and pregnant animals. Stein (1933a) extended his studies on the influence of pregnancy on pituitary size with much the same results as mentioned in his earlier work.

Kolde (1912) found that the pituitary enlarged during pregnancy in guinea pigs and rabbits. Allanson (1932) found that the pituitary glands from five pregnant rabbits varied greatly. However, all were within the weight range of pituitaries from normal rabbits.

Atwell (1930) observed no significant change in the relative volume of any of the epithelial lobes during the progress of pregnancy in the cat. He was of the opinion that the enlargement of the pituitary gland during pregnancy was due to hypertrophy or hyperplasia of all its lobes.

If one takes age and body weight into consideration in the observations made by Wittek (1913) on pregnant cows there appears to be no significant change in the pituitary gland during pregnancy. Blickenstaff (1934) reported that the average pituitary weight of open cows exceeded the average weight of glands from pregnant cows. He found a greater variation in the pituitary weights of open cows than in pregnant cows.

Saito's (1923) recorded pituitary weights on pregnant and non-pregnant mares seem to indicate an increase in pituitary size during pregnancy.

Kolde (1912) reported an enlargement of the pituitary gland in woman during pregnancy.

Although of a different nature, the observations of Stein (1934) and Wittek (1913) on the influence of recurring pregnancies on pituitary size are of interest. Stein (1934) determined the pituitary weights of a group of ten virgin white rats and a group of ten litter-mate test animals which had had three pregnancies in rapid succession. The controls had an average body weight of 212 gm. and an average pituitary weight of 13.05 mg., while the test animals' average body weight was 250 gm. and average pituitary weight was 13.52 mg. Wittek's (1913) observations on nulliparae and multiparae cows show about equal amounts of pituitary tissue per unit of body weight.

A resume of these observations is given in Table 7.

TABLE 7.—INFLUENCE OF PREGNANCY ON PITUITARY SIZE.

No. of animals	Age	Body weight	Pituitary weight (mg.)	Authority
		Rat—Albino		
9	----------	129 gm.----------	8.2	Herring (1920)
9	----------	129 gm.----------	6.2	Herring (1920)
20	----------	185 gm.----------	10.67	Stein (1931)
28	----------	175 gm.----------	10.14	Stein (1931)
22	108 days----------	184 gm.----------	10.5	Stein (1933a)
22	109 days----------	201 gm.----------	10.1	Stein (1933a)
22	153 days----------	198 gm.----------	10.3	Stein (1933a)
		Cows		
41	2.5—7 years-------	253 kg.----------	2630.0	Wittek (1913)
40	8.0—15 years------	235 kg.----------	2910.0	Wittek (1913)
		Mare		
6	----------	383 kg.----------	2060.0	Saito (1923)

The influence of pregnancy on bovine pituitaries collected in this study shows that the pituitary glands from pregnant heifers weighed, on the average, 42.6 mg. more than those taken from sexually mature and open heifers. Three of four groups of pregnant cows two years old or over had pituitary glands which averaged less than similar groups of non-pergnant cows. The observations on the heifer groups, which are the more extensive, indicate no significant weight change of the pituitary gland during pregnancy (refer to Table 2).

THE HORMONE CONTENT OF THE ANTERIOR LOBE OF THE PITUITARY GLAND

Fetal Pituitaries

Schultze-Rhonhof and Niedenthal (1929) implanted the entire pituitary gland of a fetus of man (41 cm. long and weighing 1,600 gm.) into an immature mouse weighing 7 gm. Sixty hours after the implantation the vagina of the mouse was opened and 84 hours later an estrum lasting 24 hours began. The mouse was sacrificed five days later and both ovaries showed large follicles.

Smith and Dortzbach (1929) implanted anterior pituitaries from pig fetuses into immature mice and into hypophysectomized rats in order to determine at which stages the principles stimulating ovarian maturity and body growth first appeared. The ovarian stimulating hormone could not be detected in fetal pituitaries until the fetuses reached a crown-rump length of 17 to 18 cm. It was present in amounts sufficient to give a constant maturity effect with moderate dosages only after the 20 to 21 cm. stage. They did not find the hormone stimulating general body growth in pituitaries of fetuses of 7 to 9 cm. in crown-rump length. It was present in detectable amounts at the 9 to 11 cm. stage and in larger amounts at the 11 to 13 cm. and later stages.

Nelson (1933) observed an augmentation of the ovaries and the opening of the vagina when the pituitaries of pig fetuses of 20 cm. and above were implanted into immature mice.

Catchpole and Lyons (1934) reported that the fetal horse pituitary, fetuses varying in crown-rump length from 50 to 90 cm., only infrequently contained the gonadotropic hormones. However, Hellbaum (1935) found that the pituitary gland from a 20 cm. horse fetus contained a sufficient amount of gonad stimulating factors to bring about definite ovarian stimulation when injected into a 21 day old female rat. From the larger fetuses and those at term little change was noted in the amount or character of the resultant ovarian response.

Bates, Riddle, and Lahr (1935) in assaying seven types of cattle pituitaries reported that fetal pituitaries contained more than twice as much lactogen as pituitaries from any other cattle type. The follicle simulating hormone content of fetal pituitaries was about the same as that which was found in pituitaries from adult bulls and non-pregnant cows. The thyrotropic content of fetal and steer pituitaries was about equal. The fetuses from which the pituitaries were taken, at least most of them, were between the ages of five and seven months.

The determined lactogen content of bovine fetal pituitaries.—The acetone dried powder from 31 fetal pituitaries was assayed against the acetone dried powder from 12 calf pituitaries. The average weight of the fetal pituitaries was 0.0444 gm., while the calf pituitaries averaged 0.7361 gm. The fetal pituitaries contained, on the average, 0.74 bird unit, the calf glands contained 111.4 bird units per pituitary. The degree of concentration of the hormone within the gland is indicated when one figures the number of bird units per unit weight of anterior lobe tissue. Accordingly, one gram of fresh fetal anterior pituitary tissue contained 22.5 bird units and one gram of fresh calf anterior lobe tissue contained 195.7 bird units.

As the work progressed upon the assay of cattle pituitaries it became obvious that the fetal pituitaries were the least potent for the lactogenic hormone. It was thought that perhaps the chemical treatment of the pituitary glands was altering their lactogen content. Consequently, six fetal pituitary glands were assayed without receiving any previous treatment whatsoever. These six glands contained 0.58 of a bird unit per pituitary gland, a smaller amount than was found in the composite fetal powder. The assay results on fetal pituitaries are summarized in Table 8.

TABLE 8.—LACTOGEN CONTENT OF FETAL PITUITARIES.

Class of animals	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average B.U.* per mg. of acetone dried powder	Average No. of B.U. per pituitary	Average B. U. per mg. of fresh pituitary weight	Average B.U. per mg. of fresh anterior pituitary weight
Fetal	31	0.0444	0.0329	6.2	0.12	0.74	16.6	22.5
Calf	12	0.7361	0.5693	116.0	0.96	111.4	151.3	195.7

*B. U.—Bird units.

Fetus Number	Estimated age (days)	Weight of pituitary gland (mg.)	Weight of anterior lobe (mg.)	Body wt. and sex of assay pigeon	Crop gland rating
368AA	109	19.8	16.1	326M	0.00
395AA	158	50.3	45.0	314F	0.25
385AA	155	64.4	50.7	312M	0.50
426AA	175	68.1	53.7	293F	0.75
471AA	208	96.4	82.1	361M	1.00
486AA	189	61.2	43.6	280F	1.00
Average of six	---	60.0	48.5	-----	0.58

Pituitaries From Growing and Mature Animals

Investigations by Domm (1931a, b) indicated that the pituitaries from cocks were more effective in inducing precocious development of sexual characters in the fowl than pituitaries from hens. Lipschütz, Kallas, and Paez (1929) found that the pituitary of the infantile mouse was rich in gonad stimulating hormone.

Smith and MacDowell (1931) reported that the pituitaries of dwarf mice contained a high concentration of the gonad stimulating hormone as was revealed by implants, whereas the growth hormone was not present in a sufficient amount to be detected.

Smith and Engle (1927) reported that glands from 5 to 30 day old rats induced sexual maturity in immature 17 day old mice when two to six glands were implanted daily for three days. The magnitude of the gonadal response was about equal to that induced by the administration of an adult gland. As shown by transplantation, the pituitary of a senile female rat contained sufficient gonadal-stimulating hormone to bring about premature sexual maturity. Evans and Simpson (1929b) observed that four pituitary glands taken from male rats when implanted into immature rats produced ovaries weighing 70 mg., whereas four pituitary glands from female rats often failed or barely succeeded in stimulating the ovaries, the ovaries weighing 20 mg. Other work by these investigators (1929a) showed that three male rat pituitaries produced changes in the immature ovary as great as those produced with five or six female pituitary implants. Employing the rabbit ovulation test, Ellison, Campbell, and Wolfe (1932) found that the anterior lobes of adult male rats were twice as potent as the same quantity of tissue from females. Lipschütz (1932) proposed a coefficient of luteinization by counting the number of corpora lutea in a single section of the ovary following a dose of anterior pituitary extract in immature rats. The relative activity of different preparations was then expressed on this basis. By this method it appeared that pituitaries from infantile female rats were 40 times as potent as those from normal adult females and twice as potent as those from castrated animals. Lipschütz and Reyes (1932a) compared male and female rat pituitaries for both follicle-stimulating hormone and luteinizing hormone. The pituitary of the adult female contained less of the follicle-stimulating hormone than the adult male. If a greater quantity of the anterior lobe of the adult female was injected, estrum was established, but there was no luteinization. Luteinization was sometimes absent when as much as 72 mg. of adult female pituitary tissue was administered, as against 21 mg. of male pituitary tissue given to a litter-mate. Somewhat later these two investigators (1932b) injected six young rats with known amounts of anterior lobe tissue from young female rats. Fifteen others received injections of material from adult females. The number of corpora lutea in the ovaries were estimated. The one to 1.5 mg. gland of the young rats had as much luteinizing action as the 6 mg. gland of the adult. Magistris (1932) reported that the pituitary glands from male and female rats contained about the same amount of gonadotropic hormones, while the glands from immature rats contained much smaller amounts. Hill (1934a) using the rabbit ovulation test demonstrated that male rat pituitary glands were more potent than glands from females. Clark (1935a) made an extensive study of the gonadotropic hormone content of the rat pituitary gland. She made implants into immature mice and found that from birth to 20 days of age the gonatropic hormone content of the anterior pituitary was markedly greater in females than in males of the same litter. At the time of puberty the male glands were more potent than those from females and when the rats were between the ages of four to six months the sex difference in anterior pituitary content was negligible. By the seventh month the male pituitary glands were more potent. In the female a marked increase in the gonadotropic content of the pituiary occurred when the animals were 13 to 20 days old. The rise in content reached a point that was not surpassed, even after full maturity was reached. In the males, a more gradual increase continued up to puberty. This level was then fairly evenly maintained throughout the period of the experiment, which was ten months. McQueen-Williams (1935b) determined the gonadotropic content of rat anterior pituitaries by implanting them into immature females. Seven pituitaries were usually implanted. In 18 to 23 day old rats, the female pituitaries were very potent, while the hormone was much lower in the male. In 27 to 30 day old rats, the female glands still showed a high hormone content. A prepuberal rise in gonadal-stimulating capacity took place in the male. In 35 to 38 day old rats, a sudden prepuberal drop in the amount of hormone had occurred in both females and males. Although the pituitaries were heavier, they had lost two-thirds of their potency in the period of one week. In 42 to 44 day old rats, the female pituitary potency had decreased. More than half the female rats in this group had just matured. The potency of the male pituitaries remained the same. In rats over four months old the female pituitaries caused but slight ovarian enlargement and rarely produced corpora, whereas male glands induced ovaries three and one-half times as heavy. Lipschütz and Villagran (1936) reported that the anterior pituitary gland of male rats had an average luteinizing coefficient of one while glands from females had no luteinizer.

Severinghaus (1932a) found no sex difference in the potency of pituitary glands of normal guinea pigs when they were implanted into immature mice. However, Hill (1934a) using the estrus rabbit as the assay animal, found that the pituitaries from female guinea pigs contained three times as much gonadotropic hormone as did pituitary glands from male guinea pigs. Nelson (1935) reported that the ovarian response following implantation of equal amounts of normal male and female pituitaries into immature female rats and mice was 50 per cent greater in the case of the male.

Wolfe and Cleveland (1931) tested the capacity of anterior pituitary tissue, taken from mature and immature female rabbits, to induce ovulation in sexually mature rabbits. They concluded that anterior-lobe tissue from immature female rabbits, as young as three months of age, and from mature females, had equal capacity to induce ovulation. Magistris (1932) found that the male rabbit pituitary contained more gonadotropic power than glands from females. This was especially true of their ability to cause luteinization of the immature mouse ovary. The work of Smith, Severinghaus, and Leonard (1933) indicated that the normal male rabbit pituitary gland, per unit weight of tissue, was about twice as potent as the pituitary gland from a normal female rabbit, as judged by their ability to stimulate the sexually immature mouse ovary. Employing the estrus rabbit for assay purposes, Hill (1934a) reported the mature female rabbit pituitary most potent, with male pituitaries and pituitaries from three to four month old females to be of like potency.

Hill (1934a) found that male cat pituitaries contained nearly four times as many units of gonadotropic hormone as did glands from female cats. On the other hand, Magistris (1932) employing the immature mouse for assay purposes reported that pituitaries from female cats were about twice as potent as glands from male cats.

Hill (1934a) observed that pituitaries from female dogs contained more gonadotropic hormone than the pituitaries from male dogs.

According to Magistris (1932) pituitaries from cows and bulls compared quite favorably in regard to their gonadotropic content. Calf pituitaries contained a smaller number of follicle-stimulating hormone units, but they contained nearly as many luteinizing hormone units. Pituitaries from the fattened ox were nearly devoid of gonadal-stimulating properties.

Hellbaum (1935) assayed horse pituitaries for their gonadotropic properties by injecting the acetone dried powder into immature female rats. This work was based upon the potency per unit weight of dried acetone powder and not upon potency per pituitary gland. The average weight of the ovaries from control animals was 12.5 mg., the ovaries from rats injected with colt pituitary powder averaged 33.0 mg., those from rats injected with stallion powder 22.9 mg., those from rats injected with non-pregnant mare powder 88.2 mg., and those from rats injected with old mare pituitary powder 114.5 mg.

Ueno and Murata (1931) measured the hormone content of human anterior pituitary tissue by the degree of appearance of puberty phenomena in mice after the injection of its aqueous extract. The hormone increased with age to the adult stage and then began to decrease during senility. The hormone was present in man and woman in the same quantities. Lipschütz and Del-Pino (1936) concluded that the coefficient of luteinization of the human pituitary was less in the female, but the difference between the two sexes was not very great.

Emery and Winter (1934) made a study of adrenal hypertrophy produced by pituitary implants or extracts in about 100 rats. The hypertrophy was not obtained in rats under 30 days of age, but in older rats, three to 24 months of age, age was not a factor in determining the amount of hypertrophy. The sex of the donor was an important factor in determining the amount of adrenal hypertrophy in the recipient. This was shown by the fact that a greater effect was obtained with pituitaries from female rats, guinea pigs, and cattle than with pituitaries from males.

Bates, Riddle, and Lahr (1935) reported on the lactogen and thyrotropic content of cattle pituitaries. They assayed their pituitary extracts with immature doves and found the following number of lactogen units per gram of fresh tissue: veal calves, 26 units; adult bulls, 34 units; and non-pregnant cows, 26 units. The results of their thyrotropic assay were expressed as per cent increase in the weight of the thyroid glands. Pituitaries from veal calves elicited a 63 per cent increase in thyroid weight, bull pituitaries a 93 per cent increase and non-pregnant cows an 103 per cent increase.

Loeb and Bassett (1930) reported that the subcutaneous inoculation of cattle pituitary was much more effective in causing hypertrophic changes in the thyroid than was the guinea pig pituitary.

Loeb (1932) observed that the daily injection of four pituitaries from adult male guinea pigs (weighing 350 to 500 grams) into female guinea pigs weighing 170 to 200 grams produced no hypertrophic change. Moreover, there was no indication that the liquefaction and absorption of the colloid was accelerated in any noticeable way by this procedure. When the pituitaries of male rabbits (weighing 1400 and 2000 grams) were injected a more distinct hypertrophy of the thyroid was produced than from the four guinea pig pituitaries. The effect of one or two cat pituitaries on the guinea pig thyroid gland was relatively slight and on the whole much colloid was retained in the thyroid acini. The daily intraperitoneal injection of one to three cubic centimeters of an acid extract from cattle anterior pituitary stimulated the thyroid gland very strongly. The hypertrophy and hyperplasia of the acinar epithelium was accompanied by partial or almost complete solution and absorption of the colloid.

Junkmann and Schoeller (1932) reported that the rat pituitary contained much more of the thyrotropic hormone than the guinea pig pituitary.

Rowlands and Parkes (1934) found that one gram of fresh pituitary tissue from cows contained about six units or 30 units per gram of dessicated tissue. Anderson and Collip (1934) reported that pituitary glands of cattle, pigs, and sheep were excellent sources of the thyrotropic principle.

In 1934 Stein reported that the anterior lobes of adult fowl and chick pituitaries, provided a sufficient number of glands were implanted, caused ovulation out of season in Triturus viridescens females and the thyroids of these females were stimulated beyond the normal condition.

Kunkel and Loeb (1935) implanted one-fourth of a human pituitary into guinea pigs for four days and noted a stimulation of the thyroid and adrenal glands. Injection of one cubic centimeter of an acid extract daily for eight days produced a less marked effect. Müller, Eitel, and Loeser (1935) found that the human pituitary gland, like animal pituitaries, contained the thyrotropic hormone. There was considerable variation in thyrotropic content and it seemed to be independent of age and sex. The highest content was found in tubercular patients and those with other infections.

McQueen-Williams (1935c) reported that the ratio of the amount of thyrotropic hormone in rat and beef pituitaries was entirely different from the ratio of the adrenotropic content in pituitaries of these two forms. Per unit of weight, adult male rat pituitaries were seven to nine times as potent in thyrotropic hormone as beef pituitary glands, whereas beef pituitaries exceeded male rat pituitaries in their ability to cause an hypertrophy of the adrenal cortex of adult male rats. Intramuscular implants into immature male guinea pigs and subsequent histological study of the thyroids showed that as good a response could be obtained with

6 mg. of male rat pituitary as with 50 mg. of pituitary tissue from beef animals.

Chen and van Dyke (1936) compared the thyrotropic potencies of the anterior pituitary of normal, litter-mate male, and female rabbits. The doses were based upon the total weights of the anterior pituitaries. They observed no difference in the thyrotropic content of male and female anterior pituitaries.

Loeb, Saxton, and Hayward (1936) observed that the strongest action on the thyroid was exerted by extracts of cattle, sheep, and hog anterior pituitaries. The implantation of a rat or rabbit pituitary produced a response that was intermediate when compared with cattle and guinea pig pituitaries. The implantation of a human pituitary produced a marked effect, however, this might have been due to the relatively large size of this gland as compared with the anterior pituitaries of rabbits and rats. When equal amounts of cattle and human anterior pituitaries were injected, it was found that the effects exerted by cattle anterior pituitaries, as a rule, were greater.

Rowlands (1936) determined the thyrotropic activity of pituitaries of various species. An alkaline extraction was made and the extracts were assayed on immature guinea pigs. The following number of units per gram of desiccated powder were found: cow, 20; horse, 5; sheep, 8; pig, 40; and dog, 43. By pyridine extraction one gram of desiccated powder of cow, sheep, and pig pituitary contained 28, 16 and 54 units, respectively.

The determined lactogen content. *Mice.* Twenty-four pituitaries from normal female mice were assayed. These pituitaries had an average weight of one milligram and contained one-fourth of a pigeon unit of lactogen per gland.

Rats. Ten immature female rats with an average body weight of 76 gm. had pituitaries that weighed 3.3 mg. per gland. These ten pituitary glands contained 0.94 of a pigeon unit per gland. As the female rat matures there is an increase in the size of the pituitary gland and this is accompanied by an increase in the number of bird units of lactogen per pituitary gland. This increase in lactogen unitage is due to two factors: first, an increase in pituitary size; and secondly, an increase in the concentration of the lactogenic hormone within the gland. In four old non-breeding females the lactogen content had decreased somewhat.

The pituitary glands from ten immature male rats, having an average body weight of 84 gm. and an average pituitary weight of 2 mg., contained 0.3 of a bird unit per gland. The greater lactogen content of the immature female pituitary gland in comparison with the immature male pituitary gland is due to greater pituitary size and greater hormone concentration. An increase in body weight in the male is also accompanied by an increase in pituitary size. Increase in pituitary size resulted in an increased amount of lactogen per pituitary gland, but the concentration of the hormone within the gland remained nearly constant.

Guinea pigs. The lactogen content of the pituitary glands taken from growing female guinea pigs follows much the same course as in growing rats. With increase in body weight there is an increase in pituitary weight and an increase of hormone concentration in the glandular tissue. Seven guinea pigs with an average body and pituitary weight of 112 gm. and 4.1 mg., respectively, assayed 0.89 of a bird unit per pituitary gland. Another group of 11 guinea pigs averaged 335 gm. in body weight. Their pituitaries averaged 9 mg. and contained 4.68 bird units per pituitary. The lighter group guinea pig pituitaries assayed 0.217 of a bird unit per one mg. of fresh pituitary tissue while the glands from the heavier group contained 0.520 of a bird unit.

Pituitary glands from male guinea pigs were considerably richer in the lactogenic hormone than were the glands from male rats. The glands from immature male guinea pigs contained slightly more lactogen per milligram of fresh pituitary tissue than those from immature females. In medium sized guinea pigs the pituitaries from females were more potent for the lactogenic hormone than those from males. Pituitary glands from old male guinea pigs were very high in lactogen content. As will be presented in a later section, the pituitaries from lactating females compared favorably in lactogen content with those glands secured from old males.

Rabbits. The pituitary glands of two immature female rabbits weighed 9.3 mg. per gland and contained 1.31 bird units per gland. In three estrus rabbits, pituitaries weighed 22.1 mg. each and on assay gave 9.29 bird units per gland. This greater unitage of lactogen in the pituitaries of mature animals is brought about by an increase in pituitary size and an increase in the amount of hormone per unit weight of fresh pituitary tissue. The concentration of the lactogenic hormone per milligram of pituitary tissue is somewhat lower in the estrus rabbit than in the normal guinea pig.

The pituitary glands of three immature male rabbits contained 1.42 bird units of lactogen per pituitary, while one solitary gland from a mature male rabbit contained only 1.63 units. Inas-

TABLE 9.—THE LACTOGEN CONTENT OF PITUITARIES COLLECTED FROM LABORATORY ANIMALS.

Sex	No. of animals	Average body weight when sacrificed (gm)	Average pituitary weight (mg.)	B.U.* per pituitary gland	B.U. per mg. pituitary tissue	B.U. per 100 gms. bocy weight
			Mice			
F	24	19	1.0	0.25	0.250	1.32
			Rats			
F	10	76	3.3	0.94	0.284	1.24
F	14	128	5.3	1.98	0.373	1.54
F	21	176	8.9	4.29	0.482	2.43
F	10	220	10.1	4.76	0.471	2.16
F	4	271	11.5	4.03	0.350	1.48
M	10	84	2.0	0.30	0.150	0.35
M	7	143	4.2	0.82	0.195	0.57
M	44	177	5.0	0.84	0.168	0.47
M	21	221	5.5	0.89	0.161	0.40
M	26	270	6.9	0.99	0.143	0.36
M	14	318	8.1	1.31	0.161	0.41
			Guinea Pigs			
F	7	112	4.1	0.89	0.217	0.79
F	11	335	9.0	4.68	0.520	1.40
M	15	156	5.7	1.81	0.317	1.16
M	16	243	6.4	2.23	0.348	0.91
M	4	504	12.8	10.73	0.838	2.12
M	3	779	17.2	15.92	0.925	2.04
			Rabbits			
F	3	2822	22.1	9.29	0.420	0.32
F	2	656	9.3	1.31	0.140	0.19
M	1	2951	21.9	1.63	0.074	0.05
M	3	547	6.5	1.42	0.218	0.25
			Cats			
F	12	1858	20.0	1.31	0.065	0.07
F	1	2043	34.6	7.75	0.224	0.38
M	7	2233	23.8	0.89	0.037	0.03

*Bird units

much as the mature male's pituitary gland was slightly over three times as large as the average of the glands from immature males, there followed a marked decrease in lactogen concentration.

Cats. The pituitaries from cats proved interesting. The pituitary glands from 12 anestrus cats weighed 20.0 mg. on the average, and upon assay yielded only 1.31 bird units per gland. The pituitary gland from a female cat that was collected during the breeding season contained six times as much lactogen as was present in the pituitary of an anestrus cat.

Pituitary glands from males that were collected at a time when female cats were in anestrum were nearly devoid of the lactogenic hormone. These seven pituitary glands weighed 23.8 mg. on the average and assayed 0.89 of a bird unit per gland. Of the pituitaries assayed from laboratory animals those from male cats had the lowest concentration of lactogen.

The assay results of pituitaries from laboratory animals are given in Table 9.

Cattle. Pituitaries from calves were assayed against pituitaries from bulls and heifers between the ages of four and ten months, two such comparisons being made. In the first comparison the calf pituitaries contained 124 bird units per gland and the

TABLE 10.—THE LACTOGEN CONTENT OF IMMATURE AND MATURE CATTLE PITUITARIES.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average B. U.* per mg. of acetone dried powder	Average No. of B. U. per pituitary	Average No. of B. U. per gm. of fresh pituitary	Average No. of B. U. per gm. of fresh anterior pituitary
Calves—under 4 months	12	0.7361	0.5693	116.0	1.07	124.1	168.5	217.9
Bulls and Heifers—4 to 10 months	79	0.8797	0.6841	135.1	1.35	182.4	207.3	266.6
Calves—under 4 months	9	0.5633	0.4238	84.2	1.86	156.6	278.0	369.5
Bulls and Heifers[3]—4 to 10 months	39	0.9655	0.7414	126.1	2.45	308.9	319.9	416.6
Bulls—4 to 10 months	3	0.9829	0.7621	138.0	2.15	296.7	301.8	389.1
Heifers[3]—4 to 10 months	36	0.9465	0.7396	125.1	2.65	331.5	350.2	448.2
Heifers[3]—4 to 10 months	36	0.9465	0.7396	125.1	1.75	218.9	231.3	296.0
Heifers[3]—11 to 23 months	12	1.1769	0.9350	146.3	3.00	438.9	372.9	469.4
Bulls—4 to 10 months	17	0.8051	0.6240	132.9	1.40	186.0	231.0	289.0
Bulls—11 to 23 months	6	1.1209	0.8847	166.6	1.50	249.9	222.9	282.4
Steers[3]—11 to 23 months	5	1.4186	1.1773	183.7	1.15	211.3	148.9	179.5
Heifers[3]—11 to 23 months	12	1.1769	0.9350	146.3	1.80	263.3	223.7	281.6
Beef cows—dry and open	7	1.5486	1.3261	213.4	3.50	746.9	482.3	563.2
Dairy cows—dry and open	13	1.8018	1.4989	267.4	5.42	1449.3	804.6	966.9

*Bird Units. [3]Herefords.

glands from bulls and heifers averaged 182 units. Per gram of fresh anterior lobe tissue the calf pituitaries contained 218 units and the bull and heifer pituitaries 267 units. In the second comparison the calf pituitaries assayed 157 and the bull and heifer glands 309 units. One gram of fresh anterior lobe tissue from calf pituitaries contained 370 units while an equal weight of fresh anterior lobe tissue from bulls and heifers gave 417 units. The increase in unitage of the older groups of animals was due to larger pituitary glands and an increase in lactogen concentration within the glandular tissue.

Bull pituitaries were then compared with heifer pituitaries, each group coming from animals between the ages of four to ten months. Heifer pituitaries, per unit weight of anterior lobe tissue, contained 15 per cent more lactogen than bull pituitaries.

Pituitaries from four to ten month old heifers were assayed against pituitaries from 11 to 23 month old heifers. In the younger group of heifers none was included that was sexually mature and in the older group none was included that was sexually immature. The pituitaries from four to ten month old heifers contained 219 bird units per gland while the glands from the older animals contained 439 units. Per unit weight of fresh anterior lobe tissue the glands from the older heifers contained 59 per cent more lactogen than glands from the younger group.

Pituitary glands collected from four to ten month old bulls were then compared with glands collected from 11 to 23 month old bulls. The glands from the older bulls were heavier and contained 64 more units per gland. However, the concentration of the hormone within the glands in the two groups was practically identical.

Pituitaries from heifers 11 to 23 months old were run against pituitary glands from a group of steers of the same age. This was done because glands from bulls were not available at that time. The heifer pituitaries were lighter in weight, nevertheless, they contained 52 more units of lactogen per gland and 102 more units per gram of fresh anterior lobe tissue.

Of primary interest was the comparison made of dairy and beef pituitaries. Pituitaries from dairy and beef cows two years old or over were assayed. Three other group comparisons were made between dairy and beef pituitaries, but the comparison between groups of dry and non-pregnant cows should indicate the true difference of lactogen content of dairy and beef cattle pituitaries inasmuch as these groups are not complicated by lactation and pregnancy. The pituitary glands from the dairy cows were heavier and contained nearly twice as many bird units of

TABLE 11.—THE THYROTROPIC CONTENT OF PITUITARIES FROM IMMATURE AND MATURE CATTLE.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average G.P.U.* per mg. of acetone dried powder	Average No. of G.P.U. per pituitary	Average No. of G.P.U. per gm. of fresh pituitary	Average No. of G.P.U. per gm. of fresh anterior pituitary
Calves—under 4 months	9	0.5633	0.4238	84.2	0.133	11.20	19.86	26.40
Heifers[3]—4 to 10 months	36	0.9465	0.7396	125.1	0.190	23.77	25.11	32.13
Bulls[3]—4 to 10 months	3	0.9829	0.7626	138.0	0.212	29.26	29.76	38.36
Heifers[3]—11 to 23 months	12	1.1769	0.9350	146.3	0.157	22.99	19.53	24.59
Bulls—11 to 23 months	6	1.1209	0.8847	166.6	0.186	30.99	27.64	35.02
Beef cows—dry and open	7	1.5486	1.3261	213.4	0.140	29.88	19.29	22.53
Dairy cows—dry and open	13	1.8018	1.4989	267.4	0.202	54.01	29.98	36.03

*Guinea pig units. [3]Herefords.

lactogen as was contained in the glands from beef cows. Per gram of fresh anterior lobe tissue the tissue from dairy cows contained 967 units and that from beef cows 563 units, or 72 per cent more lactogen in dairy cow pituitaries.

The assay results of cattle pituitaries are summarized in Table 10.

In the species studied one can see that there is an increase in the number of bird units of lactogen per pituitary gland as the animals mature. In the case of males this lactogen increase is due to the increase in pituitary size while in the female two factors are operating to bring about an increase in lactogen content of the pituitary glands of mature animals; first, an increase in pituitary size and secondly, an increase in the concentration of the hormone per unit weight of pituitary tissue. The superiority of female pituitaries over male pituitaries became evident in immature animals and more pronounced after the females reached sexual maturity.

The determined thyrotropic content. *Cattle.* Pituitary glands from calves contained 11.2 guinea pig units per gland or 26.4 units per gram of fresh anterior lobe tissue. The pituitaries from four to ten month old bulls contained 38 guinea pig units of the thyrotropic hormone and those from a similar aged group of heifers contained 32 units. Per unit weight of fresh anterior lobe tissue the bull pituitaries were 19 per cent more potent than those from heifers. In older animals the difference in thyrotropic content of the pituitaries of males and females becomes more apparent, the anterior lobes from bulls containing 42 per cent more than the heifer lobes. Pituitary glands from four to ten month old heifers contained more thyrotropic hormone than those from 11 to 23 month old heifers. Per unit weight of anterior lobe tissue, the tissue from the younger group of heifers contained 31 per cent more of the hormone than that from the older group of heifers. However, in the two age groups of bulls there was only a very slight advantage in favor of the younger group. The thyrotropic content of pituitaries from 11 to 23 month old heifers, when compared on the basis of equal pituitary weight, was about the same as that in glands from dry and open beef cows.

The profound influence of desiccated thyroid feeding and thyroxine injection upon milk and milk fat production has been well established. Inasmuch as dairy and beef cattle differ tremendously in their ability to secrete milk it is exceedingly interesting to note that pituitary glands from dry and open dairy cows yielded more thyrotropic hormone upon assay than those from dry and open beef cows. One gram of anterior pituitary tissue from dry and open dairy cows contained 36 units of the thyrotropic principle, while one gram of tissue from dry and open beef cows contained 22.5 units, or a 60 per cent increase in favor of the glands from dry and open dairy cows.

Thus, the thyrotropic concentration in the pituitary gland increases until the animals reach four to ten months, after which it decreases. Pituitaries from bulls are definitely more potent for the thyrotropic hormone than glands from heifers and pituitary glands from dry and open dairy cows are a richer source of the thyrotropic hormone than pituitaries from dry and open beef cows. For a resume of the assay results see Table 11.

Pituitaries From Animals in Various Stages of the Estrus Cycle

Smith and Engle (1929) reported their work which gave evidence of a correlation between the amount of gonadal stimulating hormone present in the pituitary of the guinea pig and the stage of the reproductive cycle. Only a slight ovarian response was secured from donors sacrificed during the stage when the vaginae were opened, while those sacrificed during diestrum gave a satisfactory response.

Schmidt (1937) studied the gonadotropic content of the guinea pig pituitary gland during various stages of the estrus cycle. The pituitary glands from normal sexually mature guinea pigs were removed at different stages of the estrus cycle and implanted into immature female guinea pigs. She found that pituitaries from donors in estrum were ineffective. Pituitaries from donors 5 to 6 days following estrum caused little or no follicular stimulation, but the vaginal membranes ruptured. Pituitaries from guinea pigs 7 to 10 days after estrum caused growth of the follicles but the vaginae remained closed. Maximum follicle-stimulating effect was secured with pituitaries from donors 11 to 15 days following estrum. The recipients of these glands showed all signs of estrum.

Wolfe (1930, 1931) collected pituitaries from sows which were in various periods of the estrus cycle. A saline suspension of the fresh tissue was injected intravenously into sexually mature female rabbits, in an attempt to induce ovulation. He found that one milligram of fresh tissue would induce ovulation in the rabbit when the pituitary was taken from an animal whose ovaries contained follicles, measuring six or seven millimeters in diameter, and degenerate corpora lutea. When the sows were in heat 10 mg. of anterior lobe tissue were necessary to induce ovulation. Forty milligrams of anterior lobe tissue were required to induce ovulation when the donors' ovaries contained active corpora lutea.

Siegert (1933a, b) found that the pituitaries taken from rats during diestrum caused a positive response in 90 per cent of the recipients. Pituitaries collected when the donors were in estrum elicited a postive response in only 5 per cent of the recipients, the recipients being infantile rats.

Hill (1934b) employing the rabbit ovulation technique, assayed the pituitaries of rabbits. He found that pituitaries from estrus rabbits contained 12 units per gland while pituitaries taken from rabbits one-half hour after copulation contained 10 units per gland. Twenty-four hours after mating the gonadotropic potency was greatly decreased, pituitaries of animals at this time containing only 1.5 units.

The determined lactogen content of rat pituitaries.—During the course of this study normal female rats have been sacrificed so that their pituitaries could be compared with those from experimental animals. Vaginal smears have been made on a part of these normal females and the assay results of their pituitaries have been grouped according to the stage of the estrus cycle of the donors.

The pituitary glands taken from rats that were in diestrum contained 3.8 bird units per pituitary gland, those from rats in proestrum 4.4 units, those from rats in estrum 4.8 bird units, and those from rats in metaestrum 3.3 units per gland. Thus, as the

TABLE 12.—THE LACTOGEN CONTENT OF RAT PITUITARIES COLLECTED DURING VARIOUS STAGES OF THE ESTRUS CYCLE.

No. of animals	Stage of estrus cycle when sacrificed	Average body weight when sacrificed (gm.)	Average pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
5	Diestrum	211	9.1	3.85	0.423	1.82
12	Proestrum	186	9.8	4.42	0.451	2.37
13	Estrum	200	9.2	4.83	0.525	2.41
7	Metaestrum	189	8.7	3.36	0.386	1.77

*Bird units

animals approach estrum there appears to be an increase in the lactogen content of their pituitary glands, this content reaching its height during estrum and decreasing thereafter. These assay results are summarized in Table 12.

Pituitaries From Pregnant Animals

Evans and Simpson (1929c) reported that immature rats which had received anterior lobe implants from pregnant rats exhibited slightly heavier ovaries than their litter-mate sisters which had received implants from non-pregnant rats. Siegert's (1933a, b) results demonstrated that pituitaries from pregnant rats contained slightly more gonadal-stimulating power than pituitaries from rats which were in estrum. However, pituitaries from rats which were in diestrum contained six times as much gonadal-stimulating principle as those from pregnant rats.

Hill (1934b) demonstrated that the gonadotropic content of the rabbit pituitary gland increased rapidly as pregnancy continued. During late pregnancy the potency of the pituitaries decreased to a figure below the content of estrus rabbit pituitaries.

Anterior lobe tissue taken from pregnant sows has a great variation in its capacity to induce ovulation as was reported by Wolfe (1931). Considered as a whole, his data indicate that the anterior lobe of the pregnant sow has a decreased capacity to induce ovulation in the sexually mature rabbit when compared with glands taken from non-pregnant donors sacrificed in the proestrus period. Magistris (1932) reported that pituitaries from non-pregnant sows contained 12.5 mouse units of the follicle stimulating hormone and seven mouse units of the luteinizing hormone. Pituitaries from pregnant sows contained 20 units of the follicle stimulating hormone and 14 units of the luteinizing hormone.

In 1929c Evans and Simpson observed that immature rats which had received anterior lobe implants from pregnant cows exhibited slightly heavier ovaries than their litter-mate sisters which had received implants from non-pregnant cows. Bacon (1930) employing pituitaries obtained from pregnant cows, implanted immature mice with pieces of anterior lobe tissue varying in weight from 5.0 to 50 mg. Most of the material from pregnant donors contained less of the follicle stimulating hormone than that from non-pregnant donors. The greatest difference was found when the implants weighed less than 20 mg. Magistris (1932) found considerably more gonadal-stimulating hormone in the pituitaries of pregnant cows than in non-pregnant cows. There were 537 mouse units of the follicle stimulating hormone and 188 mouse units of the luteinizing hormone in pregnant cow pituitaries as compared with 137 and 62.5 mouse units in non-pregnant cow pituitaries, respectively.

Catchpole and Lyons (1934) found that the pituitary of the mare passed through a very definite variation in activity during pregnancy. The pituitary tissue was assayed on 21 day old female rats. Fifty milligrams of pituitary tissue from mares with fetuses from 0.6 to 2.9 cm. in crown-rump length evoked either no reaction or a borderline one only. In mares with fetuses over 2.9 cm. in crown-rump length, 50 mg. usually gave an undoubted ovarian response. They thought this might be due, in part, to the activity of contained blood. Considerable amounts of pituitary tissue from mares with fetuses more than 34 cm. in crown-rump length seldom showed any reaction. Hellbaum (1935) assayed the pituitaries from pregnant and non-pregnant mares for their gonadotropic potency. The average response elicited by the acetone dried powder of these two groups was nearly identical when injected into immature rats. However, the pituitaries of pregnant mares showed greater individual differences than those of non-pregnant mares. The pituitary glands from mares in early pregnancy, as compared with the average potency of non-pregnant mare pituitaries, showed considerable decline, the gonadotropic potency being lowest at this time. As pregnancy advanced, the gonadal-stimulating powers increased and reached a maxium when the fetus was about 15 cm. in crown-rump length. The hormone content then decreased so that just before parturition the gonadotropic content was about the same as the average value secured for the non-pregnant mare.

Philipp (1930) found that as a whole the material from pregnant human donors exhibited less of the follicle stimulating hormone than that from non-pregnant women. Ehrhardt and Mayes (1930) reported that the injection of an extract of anterior lobe tissue from 32 non-pregnant patients gave a positive reaction in 30 cases, while an extract of anterior lobe tissue from eight pregnant or puerperal women was negative. Zondek (1931) reported that the anterior lobe of four women contained 100 to 160 mouse units of the follicle stimulating hormone and 23 to 50 mouse units of the luteinizing hormone: On the other hand, pituitaries from five pregnant or puerperal women contained little or none of the gonadal-stimulating substance.

Bates, Riddle, and Lahr (1935) reported that pituitaries of cows in either early or late pregnancy contained considerably more of the lactogenic hormone than pituitaries from non-pregnant cows. Pituitary extracts made from non-pregnant cows increased the immature dove's thyroid 103 per cent, extracts of pituitaries from cows in early pregnancy increased the thyroid weight 124 per cent, while an extract of pituitaries collected from cows in late pregnancy elicited an 82 per cent increase in thyroid weight.

The determined lactogen content. *Rats.* Virgin female rats were placed with males and vaginal smears made daily. The day that sperms were found in the vaginal smear was considered as the first day of pregnancy. Nine rats were sacrificed on the 12th day of pregnancy and eight rats on the 21st day of pregnancy. The pituitaries from the rats which were pregnant for 12 days contained 2.9 units per gland as compared with 3.5 bird units per gland just before parturition. Inasmuch as pituitaries from normal female rats, whose average body weight was 176 gm., contained 4.29 bird units of lactogen per gland (Table 9), one notices that there was a slight decrease in lactogen content of glands taken from rats at the middle of pregnancy. Just prior to parturition the lactogen content of the rat pituitary gland was slightly greater than the content at the middle of pregnancy. The assay results of pituitaries from pregnant rats are summarized in Table 13.

TABLE 13.—LACTOGEN CONTENT OF PREGNANT RAT PITUITARIES.

No. of animals	Stage of pregnancy (days)	Average body weight when sacrificed (gm.)	Average pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B U. per 100 gm. body weight
9	12	180	8.1	2.90	0.358	1.61
8	21	214	7.7	3.50	0.455	1.64

*Bird units

The mammary glands of rats which were pregnant for 12 days consisted of nests of alveoli and considerable fatty tissue, none of the alveoli containing secretory products (Fig. 1). At the 21st day of pregnancy the alveoli have begun to unfold and there is evidence of beginning secretory activity at this time (Fig. 2). Hence, as pregnancy continues there is a slight increase in the lactogen content of the pituitary gland and this is followed by secretory activity of the alveoli of the mammary glands.

Cattle. The pituitary glands of heifers which had been pregnant from one to 140 days contained 350.7 bird units per gland while those from heifers pregnant from 141 to 283 days contained 297.6 bird units of lactogen per gland. This greater lactogen content in the first group of heifers is due to slightly greater pituitary size and greater concentration of the hormone within the gland.

Pituitary glands from lactating and open beef cows contained about the same number of bird units of lactogen per unit weight of anterior lobe tissue as those from lactating and pregnant beef cows. However, pituitary glands from dry and pregnant

Fig. 1.—Photomicrograph of a section of the mammary gland of a rat (15) which was pregnant for 12 days. The gland consisted of nests of alveoli and considerable fatty tissue. None of the alveoli contained secretory products. The pituitaries from a group of rats pregnant for 12 days contained an average of 2.9 bird units of lactogen. X60.

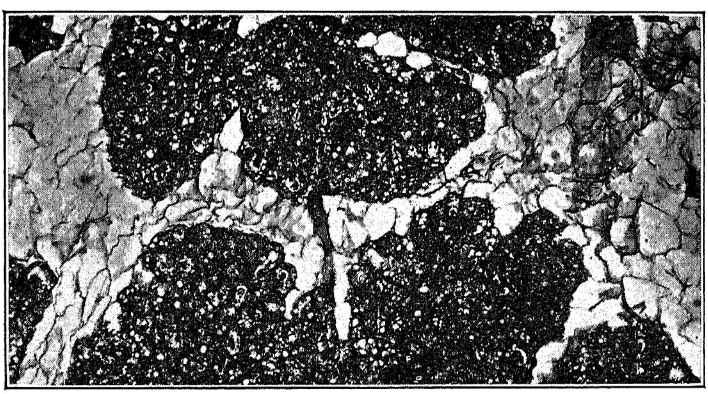

Fig. 2.—Photomicrograph of a section of the mammary gland of a rat (19) which was pregnant for 21 days. The alveoli have begun to show evidence of secretory activity. The pituitaries from a group of rats pregnant for 21 days contained an average of 3.5 bird units of lactogen. X60.

Table 14.—The Lactogen Content of Pituitaries from Pregnant Cattle Compared with Non-Pregnant Cattle Pituitaries.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average B. U.* per mg. of acetone dried powder	Average No. of B. U. per pituitary	Average No. of B. U. per gm. of fresh pituitary	Average No. of B. U. per gm. of fresh anterior pituitary
Heifers—11 to 23 months; pregnant 1-140 days	14	1.0798	0.8772	198.5	1.55	307.7	285.2	350.7
Heifers—11 to 23 mo.; pregnant 141-283 days	6	1.0601	0.8274	153.9	1.60	246.2	232.2	297.6
Beef cows—lactating and open	3	2.3524	1.9923	274.4	3.75	1029.0	437.4	516.5
Beef cows—lactating and pregnant	3	2.0223	1.7489	267.2	3.25	868.4	429.4	496.5
Beef cows—dry and open	7	1.5486	1.3261	213.4	2.16	460.9	297.6	347.6
Beef cows—dry and pregnant	8	1.8846	1.5674	232.4	3.83	890.1	472.3	567.8
Dairy cows—lactating and open	15	1.9570	1.6804	281.1	3.00	843.3	430.9	501.8
Dairy cows—lactating and pregnant	7	1.6232	1.3422	203.7	4.16	847.3	522.0	631.3
Dairy cows—dry and open	13	1.8018	1.4989	267.4	3.00	802.2	445.2	535.2
Dairy cows—dry and pregnant	9	1.7338	1.4347	226.3	3.75	848.6	489.4	591.5
Beef cows—dry and pregnant	8	1.8846	1.5674	232.4	3.83	890.1	472.3	567.8
Dairy cows—dry and pregnant	9	1.7338	1.4347	226.3	4.92	1113.4	642.1	776.1

*Bird Units.

beef cows contained 64 per cent more lactogen than pituitaries from dry and open beef cows.

Pituitary glands from lactating and open dairy cows gave upon assay practically the same number of bird units of hormone as glands from lactating and pregnant dairy cows, but the glands from the lactating and open cows were considerably heavier than those from lactating and pregnant cows. Per unit weight of anterior lobe tissue the pituitaries from the pregnant animals contained 130 more units of lactogen than glands from open cows.

There were 802 bird units of the lactogenic hormone in pituitaries taken from dry and open dairy cows and 848 units in glands from dry and pregnant dairy cows. This greater unitage in favor of the latter group is entirely due to greater hormone concentration.

The pituitary glands from dry and pregnant dairy cows were somewhat lighter in weight than those from beef cows. Nevertheless, these dairy cattle pituitaries upon assay yielded 223 more bird units of lactogen than those from beef cows. Per unit weight of anterior lobe tissue the dairy cattle pituitaries contained 37 per cent more of the lactogenic hormone than pituitaries from beef cattle.

Therefore, in three out of four paired assays, where pituitaries from pregnant animals were compared with those from open animals, the glands from the pregnant cattle contained more lactogen than those from open cattle. A summary of these assays is given in Table 14.

The determined thyrotropic content. *Cattle.* Pituitaries collected from heifers in the first half of pregnancy contained more of the thyrotropic hormone than glands collected from heifers during the second half of pregnancy. It is of interest to note that the lactogen content of these glands was in this same relationship.

In the pituitaries from cows there seems to be no relationship between the thyrotropic content and the lactogen content. However, the beef cow groups and the dairy cow groups are in accord regarding the influence of pregnancy upon the thyrotropic content of pituitary glands. The pituitaries from lactating and pregnant beef cows contained slightly more of the thyrotropic principle than glands from lactating and open beef cows and this same relationship holds true when comparing pituitary glands from similar groups of dairy cows. The influence of pregnancy on the pituitary thyrotropic content is reversed from the above mentioned influence when comparing pituitaries from dry and open cows with those from dry and pregnant cows. Pituitary glands from dry and open

TABLE 15.—THE THYROTROPIC CONTENT OF PITUITARIES FROM PREGNANT CATTLE COMPARED WITH THOSE FROM NON-PREGNANT CATTLE.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average G.P.U.* per mg. of acetone dried powder	Average No. of G.P.U. per pituitary	Average No. of G.P.U. per gm. of fresh pituitary	Average No. of G.P.U. per gm. of fresh anterior pituitary
Heifers—11 to 23 months; pregnant 1-140 days	14	1.0789	0.8772	198.5	0.190	37.72	34.95	42.99
Heifers—11 to 23 mo.; pregnant 141-283 days	6	1.0601	0.8247	153.9	0.172	26.46	24.96	31.98
Beef cows—lactating and open	3	2.3524	1.9923	274.4	0.180	49.39	20.77	24.79
Beef cows—lactating and pregnant	3	2.0223	1.7489	267.2	0.196	52.37	25.89	29.94
Beef cows—dry and open	7	1.5486	1.3261	213.4	0.140	29.88	19.29	22.52
Beef cows—dry and pregnant	8	1.8846	1.5674	232.4	0.135	31.37	16.64	20.01
Dairy cows—lactating and open	15	1.9570	1.6804	281.1	0.194	54.53	27.86	32.45
Dairy cows—lactating and pregnant	7	1.6232	1.3422	203.7	0.232	47.26	29.11	35.20
Dairy cows—dry and open	13	1.8018	1.4989	267.4	0.202	54.01	29.97	36.03
Dairy cows—dry and pregnant	9	1.7338	1.4347	226.3	0.150	33.95	19.58	23.66
Beef cows—dry and pregnant	8	1.8846	1.5674	232.4	0.135	31.37	16.64	20.01
Dairy cows—dry and pregnant	9	1.7338	1.4347	226.3	0.150	33.95	19.58	23.66

*Guinea pig units.

beef cows contained 13 per cent more of the thyrotropic hormone than those from dry and pregnant beef cows per unit weight of anterior lobe tissue. Upon assay the pituitary glands from dry and open dairy cows yielded 54 guinea pig units per gland and those from dry and pregnant cows 34 units. Per unit weight of anterior lobe tissue the dry and open dairy cow pituitaries contained 52 per cent more of the thyrotropic hormone than pituitary glands from dry and pregnant dairy cows.

The thyrotropic content of dry and pregnant dairy cow pituitaries was only slightly greater than that of dry and pregnant beef cow pituitaries. On the basis of equal weights of anterior lobe tissue the glands from the dairy cows contained 18 per cent more of the thyrotropic hormone than those from beef cows.

These assay results indicate that pregnancy in the dry cow tends to lower the thyrotropic content of the pituitary gland while pregnancy in the lactating cow was associated with a higher thyrotropic content of the pituitary gland (Table 15).

Pituitaries From Lactating Animals

Hill (1934b) assayed the pituitaries from rabbits 50 minutes after parturition for their ovulating principle. The potency of these glands was directly between that for the 25 day pregnant stage and that for the estrus condition. In this connection it should be recalled that in the absence of suckling the post-partum rabbit returns to estrum in two to three days.

The determined lactogen content. *Rats.* The pituitary glands from 11 rats were removed 48 hours following delivery. The young were with the mother continually up until the time when she was sacrificed. This condition is mentioned here because as will be shown in a later section the stimulus of suckling has a marked influence upon the lactogen content of the post-partum rat pituitary gland. These glands averaged 10.6 mg. in weight and 7.72 bird units of lactogen. The pituitary glands from nine rats ten days after parturition averaged 9.7 mg. in weight and 5.86 bird units of lactogen.

Guinea pigs. At the time when the guinea pig pituitaries were assayed the rat assay work had already demonstrated the influence of suckling upon the lactogen content of the post-partum pituitary. Consequently, in order to control the influence of suckling a definite procedure was followed. The young were left with the mothers until 36 hours after delivery. The young were then removed from their mothers and the mothers sacrificed 15 hours later. The pituitary glands from eight lactating guinea pigs handled in such a manner averaged 18.2 mg. in weight and 14.95 bird units of lactogen. On the basis of equal weights of pituitary tissue these glands compared very favorably with those from 48 hour post-partum rats.

Thus, in rats and guinea pigs there is an augmentation in the lactogen content of the pituitary gland following parturition. The assay figures are given in Table 16.

TABLE 16.—THE LACTOGEN CONTENT OF PITUITARIES FROM LACTATING RATS AND GUINEA PIGS.

No. of animals	Stage of lactation	Average body weight when sacrificed (gm.)	Average pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B U. per 100 gm. body weight
			Rats			
11	48 hours postpartum	201	10.6	7.72	0.728	3.84
9	10 days postpartum	206	9.7	5.86	0.600	2.84
			Guinea Pigs			
8	51 hours postpartum	564	18.2	14.95	0.876	2.71

*Bird units

Cattle. There were four groups where the lactogen content of pituitaries from lactating cows was compared with that of pituitaries from dry cows. In three out of these four groups the lactogen content was higher in the pituitaries from dry cows, in the fourth comparison the lactogen content was higher in the pituitary glands from the lactating cows. These assay results which showed a higher lactogen content in the pituitaries from dry cows are opposed to the findings in the rat and the guinea pig. The exact stage of lactation and the length of time from the last milking were unknown and it is possible that these uncontrollable factors may have altered the assay results of the pituitaries from lactating animals.

On the average, the pituitary glands from lactating and open beef cows contained 870 bird units while those from lactating and open dairy cows contained 1243 units. Per unit weight of anterior lobe tissue the dairy pituitary glands contained 69 per cent more of the lactogenic hormone than beef pituitary glands. Upon assay, the pituitaries from lactating and pregnant beef cows yielded 780 bird units per gland and those from lactating and pregnant dairy cows 1035 units. The pituitaries from the beef cows were considerably larger than those from the dairy cows and when one compares the lactogen content per unit weight of anterior lobe tissue the dairy pituitaries contained 73 per cent more of the lactogenic hormone than those from beef cows. The difference between the lactogen content of dairy and beef pituitaries has

TABLE 17.—THE LACTOGEN CONTENT OF PITUITARIES FROM LACTATING COWS COMPARED WITH PITUITARIES FROM DRY COWS.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average B. U.* per mg. of acetone dried powder	Average No. of B. U. per pituitary	Average No. of B. U. per gm. of fresh pituitary	Average No. of B. U. per gm. of fresh anterior pituitary
Beef cows—lactating and open	3	2.3524	1.9923	274.4	3.91	1072.9	456.1	538.5
Beef cows—dry and open	7	1.5486	1.3261	213.4	4.16	889.9	574.6	678.6
Beef cows—lactating and pregnant	3	2.0223	1.7489	267.2	3.25	868.4	429.4	496.5
Beef cows—dry and pregnant	8	1.8846	1.5674	232.4	3.50	813.4	431.6	518.9
Dairy cows—lactating and open	15	1.9570	1.6804	281.1	4.91	1380.2	705.3	821.4
Dairy cows—dry and open	13	1.8018	1.4989	267.4	4.25	1136.4	630.7	758.2
Dairy cows—lactating and pregnant	7	1.6232	1.3422	203.7	3.50	712.9	439.2	531.1
Dairy cows—dry and pregnant	9	1.7338	1.4347	226.3	3.83	866.7	499.8	604.1
Beef cows—lactating and open	3	2.3524	1.9923	274.4	3.17	869.8	369.7	436.6
Dairy cows—lactating and open	15	1.9570	1.6804	281.1	4.42	1242.5	634.7	739.4
Beef cows—lactating and pregnant	3	2.0223	1.7489	267.2	2.92	780.2	385.8	446.1
Dairy cows—lactating and pregnant	7	1.6232	1.3422	203.7	5.08	1034.7	637.4	770.9

*Bird units.

been the most outstanding one observed in the assay of cattle pituitaries. Table 17 gives a resume of the results.

The determined thyrotropic content. *Cows.*—If the thyrotropic assay results of pituitaries from lactating cows are compared with the results from dry cows four such comparisons are available when other factors are held constant. In three of four such comparisons the pituitary glands from lactating cows contained more of the thyrotropic hormone than glands from dry cows. In the fourth comparison the opposite condition was found and this was in the comparison of pituitaries from lactating dairy cows with those from non-lactating dairy cows. In these group comparisons it is of interest to note that the pituitaries from the group containing the higher lactogen content, per unit weight of anterior lobe tissues, contained the lower thyrotropic content. For example, pituitary glands from lactating and pregnant dairy cows contained 531 units of lactogen and 35 units of the thyrotropic principle, while the glands from dry and pregnant dairy cows contained 604 units of lactogen and 24 units of the thyrotropic hormone.

Per unit weight of anterior lobe tissue the pituitaries from lactating and open dairy cows contained 31 per cent more of the thyrotropic hormone than glands from lactating and open beef cows and glands from lactating and pregnant dairy cows 18 per cent more than those from lactating and pregnant beef cows.

Hence, there was a tendency for pituitaries from lactating cows to contain more of the thyrotropic hormone than those from dry cows and on equal weight of pituitary tissue the tissue from dairy cows contained decidedly more of the thyrotropic hormone than that from beef cows (Table 18).

TABLE 18.—THE THYROTROPIC CONTENT OF PITUITARIES FROM LACTATING COWS COMPARED WITH PITUITARIES FROM DRY COWS.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average G.P.U.* per mg. of acetone dried powder	Average No. of G.P.U. per pituitary	Average No. of G.P.U. per gm. of fresh pituitary	Average No. of G.P.U. per gm. of fresh anterior pituitary
Beef cows—lactating and open	3	2.3524	1.9923	274.4	0.180	49.39	20.99	24.79
Beef cows—dry and open	7	1.5486	1.3261	213.4	0.140	29.88	19.29	22.52
Beef cows—lactating and pregnant	3	2.0223	1.7489	267.2	0.196	52.37	25.89	29.94
Beef cows—dry and pregnant	8	1.8846	1.5674	232.4	0.135	31.37	16.64	20.01
Dairy cows—lactating and open	15	1.9570	1.6804	281.1	0.194	54.53	27.86	32.45
Dairy cows—dry and open	13	1.8018	1.4989	267.4	0.202	54.01	29.97	36.03
Dairy cows—lactating and pregnant	7	1.6232	1.3422	203.7	0.232	47.26	29.11	35.20
Dairy cows—dry and pregnant	9	1.7338	1.4347	226.3	0.150	33.95	19.58	23.66
Beef cows—lactating and open	3	2.3524	1.9923	274.4	0.180	49.39	20.99	24.79
Dairy cows—lactating and open	15	1.9570	1.6804	281.1	0.194	54.53	27.86	32.45
Beef cows—lactating and pregnant	3	2.0223	1.7489	267.2	0.196	52.37	25.89	29.94
Dairy cows—lactating and pregnant	7	1.6232	1.3422	203.7	0.232	47.26	29.11	35.20

*Guinea pig units.

Pituitaries From Gonadectomized Animals

Novelli (1932) injected female toads (Bufo arenarum) with anterior pituitaries obtained from male toads 30 to 90 days after castration. Ovulation occurred in these injected toads showing that no appreciable modification had taken place in the male toad's pituitary glands 30 to 60 days following castration.

Domm (1931a, b) found the pituitaries from capons to be more effective in stimulating precocious development of sexual characters in the fowl than pituitaries from cocks.

Engle (1929) was the first to demonstrate that spaying or castrating rats increased the gonadotropic potency of their pituitary glands, as shown by the effects of implants on the ovaries of immature mice and rats. He reported no sex difference in the biological potency of the glands of gonadectomized donors and no significant difference as regards the age of the donors at the time of gonadectomy. Evans and Simpson (1929d, e) reported that pituitaries from spayed female rats produced ovaries in immature rats that weighed six times as much as the ovaries from immature rats implanted with pituitary tissue from normal female rats. Pituitaries from castrated males produced ovaries that weighed about two and one-half times the average obtained when normal male implants were made. These same investigators (1929e) were able to demonstrate that the gonadotropic potency of the pituitary gland increased as the length of the spayed period increased, the greatest increase occurring during the first month. Emanuel (1931) found that the effect produced by the pituitaries obtained from castrated rats was many times stronger than that obtained from normal rat pituitaries. Upon microscopic examination, the ovaries presented a development much greater in all their elements.

Wolfe (1932) observed that the removal of the ovaries of female rats caused hypertrophy of the anterior lobe of the pituitary gland and this was associated with an increase in its capacity to induce ovulation in the rabbit. Five milligrams of anterior lobe tissue from normal female rats were required to elicit ovulation in the rabbit while 0.5 mg. of anterior lobe tissue from female rats which had been spayed two to eight weeks caused ovulation.

Although of a different type of evidence, Emery (1932) tested the amount of gonadotropic hormones in the blood of several types of rat donors by injecting it into immature female rats. Positive results, as represented by enlarged ovaries and signs of estrus, were obtained with castrated male and spayed female sera but not with sera of normal males and normal females.

Siegert (1933a, b) reported that the gonadotropic content of pituitaries from rats was considerably augmented following castration. Lipschütz (1933) found that following spaying in the adult rat there was an enormous augmentation in the luteinizing power of its pituitary gland. Clark (1935b) gonadectomized a series of male and female rats on the first day of life. These animals were sacrificed 16 to 18 days later and their pituitary glands assayed for their gonadotropic power by the implantation of a single gland into immature female mice. She found that the pituitaries from gonadectomized animals showed a negligible sex difference in gonadal stimulating capacity. The castrated males always showed increased pituitary potency when compared with normal males, the average increase being 102 per cent. The spayed females showed variable pituitary potencies, fluctuating both above and below the normal female's potency. On the average the pituitaries from the spayed rats showed a 19 per cent greater potency than the normals. Stein (1935) gonadectomized a series of male and female rats between the ages of seven and nine days. The animals were sacrificed 17 to 19 days later and their pituitaries injected into immature female rats, litter-mates serving as controls. The ovaries of the rats injected with normal male pituitaries weighed 13.98 mg.; those receiving the normal female pituitaries 15.5 mg.; those receiving the castrated male pituitaries 19.9 mg.; and those receiving the spayed female pituitaries 48.5 mg. Lipschütz and Villagran (1936) summarized their work upon the ability of normal and castrated rat pituitaries to luteinize the immature ovary. They showed that the luteinizing coefficient of castrated male pituitary glands varied in the neighboring limits of that of normals, and the luteinizing ability of the anterior pituitary of the adult female was enormously elevated after spaying, the luteinizing coefficient reaching the characteristic value of the male, normal or castrated. On the other hand, Leonard (1936) found that castration of the adult female rat markedly increased the follicle stimulating hormone content of the pituitary gland but only slightly increased the luteinizing hormone content.

Smith and Engle (1929) found that castration augmented the amount of the gonadal-stimulating hormone in the anterior pituitary of guinea pigs. In 1932b Severinghaus demonstrated that pituitaries from castrated male and female guinea pigs effected a greater growth response in the ovaries of recipient immature mice than was produced by similar implants from normal male and female guinea pigs. Nelson (1935) implanted the anterior lobes from normal and castrated adult male and female guinea pigs intramuscularly into immature female rats and mice. Pituitaries from castrated males and females were of equal potency, being 70 per cent greater than normal male glands.

Wolfe (1932) reported that in the female rabbit there was no appreciable hypertrophy of the anterior lobe after spaying and no increase in the capacity of the anterior lobe to induce ovulation in the rabbit. These animals had been spayed two to nine months before they were sacrificed. Smith, Severinghaus, and Leonard (1933) employing 22 day old female mice assayed the pituitaries of normal and castrated male and female rabbits. Taking the untreated control as 100, the average relative weight of the ovaries stimulated by normal female anterior pituitary was 207, by spayed female anterior pituitary 462, by normal male anterior pituitary 462, and by castrated male anterior pituitary 535. Since the amount of implanted tissue was quite constant, the greater ovarian effect with the implanted pituitaries from castrated rabbits was due to a higher gonadal-stimulating hormone content per unit amount of tissue. Using ovulation in the rabbit as the method of determining the potency of pituitaries their results showed an increased capacity of the anterior pituitary of spayed rabbits to induce ovulation. Animals were castrated from 33 to 123 days before sacrifice. Hill (1934a) employing the ovulation response in the estrus rabbit as his criterion of gonadotropic potency, reported that pituitaries from castrated rabbits and cats and spayed rabbits contained fewer units per gram of acetone dried powder than did those from normal animals.

Hellbaum (1935) assayed the pituitaries from stallions and geldings for their gonadotropic factors. Twelve and one-half milligrams of the acetone dried powder were injected into immature female rats as a constant total dose. The ovaries from the rats injected with the stallion pituitary powder weighed 22.9 mg. on the average and the response was of the follicular type. The ovaries from the recipients of the gelding pituitary powder averaged 76.5 mg. in weight and there was a stimulation of the follicles and the corpora lutea.

Emery and Winter (1934) reported that the pituitaries from castrated rats had no greater adrenal hypertrophying power than glands from normal rats.

Bates, Riddle, and Lahr (1935) compared the hormone content of pituitaries from mature bulls and steers. Bull pituitaries contained 34 dove units of lactogen per gram of fresh tissue while an equal amount of pituitary tissue from steers contained 29 units.. Thyrotropic and follicle stimulating hormones were found in greater quantities in pituitaries from bulls than in those from steers. Doves injected once daily for four days with 10 mg. (equivalent to 455 mg. of anterior lobe tissue) of a pituitary extract made from steer pituitaries and sacrificed 96 hours after the first injection had testes that had been increased 686 per cent in weight and thyroids that had been increased 61 per cent in weight. The doves injected once daily for four days with 10 mg. (equivalent to 345 mg. of anterior lobe tissue) of an extract made from bull pituitaries had testes that had been increased 850 per cent in weight and thyroids that had been increased 93 per cent in weight.

The determined lactogen content. *Rats.* The assay results of the lactogen content of pituitary glands from two groups of rats which had been ovariectomized for 18 and 20 days, are presented here even though they were not assayed against glands from normal females. These two groups of ovariectomized females served as controls in another phase of this work. The pituitaries from five females that had been ovariectomized for 18 days averaged 7.5 mg. in weight and 3.4 bird units of lactogen. A group of ten rats was ovariectomized and sacrificed 20 days later. Their pituitary glands weighed 7.9 mg. and contained 2.73 bird units of lactogen, these being average values. Twenty rats were paired upon the basis of body weight and the ovaries of one of each pair removed when the average body weight of each group was 42 gm. The pituitaries from these two groups of rats, which were removed 60 days after the one group had been ovariectomized, showed a considerable difference in their lactogen content. The pituitaries from normal female rats weighed 5.1 mg. and contained 1.4 units of lactogen when the figures were averaged. The average weight and average lactogen content of the glands from the ovariectomized rats were 7.3 mg. and 0.86 of a bird unit, respectively.

Three groups of male rats were castrated at three different stages of growth. The average body weights at the time of castration were 45 gm., 109 gm., and 202 gm. All three groups were sacrificed at the end of a 60 day castration period and their pituitaries assayed against glands from normal male rats. In general, the assay results were uniform. The pituitaries from the castrated rats contained practically the same quantity of the lactogenic hormone as those from normal rats. Inasmuch as castration caused an hypertrophy of the pituitary glands there was a decrease in the concentration of the lactogenic hormone in these pituitary glands.

This assay work clearly demonstrates that the removal of the ovaries in rats markedly decreases the lactogen content per pituitary gland and that castration of the male rat does not alter the lactogen unitage per pituitary gland. The averaged figures are recorded in Table 19.

Steers.—Two groups of steer pituitaries were compared with pituitaries from bulls of the same age, with two comparisons being made of pituitaries from four to ten month old steers and bulls and one comparison of pituitaries from 11 to 23 month old steers and bulls.

In the first comparison of the four to ten month old groups the bull pituitaries, per unit weight of anterior lobe tissue, contained 36 per cent more lactogen than the steer pituitaries and in the second comparison there was a 47 per cent difference in favor of the bull pituitaries.

Pituitary glands from 11 to 23 month old steers contained 218 units of lactogen and those from bulls of the same age contained 333 units. In these older steer-bull comparisons there was a greater difference in hormone concentration within the glands than in the younger steer-bull comparisons. These bull pituitaries contained 58 per cent more lactogen per unit weight of anterior lobe tissue than the pituitaries from steers.

When pituitaries from four to 10 month old steers were compared with glands from 11 to 23 month old steers it was found that the pituitaries from the latter group contained 404 units and the former group 331 units. However, this difference was entirely due to a greater pituitary size in the older group, one gram of anterior lobe tissue from the four to ten month old steers containing 21 per cent more of the lactogenic hormone than one gram of tissue from the 11 to 23 month old steers.

Thus, the removal of the testes from bulls brings about a decrease in the lactogen content of the pituitary gland and the longer the castration period the greater the decrease in hormone concentration (Table 20).

The determined thyrotropic content. *Steers.*—Pituitaries from four to ten month old steers contained 20 guinea pig units per gland, those from four to ten months old bulls contained 29 units per gland. Per unit weight of anterior lobe tissue that from bulls contained 50 per cent more of the thyrotropic hormone than that from steers.

In the pituitary glands from older animals there was a greater difference in thyrotropic content of glands from steers and bulls than from younger animals. Pituitaries from 11 to 23 month old steers contained 15 guinea pig units per pituitary while glands from bulls contained 31 units. The relative concentration of the

TABLE 19.—A COMPARISON OF THE LACTOGEN CONTENT OF PITUITARIES FROM NORMAL AND GONADECTOMIZED RATS.

Sex	No. of Animals	Physiological Condition	Average body weight at beginning of experiment (gm.)	Average body weight when sacrificed (gm.)	Average pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
F	5	Ovariectomized—18 days	144	152	7.5	3.40	0.453	2.24
F	10	Ovariectomized—20 days	150	174	7.9	2.73	0.346	1.57
F	10	Normal	42	123	5.1	1.40	0.275	1.14
F	10	Ovariectomized—60 days	42	149	7.3	0.86	0.118	0.58
M	10	Normal	45	172	5.7	1.03	0.181	0.60
M	10	Castrated—60 days	45	166	9.9	0.99	0.100	0.60
M	10	Normal	109	208	4.9	0.60	0.122	0.29
M	10	Castrated—60 days	109	194	10.2	0.71	0.070	0.37
M	14	Normal	201	239	5.4	1.04	0.193	0.44
M	14	Castrated—60 days	202	239	9.9	1.00	0.101	0.42

*Bird units

TABLE 20.—A COMPARISON OF THE LACTOGEN CONTENT OF PITUITARIES FROM STEERS AND BULLS.

Group	No. of animals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average B. U.* per mg. of acetone dried powder	Average No. of B. U. per pituitary	Average No. of B. U. per gm. of fresh pituitary	Average No. of B. U. per gm. of fresh anterior pituitary
Steers—4 to 10 months	23	0.9013	0.7016	143.2	1.30	186.2	206.5	265.3
Bulls—4 to 10 months	17	0.8051	0.6240	132.9	1.70	225.8	280.4	361.8
Steers[3]—4 to 10 months	12	1.0566	0.7965	135.0	1.55	240.3	227.4	301.7
Bulls[3]—4 to 10 months	3	0.9829	0.7626	138.0	2.45	338.0	343.9	443.2
Steers—11 to 23 months	14	1.1540	0.9147	124.3	1.75	217.5	188.4	237.7
Bulls—11 to 23 months	6	1.1209	0.8847	166.6	2.00	333.2	297.2	376.6
Steers[3]—4 to 10 months	12	1.0566	0.7965	135.0	2.45	330.8	313.1	415.3
Steers[3]—11 to 23 months	5	1.4186	1.1773	183.7	2.20	404.1	284.9	343.2

*Bird units. [3]Herefords.

thyrotropic hormone is indicated when equal weights of anterior lobe tissue are compared. On this basis the pituitaries from bulls contained 115 per cent more of thyrotropic hormone than glands from steers.

This assay work demonstrates that pituitaries from bulls contain more thyrotropic hormone than pituitary glands from steers and that this difference is more pronounced in older animals. The results are summarized in Table 21.

Pituitaries From Experimental Cryptorchid Animals

Evans and Simpson (1929 d, e) reported that the pituitary glands from male rats, which had been rendered cryptorchid by abdominal anchorage of the testes, produced ovaries in immature rats that weighed intermediate between the weights of ovaries produced when normal and castrated male pituitary glands were employed.

Nelson (1935) assayed the pituitaries from experimental cryptorchid guinea pigs for their gonadotropic potency, the assays being made with immature rats and mice. His results showed that pituitaries from cryptorchid males were equal in potency to those from castrated males and spayed females, or 70 per cent greater than normal male glands.

The determined lactogen content. *Rats.* Twenty-two rats were paired upon the basis of body weight and in one of each pair the testes were anchored to the abdominal wall. Sixty days later these rats were sacrificed, their pituitaries removed and assayed. The average pituitary weight of the normal group was 7.7 mg., of the experimental cryptorchid group it was 8.3 mg. The lactogen content of the pituitary glands from the two groups was nearly identical, demonstrating that testis location had no influence upon the lactogen content of the pituitary gland (Table 22).

TABLE 21.—A COMPARISON OF THE THYROTROPIC CONTENT OF PITUITARIES FROM STEERS AND BULLS.

Group	No. of ani-mals	Average whole pituitary weight (gm.)	Average anterior pituitary weight (gm.)	Average mg. of acetone dried powder	Average G.P.U.* per mg. of acetone dried powder	Average No. of G.P.U. per pituitary	Average No. of G.P.U. per gm. of fresh pituitary	Average No. of G.P.U. per gm. of fresh anterior pituitary
Steers—4 to 10 months	12	1.0566	0.7965	135.0	0.151	20.39	19.29	25.59
Bulls[3]—4 to 10 months	3	0.9829	0.7626	138.0	0.212	29.26	29.76	38.36
Steers—11 to 23 months	14	1.1540	0.9147	124.3	0.120	14.92	12.92	16.30
Bulls—11 to 23 months	6	1.1209	0.8847	166.6	0.186	30.99	27.64	35.02

*Guinea pig units. [3]Herefords.

TABLE 22.—THE LACTOGEN CONTENT OF PITUITARIES FROM CRYPTORCHID MALES.

No. of Ani-mals	Physiological Condition	Average body weight at beginning of experiment (gm.)	Average body weight when sacrificed (gm.)	Average pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. of pituitary tissue	Average No. of B.U. per 100 gm. body weight
11	Normal	259	270	7.70	1.16	0.151	0.430
11	Cryptorchids (60 days)	256	273	8.30	1.27	0.153	0.470

*Bird units

Pituitaries From Animals Under the Influence of Ovarian Hormones

Leonard, Meyer, and Hisaw (1930) injected immature female rats daily with two to four rat units of an oil-soluble estrogen for 20 to 67 consecutive days. Ovarian development was markedly decreased as was shown by the fact that the ovaries from the injected animals weighed 33 per cent less than those from controls. Upon transplantation into immature female rats, they found that the pituitaries from the non-treated rats contained more gonadal-stimulating hormone than those from litter-mate estrogen treated rats. Employing rats somewhat older, the above results were confirmed by Meyer et al. (1930).

Burch and Cunningham (1930) ovariectomized a group of female rats and after a recovery period of two weeks the animals were divided into two groups. One group was kept as controls and the other group received six daily subcutaneous injections of a commercial alcoholic extract of human placenta in which the estrogen content had been standardized. The pituitaries from these animals were then implanted into immature female mice. The ovaries of the mice receiving anterior lobe tissue from the injected rats were much larger than those receiving tissue from the control group, the ratio being about five to two.

Meyer et al. (1932) reported that the pituitaries from castrated male and female rats which had been treated with estrogens (amniotin and theelin) contained less gonadal-stimulating power than pituitaries from control recipients. The ovaries of immature rats which received the pituitaries of castrated males injected with estrogen weighed 45 per cent less than the ovaries of the control recipients and the ovaries of the rats implanted with pituitaries of ovariectomized female rats injected with estrogen weighed 47 per cent less than those of the control animals. Kraul (1932) noted that the implantation of pituitaries from rats, rabbits, and guinea pigs which had been injected with estrogens produced a strong follicle-stimulating effect. Hohlweg and Dohrn (1932) reported that appropriate doses of estrogen (progynon-B) prevented an increase in the gonadotropic potency of the pituitaries of spayed rats.

Lipschütz (1934) demonstrated that the injection of estrogen into normal adult males decreased the luteinizing power of the anterior pituitary, but augmented its follicle-stimulating power. Later work by Lipschütz (1935) showed that following estrogen treatment the follicle stimulating hormone remained unaltered. Lipschütz, Palacios, and Akel (1936) reported that the anterior pituitary of the normal male rat, under the influence of an estrogen, compared favorably in its weight and gonadotropic potency with the anterior pituitary of the normal adult female rat.

Lane (1935) injected 6.25 rat units of estrogen per day into 22 day old female rats for periods ranging from five to 39 days. At the end of the injection period a count was made of the ovarian follicles. The pituitaries of the estrogen injected rats were then implanted into normal 22 day old rats and the recipients' ovaries also studied. Estrogen injection at first stimulated the pituitary to liberate gonadotropic hormones in excess of those produced by normal animals. Later there was an inhibition of the follicle stimulating hormone. As judged by ovarian response the injections greatly enhanced the luteinizing hormone content of the donor's pituitary gland. Fevold, Hisaw, and Greep (1935) found that the first effect of estrogen injections on the anterior pituitary was to increase the secretion of the luteinizing hormone without increasing the secretion of the follicle stimulating hormone. Eight days of estrogen injection seemed to decrease the rate of secretion of the follicle stimulating and luteinizing hormone as was determined by the fact that ovarian response to the follicle stimulating hormone and pregnancy urine was greatly reduced. Nelson (1935) noted that pituitary glands from gonadectomized male and female guinea pigs which had received 25 to 100 rat units of estrogen daily were somewhat less potent than pituitaries from normal animals. Leonard (1936) reported that the estrogen treatment of castrated rats decreased both gonad stimulating hormones. With a slightly different technique, it was found that estrogen treatment lowered the luteinizing hormone content in the normal immature female rat pituitary but did not affect the follicle stimulating hormone content.

The determined lactogen content of pituitaries from animals injected with estrogens. *Female rats.* Twenty sexually mature non-pregnant rats were ovariectomized and paired upon the basis of body weight. The day following the operation one of each pair was injected with 500 i. u. of estrogen (theelin-in-oil) and these injections were continued daily for 20 days. The other rat of each pair served as a control. All of the rats were sacrificed the day following the last injection of the experimental group, their pituitaries removed, weighed, and assayed. The pituitary glands from the control animals averaged 7.9 mg. in weight, those from the injected animals 9.0 mg. There was an average of 2.73 bird units of lactogen in the control pituitaries and 3.96 bird units in the pituitaries from the injected rats. This increase in lactogen content of the pituitaries from the injected animals was not due wholly to increased pituitary size, but due in part to an increased concentration of the hormone in the gland. One milligram of pituitary tissue from control animals containing 0.346 of a bird unit compared to 0.440 of a bird unit in one milligram of tissue from the injected animals.

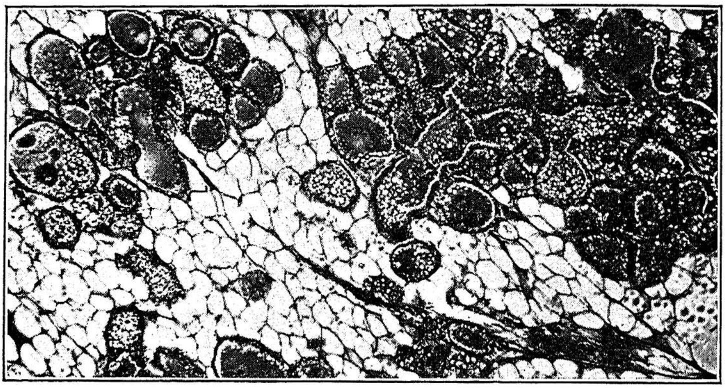

Fig. 3.—Photomicrograph of a section of the mammary gland of an ovariectomized rat (Series XX3t) which was injected with 1,000 i. u. of estrogen (progynon-B) daily for 18 days. The gland was filled with secretory products. The pituitaries from a group of rats similarly treated contained an average of 5.87 bird units of lactogen. X60.

Fig. 4.—Photomicrograph of a section of the mammary gland of an ovariectomized rat (Series XX6t) which was injected with 1,000 i. u. of estrogen (progynon-B) daily for 18 days. The secretory tissue was distended with milk. The pituitaries from a group of rats similarly treated contained an average of 5.87 bird units of lactogen. X60.

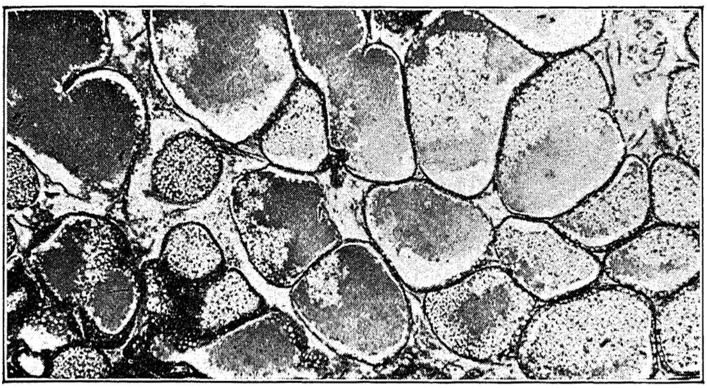

Fig. 5.—Photomicrograph of a section of the mammary gland of an ovariectomized rat (Series XX5t) which was injected with 1,000 i. u. of estrogen (Progynon-B) daily for 18 days. The secretory tissue was gorged with milk. The pituitaries from a group of rats similarly injected contained an average of 5.87 bird units of lactogen. X60.

Ten sexually mature virgin rats were ovariectomized and five of these were injected with 1,000 i. u. of estrogen (progynon-B) daily for 18 days. The remaining five rats served as controls. On the 19th day of the experiment all of the animals were sacrificed. There was a tremendous increase in pituitary size of the injected animals, their pituitaries averaging 16.3 mg., while those from the control animals averaged 7.5 mg. Upon assay the experimental pituitaries yielded 5.87 bird units of lactogen while those from the control animals yielded 3.40 units. However, this increase in the lactogen content of the experimental pituitaries was entirely due to the increase in the size of the pituitary gland as there was a decrease in hormone concentration. Histological section of the mammary glands of the injected animals revealed the fact that milk secretion had been initiated (Figs. 3, 4, and 5).

In connection with another experiment, six sexually mature virgin rats were ovariectomized and injected daily with 200 i. u. of estrogen (progynon-B). The injection period varied from 6 to 14 days. The average pituitary weight was 14.4 mg. and they contained 10 bird units of lactogen per gland. In contrast to the decreased hormone concentration of the pituitaries from rats injected with 1,000 i. u. of estrogen, the hormone concentration in these pituitaries was increased. This hormone concentration of 0.694 of a bird unit per milligram of pituitary tissue compares very favorably to the hormone concentration found in the pituitaries of lactating rats.

This assay work demonstrates that the injection of estrogens definitely increases the lactogen content of the pituitary gland of the ovariectomized female rat. Massive injections of estrogen decreased the hormone concentration, but smaller doses increased the hormone concentration. The results are summarized in Table 23.

Male rats. Eighteen normal male rats were paired according to body weight and one of each pair was injected with 500 i. u. of estrogen (theelin-in-oil) for 18 days. All of the rats were sacrificed the day after the last injection. There was an average increase of 2.7 mg. in the weight of the experimental pituitaries over that of the pituitaries from the control animals. The experimental pituitaries contained 60 per cent more lactogen than the glands from control animals.

The pituitary glands from eight normal male rats which had been injected with 500 i. u. of estrogen (oestroform-B) daily for 15 days contained 1.22 bird units of lactogen, on the average, and those from the eight control rats averaged 0.72 of a unit. The assay results of pituitaries from nine normal male rats injected daily for 7 days with 500 i. u. of estrogen (oestroform-B) when assayed against pituitaries from normal non-injected male rats revealed increases in lactogen content of about the same magnitude as those previously noted.

Inasmuch as it had been reported that lactation followed the cessation of estrogen treatment in rats and guinea pigs, it was thought desirable to assay the pituitaries of rats treated with estrogen, but sacrificed several days after the last injection. Accordingly, twenty normal male rats were paired upon the basis of body weight. The experimental group was then injected daily for 7 days with 500 i. u. of estrogen (oestroform-B). Five days after the last injection these twenty rats were sacrificed. The average weight of the experimental pituitary was 9.4 mg., that of the control pituitary was 6.9 mg. On the average, the pituitaries from the injected animals assayed 1.11 bird units while those from the non-injected animals assayed 0.64 of a bird unit. These results are nearly identical with those where the animals were sacrificed the day following the last injection.

In three out of these four groups of injected male rats there was an increase in the hormone concentration in the pituitary gland, in the fourth group the concentration was nearly identical with that of the controls.

The mammary glands from these injected rats were not sectioned. However, a histological section of a mammary gland from

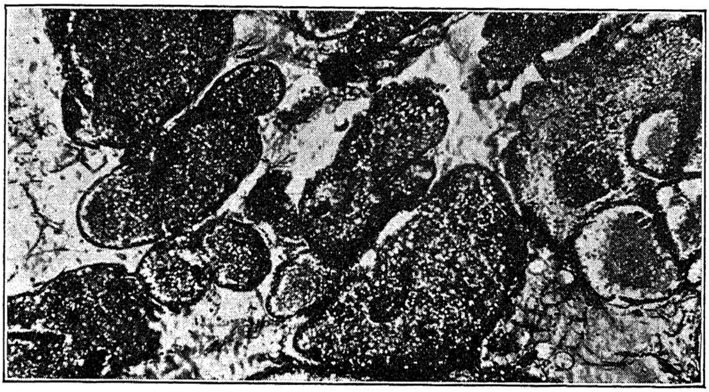

Fig. 6.—Photomicrograph of a section of the mammary gland of a castrated rat which was injected daily for 15 days with 500 i. u. of estrogen (progynon-B). The gland parenchyma was distended with milk. X60.

a castrated male rat which had been injected for 15 days with 500 i. u. of estrogen (progynon-B) indicates that milk secretion was induced (Fig. 6).

Eight immature male rats were castrated and this was followed by a castration period of 35 days, during which time no treatment was administered. Then 25 i. u. of estrogen (theelin-in-oil) were injected daily for 14 days and this was followed by 50 i. u. daily for 18 days and finally 100 i. u. daily for 12 days. The animals were then sacrificed and their pituitaries assayed against glands from normal males of like body weight. This treatment nearly doubled the pituitary weight and lactogen content, however, the hormone concentration was not altered (Table 23).

Male guinea pigs. Eleven normal male guinea pigs were injected daily with 200 i. u. of estrogen (theelin-in-oil) for 15 to 20 days. These animals were sacrificed the day after the last injection. The average pituitary weight was 10.7 mg. and there were 12.5 bird units, on the average, of lactogen. In comparison with the lactogen content of pituitaries from normal non-injected guinea pigs these estrogen injections elicited over a four-fold lactogen increase per pituitary gland and a three-fold increase in hormone concentration. These pituitaries had the highest concentration of the lactogenic hormone of any pituitaries assayed in this study. Lactation had not been initiated in these animals (Fig. 7).

TABLE 23.—THE LACTOGEN CONTENT OF PITUITARIES FROM RATS INJECTED WITH ESTROGEN, PROGESTIN, AND ESTROGEN + PROGESTIN

Sex	No. of Animals	Physiological Condition	Average body weight at beginning of experiment (gm.)	Average pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
		Rats					
F	10	Ovariectomized (20 days)	150	7.9	2.73	0.346	1.57
F	10	Ovariectomized + 500 I.U. Theelin per day (20 days)	147	9.0	3.96	0.440	2.64
F	5	Ovariectomized (18 days)	144	7.5	3.40	0.453	2.24
F	5	Ovariectomized + 1000 I.U. Progynon-B per day (18 days)	145	16.3	5.87	0.360	4.25
F	5	Ovariectomized (10 days)	156	8.0	3.00	0.375	1.92
F	5	Ovariectomized + 0.5 Clauberg Unit of Progestin per day (10 days)	156	7.6	3.25	0.428	2.08
F	6	Ovariectomized + 200 I.U. Progynon-B per day (6-14 days)	149	14.4	10.00	0.694	6.94
F	6	Ovariectomized + 200 I.U. Progynon-B and 0.5 Clauberg Unit of Progestin per day (6-14 days)	151	13.2	10.25	0.777	7.43
M	9	Normal	232	6.2	1.05	0.162	0.50
M	9	500 I.U. Theelin per day for 18 days	232	8.9	1.69	0.199	0.85
M	8	Normal	187	5.7	0.72	0.126	0.37
M	8	500 I.U. Oestroform-B per day for 15 days	184	6.3	1.22	0.194	0.75
M	9	Normal	161	4.4	0.80	0.182	0.45
M	9	500 I.U. Oestroform-B per day for 7 days	161	6.2	1.10	0.177	0.64
M	10	Normal	271	6.9	0.64	0.093	0.24
M	10	500 I.U. Oestroform-B per day for 7 days. Sacrificed on 5th day after last injection	282	9.4	1.11	0.118	0.40
M	8	Normal	---	4.1	0.91	0.222	0.55
M	8	Castrated for 35 days, then 25 I.U. Theelin per day for 18 days; 100 I.U. Theelin per day for 12 days	50	7.9	1.75	0.222	1.52
		Guinea Pigs					
M	18	Normal	308	6.9	2.59	0.375	0.84
M	11	200 I.U. Theelin per day (15-20 days)	370	10.7	12.50	1.168	3.38

*Bird units

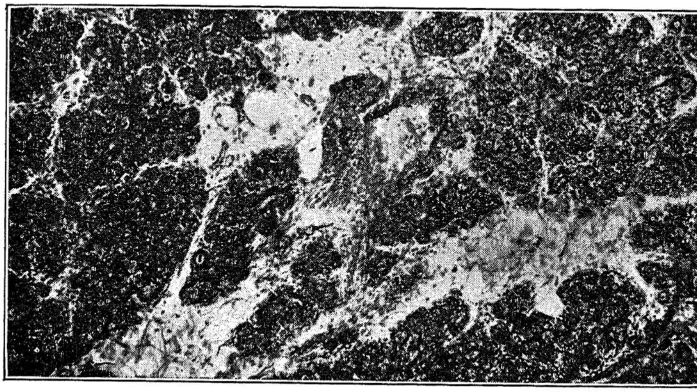

Fig. 7.—Photomicrograph of a section of the mammary gland of a male guinea pig (Series IV 17) injected with 200 i. u. of estrogen (theelin-in-oil) daily for 20 days. The gland parenchyma was well developed but there was no evidence of secretory activity. The pituitary glands from a group of male guinea pigs similarly treated contained an average of 12.5 bird units of lactogen. X60.

The determined lactogen content of pituitaries from female rats injected with progestin. Ten sexually mature virgin rats were ovariectomized and five of these were injected with one-half of a Clauberg unit of progestin daily for ten days. The remaining five animals served as controls. All of the animals were sacrificed the day after the last injection of the experimental group. Upon pituitary assay it was found that the lactogen content of these two groups of pituitaries was much the same. The average lactogen content of the experimental pituitaries was 3 bird units and that of the control pituitaries 3.25 bird units. These limited observations indicate that the injection of progestin into ovariectomized virgin rats does not alter the lactogen content of the pituitary gland (Table 23).

The determined lactogen content of pituitaries from female rats injected with estrogen and progestin. Assay work upon the pituitaries from pregnant rats (presented in a previous section) had demonstrated that the lactogen content of the pituitary gland was not elevated during pregnancy. Inasmuch as estrogen injection markedly increased the lactogen content of the pituitary gland of the ovariectomized rat it seemed as though some factor was withholding this stimulating action during pregnancy. As an attack upon this question 12 rats were ovariectomized and half of these injected with 200 i. u. of estrogen (progynon-B) and the other half injected with 200 i. u. of estrogen plus one-half of a Clauberg unit of progestin. These injections were made daily for

6 to 14 days, each pair of animals being injected the same length of time. The lactogen content of the pituitaries of these two groups of animals was nearly identical, the average of those receiving 200 i. u. of estrogen being 10 bird units and those receiving 200 i. u. of estrogen plus one-half of a Clauberg unit being 10.25 bird units. However, it was obvious that the conditions of pregnancy were not being simulated, that is, concerning the ovarian hormones, as indicated by vaginal smears and the size of the pituitary gland (Table 23).

Pituitaries From Animals Fed Thyroid Tissue or Injected With Thyroxine

Evans and Simpson (1930) reported on the effects of hyperthyroidism on the pituitary gland. Fifty young adult female rats were fed one gram of fresh beef thyroid daily for five weks. Another group of fifty rats served as controls. The animals fed thyroid tissue possessed smaller pituitary glands (8.8 mg.) than the control animals (10 mg.). Upon implantation of these pituitaries into immature female rats it was found that the pituitaries from the thyroid-fed animals were more effective in provoking precocious sexual maturity than the pituitaries from the normal animals.

Van Horn (1933) fed desiccated thyroid powder to a group of female rats. After a six weeks thyroid feeding period the pituitaries from these animals were implanted into sexually immature female rats. Control pituitaries were implanted into littermate sisters of the experimental recipients. As revealed by the weights of the ovaries from the recipients it was found that thyroid feeding caused an increase in the gonadal-stimulating power of the pituitary glands ranging from 15 to 63 per cent.

Kuschinsky (1933) injected rats with 0.35 mg. of thyroxine daily for 5 to 18 days. At the end of the injection period the pituitaries from these rats were injected into guinea pigs. From four to six pituitary glands were injected into each animal. Ten such pigs were recipients and after treatment six of them had normal thyroid glands. Of the four remaining guinea pigs two had thyroid glands which were slightly active and two had thyroid glands which were active.

Hohlweg and Junkmann (1933) observed that the changes induced in the pituitary gland following thyroidectomy could be prevented by the injection of thyroxine or the ingestion of thyroid. After thyroidectomy and the injection of thyroxine the thyrotropic hormone content was diminished.

TABLE 24.—THE LACTOGEN CONTENT OF PITUITARIES FROM MALE RATS INJECTED WITH THYROXINE.

Sex	No. of Animals	Physiological Condition	Average body weight when injections began (gm.)	Average body weight when sacrificed (gm.)	Average pituitary weight (mg.)	Average B.U.* per pituitary gland	Average No. B.U. per mg. of pituitary tissue	Average No. of B.U. per 100 gm. body weight
M	10	Normal	309	318	8.1	1.28	0.158	0.40
M	10	.01 mg. Thyroxine per day for 14 days	310	312	7.8	1.10	0.141	0.35
M	7	Normal	149	172	4.9	0.86	0.176	0.50
M	7	.01 mg. Thyroxine for 7 days; .02 mg. 7 days, .03 mg. 7 days	150	161	4.0	0.39	0.097	0.24

*Bird units

In 1935 Cohen reported the results of feeding 0.25 mg. of thyroid powder daily to immature rats for three weeks. The pituitaries from these rats and those from controls were implanted into immature female rats. She found that pituitaries from the experimental animals were more potent in the stimulating precocious sexual maturity than those from control animals.

The determined lactogen content of pituitaries from male rats injected with thyroxine. Ten male albino rats were paired upon the basis of body weight and one-half of these were injected daily with 0.01 mg. of thyroxine for 14 days. The pituitaries from the injected rats averaged 7.8 mg. in weight and those from the control animals averaged 8.1 mg. in weight. Upon assay there was found very little difference in the lactogen content of the pituitary glands from these two groups. The pituitaries from the experimental animals averaged 1.1 bird units and those from control animals averaged 1.28 bird units.

Another group of male rats were paired and one of each pair was injected with the following amounts of thyroxine daily: 0.01 mg. for 7 days; 0.02 mg. for 7 days; and 0.03 mg. for 7 days. All of the animals were sacrificed the day following the last injection. The pituitaries from the control animals weighed 4.9 mg. and those from the thyroxine injected rats weighed 4.0 mg., these being average values. The experimental pituitary glands contained 0.39 of a bird unit per gland and glands from the control animals contained 0.86 of a bird unit per gland (Table 24).

Consequently, thyroxine injections, when sufficient quantities are injected, decrease pituitary size and lactogen content.

INHIBITION OF LACTATION

In the past various factors have been suggested as being responsible for holding lactation in abeyance during pregnancy. Although such factors must be functioning in the non-lactating pregnant organism, one should not consider lactation and pregnancy as being antagonistic toward each other. It is a well known fact that lactation and pregnancy exist simultaneously in many animals. In dairy cattle the lactation curves of pregnant and open cows are identical during the first five months and after this time the lactation curve of the pregnant animal declines more rapidly than that of open animals. A lactation inhibiting hormone has been suggested as being responsible for the more rapid decline in the lactation curve of pregnant animals. Nevertheless, it would seem that the divergence of a part of the nutrients available for milk production to the fetus must be an important factor. Certainly, as pregnancy continues there must be an ever decreasing amount of blood flowing through the udder. In the making of an estimate of fetal nutrient requirements one should take into consideration not only the energy present in the new-born, as has been done in the past, but also the requirements for maintenance and organization.

It was noted by Halban (1905) that when the placenta was retained following parturition in woman, lactation did not occur during the period of retention. As an experimental attack upon this problem, Frankl (1923) implanted placental tissue into rats near term. Milk secretion did not occur following parturition as long as the grafts remained active. Smith and Smith (1933) noted the absence of lactation in four rabbits after parturition. Laparotomies indicated the presence of attached unabsorbed remnants of the placenta in the uterus. Litt (1933) reported success in transplanting placental tissue of pregnant rabbits into the anterior chamber of their eyes. The placental implants attached themselves to structures, usually the iris, and derived a blood supply sufficient for their maintenance from these structures. The implants grew steadily for a period of about 30 days, after which gradual degeneration occurred. Fetal placenta alone took part in the growth. After parturition the lactation of the rabbits could not be differentiated from normal controls. He concluded that the amounts of placental tissue which could be transplanted to the anterior chamber of the eye had no effect on milk secretion in the host. Nelson (1934) removed the ovaries and embryos from guinea pigs in advanced pregnancy. In no instance did lactation occur until from 24 to 45 hours after the expulsion of the placenta. These observations are of importance inasmuch as it has been generally accepted that the placenta is an important contributor to estrogen production during pregnancy. However, Selye, Collip, and Thompson's (1934) investigations indicate that this placental inhibition of lactation may be a mechanical one. These investigators were able to show that lactation did not occur in the rat following the removal of the fetuses by Caesarian section if the uterus was immediately distended with paraffin.

Other lines of evidence were being brought forth which aided in the establishment of a hypothesis concerning the inhibition of lactation during pregnancy. Fels (1926) reported the recovery of estrogen in the blood of pregnant women as was likewise reported the same year by Aschheim (1926). Estrogen was then detected in the urine of pregnant women by Laqueur (1927) and this work was confirmed by Aschheim and Zondek (1927). The latter investigators also reported the presence of estrogen in the urine from pregnant cows. Hisaw and Meyer (1929) reported the finding of estrogen in the urine from pregnant cows as did Turner et al. (1930). In addition to the demonstration of the presence of estrogen in the urine of pregnant cows, Turner et al. (1930) observed that during the advance of the stage of gestation there was an ever increasing amount of the hormone being excreted in the urine.

Steinach et al. (1928) injected castrated guinea pigs with a water soluble estrogen for 7 to 8 weeks. Histological section of the mammary glands showed that the lumina of the alveoli were filled with secretion. Laquer et al. (1928) injected an estrogen into a spayed guinea pig for 14 days. The mammary glands were examined three days after the last injection and were reported as showing the discharge of milk. Lactation was reported as being induced in rabbits and guinea pigs by estrogen injections followed by withdrawal of the hormone treatment (Fellner, 1931). By suitable dosage of estrogen, de Jongh and Laqueur (1930) noted that milk secretion could be evoked in adult female or normal guinea pigs and made to continue for almost six weeks. Large doses of estrogen were injected and then suddenly diminished, thus simulating that which normally happens after parturition. Experiments of a similar nature were reported by de Jongh and Dingemanse (1931). After an extensive preliminary treatment with estrogen, the dosage of the hormone was greatly reduced, then alternately increased and decreased. Turner and Gomez (1934) observed that periodic or alternate injections of estrogens into guinea pigs with a well developed gland failed to initiate milk secretion at all comparable to that induced by lactogen. Frazier and Mu (1935) injected adult male rabbits with 20 to 60 rat units of estrogen daily. After 80 days of treatment milk could be expressed from the nipples. In six animals, the secretion increased in amount and became thick and white; in two animals it remained thin and watery. Four rabbits continued to secrete some milk for as long as 200 days. Nelson (1937) reported that lactation regularly followed the cessation of treatment of guinea pigs with estrogen-in-oil.

Enzmann and Pincus (1933) observed a reduced growth rate and death of young mice when increasing amounts of gonadotropic hormone from urine were injected into the mothers. These observations suggest that the increased amount of estrogen thus stimulated suppressed milk secretion. Even more suggestive would be the stimulation of progestin secretion. De Jongh (1933) reported that estrogen treatment of nursing mice caused the death of the young. The daily injection of 5 r. u. of urinary gonadotropic hormone resulted in one of three lactating mice losing her young. When 10 r. u. were injected into four other mice all young were dead within 8 to 13 days afterward. The results obtained by Robson (1935) show that the injection of estrogen into lactating mice inhibits mammary secretion. This inhibition occurred in both normal and ovariectomized animals and the degree of inhibition was definitely related to the dose administered and to the method of administration. When the estrogen injections were given at short intervals, two hours, a complete cessation of mammary secretion rapidly followed.

De Jongh (1933) reported that the injection twice daily of 250 m. u. of crystalline estrogen in 0.1 cc. of oil was sufficient to cause the death of the majority of the young of four lactating rats. One rat injected with 50 m. u. twice daily reared her young but they did not grow well. Anselmino and Hoffman (1936) injected 1,000 i. u. of estrogen in oil daily into rats immediately after parturition. The number of young was reduced to six or eight. Of ten mothers, seven were unable to rear their young, the young dying after 5 to 9 days. The stomachs of the young showed only traces or no milk. In the other three, the young survived but were stunted. One hundred and 250 i. u. had no effect. In a second series of experiments, the ovaries from the others were removed immediately after parturition. The same level of estrogen injection was without any inhibiting influence upon lactation. Consequently, the corpus luteum hormone was suspected. A purified, but not crystallized, preparation of progestin was used. Lactating rats received one-half of a Corner-Allen unit daily for 15 days, others received one-fourth of a unit daily. In no case was lactation inhibited although there were some slight modifications. In a second report Anselmino et al. (1936) induced lactation in young rats by the daily injection of 200 r. u. of prolan for 14 to 18 days followed by castration. Twenty to 4,000 i. u. of estrogen were injected into another group of rats at the time of castration and a second injection 18 hours later. The animals were sacrificed 36 hours after castration and no inhibition of lactation was observed. In a third group of rats the same procedure was followed except that 0.2 to 2 units of progestin were injected instead of estrogen. Lactation was reduced at the lower levels of injection and at the higher levels it was suppressed entirely. These experiments were taken to indicate that estrogen does not suppress lactation but rather some factor associated with the crude corpus luteum extract.

Nelson (1936) has summarized his work which led to the development of a hypothesis concerning the inhibition of lactation during pregnancy. His hypothesis is that the ovarian hormones, chiefly estrogen, during the latter part of pregnancy inhibit the actual secretion of milk. This inhibiting influence was thought to be suppressing the secretion or excretion of lactogen from the pituitary gland and acting directly on the mammary gland. Following parturition there is a decline in the level of the ovarian hormones. This would remove the inhibitory influence, thus allowing lactogen to be secreted and discharged, and lactation would set in. A recent report by Nelson (1937) indicates that the inhibiting influence is acting directly upon the mammary glands. Lactation was induced in guinea pigs previously treated with estrogen by the administration of crude pituitary extracts. If estrogen was given, in addition to the pituitary extract, lactation was prevented. These effects were observed in hypophysectomized as well as in normal animals. Sardi (1935) observed that the simultaneous injection of estrogen and anterior hypophyseal extract produced mammary hyperplasia in five male guinea pigs but even after 16 to 30 days it did not stimulate milk secretion.

Experiments were cited by Fellner (1931) to indicate that large amounts of estrogen have an inhibiting effect on milk secretion in rabbits. Smith and Smith (1933) reported that the lactation which would normally be induced by lactogen in the rabbit could be prevented by the simultaneous administration of large amounts of estrogen. However, the injection of as much as 4,000 r. u. of estrogen over a period of six days had no pronounced inhibiting effect upon the amount or duration of secretion in two rabbits well advanced in lactation. De Fremery (1934) reported that the simultaneous injection of estrogen with lactogen was neither able to prevent nor delay the initiation of lactation in rabbits. It was thought that the inhibiting effect of estrogen was upon the pituitary.

De Fremery (1934) reported that milk production quickly decreased following the injection of 200,000 i. u. of estrogen into a virgin goat in which lactation had been induced by the injection of lactogen. When the treatment with estrogen was discontinued milk secretion reappeared.

Folley (1936a) injected intramuscularly over a period of three days a total of 500 mg. of oestrone and 50 mg. of oestradiol benzoate into a lactating Guernsey cow. The injections caused a depression of about 20 per cent in milk yield, and at the same time solids-not-fat increased more than 10 per cent. The fat content of the milk was not altered. The injection of an equal amount of oil into a control cow was without any effect on lactation. Waterman, Freud, and de Jongh (1936) treated a cow during a 33 day period with oleum olivarum containing 400,000 i. u. of oestradiol benzoate, the material being rubbed over the udder twice a day. During the treatment the milk production was reduced from 5 liters to 0.5 liter. Similar treatment with oil alone had no effect whatever in a half-sister cow. In a later report Folley (1936b) injected considerable amounts of estrogen into three Guernsey cows. In all three cows, there followed a temporary decline in milk yield. However, in only one cow was the decrease more marked than were subsequent decreases in the lactation curves of these cows when uninjected. The injection of the hormone was followed by higher fat and solids-not-fat percentages.

Attempts to Inhibit Lactation in the Albino Rat by Injections of Ovarian Hormones

The availability of a technique whereby the lactogen content of pituitary glands from small laboratory animals could be determined, made it possible to determine the influence of ovarian hormones on the pituitary glands from lactating laboratory animals. This assay work would indicate whether or not the ovarian hormones were inhibiting the secretion of the lactogenic hormone.

Accordingly, six lactating rats were injected daily with 1,000 i. u. of estrogen, the first injection being made on the day of delivery. In each case the number of young in a litter was reduced to six at the time of the first of the injections of the mother. The injections were continued for seven days, the litters then removed and the mother rats sacrificed 15 hours later. The young were weighed daily and examined to see if they had milk in their stomachs. The results were uniform and only a summary will be presented. On the seventh day of injection 35 of 36 young were alive but appeared emaciated. Judging from the average daily weights of the young, they were securing sufficient milk for maintenance and a small daily gain in weight. The average weights of the young in grams on seven consecutive days were as follows: 6.0; 6.7; 7.5; 8.3; 8.8; 9.1; and 9.2. The average body weights of the mother rats at the time of sacrifice were 176 gm., average pituitary weight 16.6 mg., and on the average the pituitary glands contained 13.3 bird units of lactogen. On section, the alveoli in five of the six mammary glands were distended with milk. In the sixth case only a small amount of milk was present in the mammary gland. These results are taken to indicate that the injection of estrogen decreases the rate of milk secretion, but in no case did it completely inhibit milk secretion. The mode of this inhibitory action of estrogen remains undetermined. However, it did not appear to be inhibiting the secretion or excretion of the lactogenic hormone of or from the pituitary gland, and inasmuch as the alveoli were distended with secretory products the estrogen was not completely inhibiting the secretory activity of the glandular epithelium.

Since the ovaries were intact in the above animals it was then decided to see what effect estrogen injections would have in the ovariectomized lactating rat. Only two such trials were made and the following are the protocols.

Lact. Rat 92. Dropped 9 March 1, 1937. The 9 young weighed 50 gm. and the 6 young which were saved weighed 34.4 gm. Mother ovariectomized and daily injections of 1,000 i. u. of estrogen begun. Average daily weights of young in grams: 5.4; 6.1; 7.1; 8.0; 9.2; 10.3; 11.0; 11.5; 12.2; 12.6; 13.0; and 13.4. Litter removed and mother sacrificed 15 hours later. Body weight 175 gm., pituitary weight 15.3 mg., and pituitary assayed 11.25 bird units of lactogen.

Lact. Rat 94. Dropped 6 March 7, 1937. The 6 young weighed 30.2 gm. Mother ovariectomized and daily injections of 1,000 i. u. of estrogen begun. Average daily weights of young in grams: 5.0; 5.6; 6.5; 7.5; 8.3; 9.3; 10.1; 11.6; 11.6; 13.0; and 13.0. Litter removed and mother sacrificed 15 hours later. Body weight 184 gm., pituitary weight 30.4 mg., and pituitary assayed 25 bird units of lactogen.

The average weight of the young until the end of the fifth day, in these two litters, is much the same as that observed of young nursing normal mothers in the same colony. However, at the end of the eleventh day of injection, the average weight of the young was only two-thirds of that observed of young nursing normal mothers. Judged by the pituitary lactogen content and the distention of the alveoli of the mammary gland with milk, there was no evidence of inhibition of lactogen secretion or excretion, and no evidence of the estrogen directly inhibiting the secretory epithelium of the mammary gland.

These results and those of Anselmino and Hoffmann (1936) suggested that the estrogen might be acting on the ovaries through the pituitary gland, lactation inhibition being more pronounced in animals injected with estrogen when the ovaries were intact. The logical step was to determine the effect of injecting progestin into lactating animals, and this was done in seven lactating rats. The effects produced by progestin injections were inconsistent, therefore, the protocols are given here.

Lact. Rat 74. Dropped 5 December 9, 1936. Mother ovariectomized and injection of one-half of a Clauberg unit of progestin twice daily begun. On December 13, 1936, the progestin dosage was doubled. Average daily body weight of young in grams: 5.8; 6.1; 7.1; 8.4; 8.7; 8.8; 8.9; 8.8; and 8.3.

Young all dead December 17, 1936, three dying December 16, 1936, and two dying December 17, 1936. Mother rat sacrificed 15 hours later. Body weight of mother was 211 gm., pituitary weight 11.8 mg., and lactogen content 5.37 bird units.

Lact. Rat. 85. Dropped 9 January 20, 1937. Number in litter reduced to 6. Mother ovariectomized and injection of one Clauberg unit of progestin twice daily begun. Average daily body weight of young in grams: 5.9; 5.6; 5.6; 5.6 (one young died); 5.8; 5.8; and 6.1. Five young were alive January 27, 1937, and they were removed from their mother. Mother rat sacrificed 15 hours later. Body weight 199 gm., pituitary weight 8.5 mg., and lactogen content of pituitary gland was 4.25 bird units.

Lact. Rat. 86. Dropped 6 February 2, 1937. Mother ovariectomized and injection of a Clauberg unit of progestin twice daily begun. Average daily body weight of young in grams: 5.9; 6.2; 6.5; 6.1; 6.3; 6.7; 7.3; 7.9; and 7.7. Five young dead February 10, 1937, remaining young removed from mother and the mother rat sacrificed 15 hours later. Body weight of mother was 163 gm., pituitary weight 8.7 mg., and pituitary assayed 6.5 bird units of lactogen.

Lact. Rat. 87. Dropped 10 February 6, 1937 and litter reduced to 6. Mother ovariectomized and injections of 1.5 Clauberg units of progestin twice daily begun. Average daily body weight of young in grams: 5.6; 5.6; 6.4; 7.1; 8.2; 9.3; 10.1; 11.6; and 12.5. Young were all alive February 14, 1937, and they were removed at this time. Mother rat sacrificed 15 hours later. Her body weight was 223 gm., pituitary weight 10.1 mg., and pituitary assayed 10.12 bird units of lactogen.

Lact. Rat 93. Dropped 6 March 17, 1937, with body weight 29.7 gm. Mother ovariectomized and injection of 1.5 Clauberg units of progestin twice daily begun. Only one young alive March 18, 1937. The young was removed from the mother and the mother rat sacrificed 15 hours later. Her body weight was 180 gm., pituitary weight 10.2 mg., and pituitary gland contained 4.25 bird units of lactogen.

Lact. Rat 95. Dropped 13 March 21, 1937. Litter number reduced to 6. Mother ovariectomized and injections of 1.5 Clauberg units of progestin twice daily begun. Average daily body weight of young in grams was: 5.0; 5.6; 6.8; 7.6; 9.1; 10.4; and 12.2. Young all alive March 28, 1937, at which time they were removed and the mother rat sacrificed 15 hours later. Her body weight was 234 gm., pituitary weight was 7.7 mg., and pituitary gland assayed 10.25 bird units of lactogen.

Lact. Rat 96. Dropped 6 March 22, 1937. Mother ovariectomized and injections of 1.5 Clauberg units of progestin twice daily begun. Average daily weight of young in grams was as follows: 5.5; 4.7; 4.9; 5.8; 6.8; 7.6; and 8.9. Young all alive March 28, 1937, at which time they were removed. The mother rat was sacrificed 15 hours later. Her body weight was 177 gm., pituitary weight 7.9 mg., and pituitary gland upon assay yielded 8.25 bird units of lactogen.

Although the effects produced by progestin injections were inconsistent, it is worthy of mentioning that in those cases where lactation was noticeably inhibited the lactogen content of the pituitary gland was low. In these cases the pituitary assays would suggest that progestin might have been inhibiting lactogen secretion. Histological sections of the mammary glands showed that two of four animals, whose lactogen content of the pituitary gland was low, had mammary glands in which the alveoli were not distended with milk. In the other three cases, where lactation was inhibited, very little, if any, the lactogen content of the pituitary glands compared favorably with those from non-injected lactating animals.

These results are not conclusive, but they do suggest several points. Inhibition of lactation by estrogen injections is more pronounced in animals with their ovaries intact than in ovariectomized animals. The lactogen content of pituitaries from lactating rats which were injected with estrogen was higher than pituitaries from normal lactating rats. Furthermore, the mammary glands from these rats were distended with secretory products. The results from progestin injections suggested, in four out of seven cases, that the injections might be inhibiting lactogen secretion.

THE RELATION OF THE NERVOUS SYSTEM TO THE PITUITARY GLAND

For many years the nervous system has been recognized as an integrator of body function. In more recent years the developments in the field of endocrinology have clearly indicated the part played by hormones in regulating body activity. Only in the last few years, especially in the field of the physiology of reproduction, has it become apparent that these two regulatory systems are also working together in the control of body function.

Kuramitsu and Loeb (1921) in making a study of the involution of the mammary gland of rats and guinea pigs observed that the suckling of the mammary gland(s) on one side sometimes caused the pressing out of milk from the glands of the other side. This observation suggested to them the possibility of the participation of reflexes which connected the separate glands in the process of suckling. They were interested in checking the effect of suckling in one mammary gland on the involutionary changes in the other gland. In several guinea pigs they ligated one nipple. In each case they found that the gland from which milk could no longer be removed retrogressed rapidly, comparable to the involution of the mammary gland in the non-nursing guinea pig. The other gland which was suckled by the young continued to be large and to produce milk. They therefore concluded that the process which maintains the active state of lactation in the mammary gland consisted solely in the local effect of the withdrawal of the milk.

Hammond and Marshall (1925) employing rabbits, devised the following experiment to determine if the cause of atrophy of the mammary glands in non-suckled rabbits was due either to the accumulation of secretory products in the alveoli and ducts or to the lack of the stimulus of suckling. The nipples on one side of the rabbit were ligated and painted over with collodion and cotton-wool. The remaining nipples were untouched. The mammary glands in the first animal from which milk was removed weighed 194 gm. and the glands from which milk was not removed weighed 32 gm. In a second rabbit the mammary glands from the side with occluded nipples weighed 56 gm. while the glands from the side with normal nipples weighed 170 gm. These investigators concluded that it was not the lack of stimulus of suckling that caused the mammary glands to atrophy when the young were removed at parturition, but that it was probably due to the accumulation of milk in the gland causing cessation of secretion and the absorption of the gland. Histological sections of these glands were not shown so that the anatomical condition of the glands remained undetermined.

Selye (1934) was able to show that the act of suckling maintained the mammary glands of the rat in the stage of active secretion over a 14 day period, even though the main galactophores were ligated and milk could not be removed. Suckling at one set of nipples maintained active secretion in the other glands where suckling had been prevented by excision of the nipples. It thus appeared that the involution of the mammary gland after weaning was due to the withdrawal of the nervous stimulation of suckling rather than by the accumulation of the products of secretion in the gland. Selye suggested that unless the guinea pig differed greatly from the rat, Kuramitsu and Loeb would have obtained different results had they examined the glands at an earlier stage after the operation.

Utilizing a series of ten rats which had been lactating at least 48 hours, Ingelbrecht (1935) sectioned the spinal cord between the last dorsal vertebra and the first lumbar vertebra. This rendered the six inferior mammary glands insensible. If the six remaining sensitive superior teats were covered with a jacket, only allowing the anesthetized group to be nursed, there was a sudden decrease in the weight of the young and they died within 48 hours. Moreover, if the jackets covered only four superior sensitive teats, milk secretion continued in all of the glands, innervated or not. Lactation could be re-established by permitting the young to nurse the sensitive teats. He concluded that the reflex arising from the teats passed through the cerebro-spinal axis and that the response was probably transmitted by a humoral method.

Turner and Reineke (1936) working with two goats, suspended milking on one side of the udder about 30 days after parturition, while the other side was milked regularly. Histological specimens taken 65 days after the beginning of the experiment revealed that the alveolar structure was still well defined on the unmilked side.

Hesselberg and Loeb (1937) repeated the earlier work of Kuramitsu and Loeb (1921). One nipple of a series of lactating guinea pigs was ligated and the other remained normal. Three to 20 days following labor, the mothers were sacrificed and both mammary glands removed for microscopic examination. The amount of milk in the non-suckled gland rapidly diminished and after 12 days and later the amount of glandular tissue left on the ligated side was very small. The mammary glands from the side where the nipples were not ligated presented a typical picture of the secreting gland during the first 18 days.

Effect of the Stimulus of Suckling Upon the Lactogen Content of the Rat Pituitary

In an earlier section results were presented which showed that pituitary glands from 48 hour post-partum rats contained, on the average, 7.72 bird units of lactogen per gland. In this experiment, the young were with the mother continually up until the time she was sacrificed.

In the light of Selye's (1934) work upon the influence of suckling on the rat mammary gland, it became exceedingly interesting to study the influence of suckling upon the lactogen content of the rat pituitary. The litters of ten rats were removed 36 hours after parturition and then returned to their mothers 12 hours later, without receiving any nourishment from other lactating rats during this 12 hour period. Frequent observations were then made to see if the young were nursing. Immediately after a nursing period of three hours the mother rats were sacrificed, their pituitaries removed, weighed, and assayed. For controls the litters of ten rats were removed 36 hours after parturition and the mother rats sacrificed 15 hours later. Their pituitaries were then removed, weighed, and assayed. The pituitaries from the suckled rats contained an average of 3.06 bird units of lactogen, those from non-suckled rats contained an average of 9.2 bird units per gland. On the basis of one milligram of fresh pituitary tissue the pituitaries from the suckled rats contained 0.30 of a bird unit and those from non-suckled rats assayed 1.04 bird units. This work demonstrates that the stimulus of suckling and/or the removal of milk from the mammary glands markedly decreased the lactogen content of the post-partum rat pituitary gland. When the mother rats were not nursed there occurred an accumulation of milk in the mammary glands and an augmentation of the lactogen content in the pituitary gland; 9.20 bird units of lactogen as compared with 7.72 units.

TABLE 25.—LACTOGEN CONTENT OF PITUITARIES OF LACTATING RATS IN WHICH THE YOUNG NURSED FOR 36 HOURS, YOUNG THEN REMOVED FOR 12 HOURS, AND THEY THEN RETURNED FOR A 3-HOUR NURSING PERIOD. MOTHERS THEN SACRIFICED.

Rat No.	Body weight (gm.)	Total pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
36	226	12.5	5.50	0.44	2.07
38	232	9.8	2.37	0.24	1.02
41	225	9.4	2.67	0.28	1.19
24	213	10.4	1.50	0.14	0.70
21	206	10.6	5.50	0.52	2.67
18	191	8.4	2.00	0.24	1.05
42	190	9.9	3.50	0.35	1.84
40	187	9.7	2.50	0.26	1.34
28	183	9.2	2.25	0.24	1.23
39	175	9.2	2.87	0.31	1.64
Average	206	9.9	3.06	0.30	1.48

*Bird units

TABLE 26.—LACTOGEN CONTENT OF PITUITARIES OF LACTATING RATS IN WHICH THE YOUNG WERE ALLOWED TO NURSE FOR 36 HOURS AND THE MOTHER RATS SACRIFICED 15 HOURS LATER.

Rat No.	Body weight (gm.)	Total pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
26	230	13.7	10.50	0.77	4.57
46	227	10.4	10.25	0.99	4.52
23	217	11.5	6.75	0.59	3.11
49	207	10.5	9.25	0.88	4.47
50	191	9.2	8.50	0.92	4.45
43	176	8.1	9.25	1.02	5.26
44	169	7.4	8.67	1.17	5.13
47	167	7.3	9.00	1.23	5.39
45	162	7.2	11.37	1.58	7.02
48	156	7.0	8.50	1.21	5.45
Average	190	9.2	9.20	1.04	4.94

*Bird units

The records on the individual rats are given in Table 25 for the suckled rats and in Table 26 for the non-suckled rats.

In order to determine which one of the above two mentioned factors was responsible for the lactogen decrease in the pituitary gland, the following experiment was carried out. The litters from 11 rats were removed 36 hours following parturition. The lactating mother rats were then placed under light ether anesthesia and the primary galactophore of each mammary gland ligated. The galactophore was approached through a dorsal-lateral skin incision made from each teat. This procedure usually made it impossible for the young to secure milk from any of the mammary glands. Twelve hours later the litters were returned to their mothers and the young allowed to nurse for three hours. In all cases the young nursed vigorously and in those instances where the young secured observable amounts of milk the results were not included in this group. Following the three hour nursing period the mother rats were sacrificed, their pituitaries removed, weighed, and assayed. These pituitary glands contained 5.20 bird units per gland. This value is somewhat higher than that obtained when the young were allowed to suckle and remove the milk, but only about one-half of the value secured in those cases where the young were not returned to their mothers for a three hour nursing period. Thus, the stimulus of suckling definitely decreases the lactogen content of the post-partum rat pituitary gland even when no milk is removed (Table 27).

TABLE 27.—LACTOGEN CONTENT OF PITUITARIES OF LACTATING RATS IN WHICH THE YOUNG NURSED FOR 36 HOURS, YOUNG THEN REMOVED FOR 12 HOURS AND THEY THEN RETURNED FOR A 3-HOUR NURSING PERIOD DURING WHICH TIME THEY SECURED NO MILK. MOTHERS THEN SACRIFICED.

Rat No.	Body weight (gm.)	Total pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
58	253	9.7	6.50	0.67	2.57
57	241	12.6	5.87	0.47	2.44
53	215	13.6	6.12	0.45	2.85
56	192	10.9	3.67	0.34	1.91
51	182	9.5	5.00	0.54	2.75
60	180	9.1	4.75	0.52	2.64
62	175	9.9	6.00	0.61	3.43
63	170	7.6	5.00	0.66	2.94
61	160	7.5	5.12	0.68	3.20
54	151	9.7	3.67	0.38	2.43
52	150	6.7	5.50	0.82	3.67
Average	188	9.7	5.20	0.56	2.80

*Bird units

After demonstrating the profound influence of the stimulus of suckling on the lactogen content of the lactating rat pituitary gland, the thought presented itself that perhaps the stimulus of suckling was responsible for the increase in lactogen content of pituitaries from post-partum rats as compared with glands from normal females or pregnant rats. Accordingly, pregnant rats were placed in cages with wire floors which were meshed so that they would withhold the pregnant females but not the young. No nesting material was provided and as a result the young dropped through the wire floor soon after being delivered, the parturient rats never being suckled. The mother rats were sacrificed 51 hours after they had delivered. The lactogen content of these pituitaries compared favorably with those taken from lactating rats where the young were with the mothers continually until the time when they were sacrificed. The average lactogen content of the pituitaries from 51 hour post-partum rats, where the young never nursed, was 7.12

bird units. Therefore, the increase in the lactogen content of the rat pituitary gland following parturition is not dependent upon the suckling stimulus. The results are given in Table 28.

TABLE 28.—LACTOGEN CONTENT OF PITUITARIES FROM 51-HOUR POST-PARTUM RATS IN WHICH THE YOUNG NEVER NURSED.

Rat No.	Body weight (gm.)	Total pituitary weight (mg.)	Average No. of B.U.* per pituitary gland	Average No. of B.U. per mg. pituitary tissue	Average No. of B.U. per 100 gm. body weight
75	242	13.2	10.0	0.76	4.13
76	232	13.1	8.00	0.64	3.45
71	213	8.7	7.13	0.82	3.35
73	205	8.9	5.25	0.59	2.56
67	198	9.3	5.88	0.63	2.97
77	185	9.5	7.50	0.79	4.05
68	182	9.3	6.68	0.72	3.67
70	181	7.6	6.00	0.79	3.31
79	180	9.4	9.75	1.04	5.42
82	155	7.7	5.00	0.65	3.23
Average	197	9.67	7.12	0.74	3.61

*Bird units

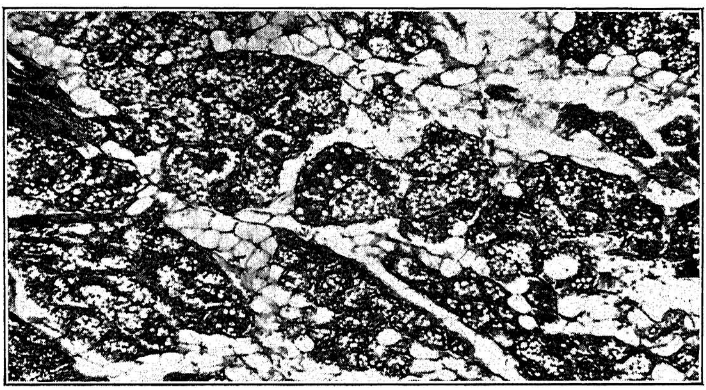

Fig. 8.—Photomicrograph of a section of the mammary gland of a lactating rat (79). The young were not permitted to nurse and the mother rat was sacrificed 51 hours after parturition. The alveoli were distended slightly with milk. The pituitary glands from a group of lactating rats sacrificed under the same conditions contained an average of 7.12 bird units of lactogen. X60.

The mammary glands from these rats in which the young never nursed were not gorged with milk, and a histological section in some cases showed a fair amount of milk being secreted (Fig. 8), and in other cases there was little evidence of secretory activity (Fig. 9). There was the possibility that either the mammary gland had been in a more active state at an earlier hour or that in the absence of nursing the lactogenic hormone was not being dis-

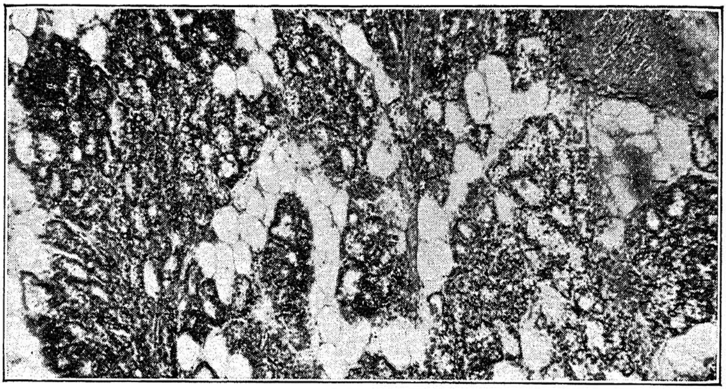

Fig. 9.—Photomicrograph of section of the mammary gland of a lactating rat (75). The young were not permitted to nurse and the mother rat was sacrificed 51 hours after parturition. The alveoli showed very little evidence of secretory activity. The pituitary glands from a group of lactating rats sacrificed under the same conditions contained an average of 7.12 bird units of lactogen. X60.

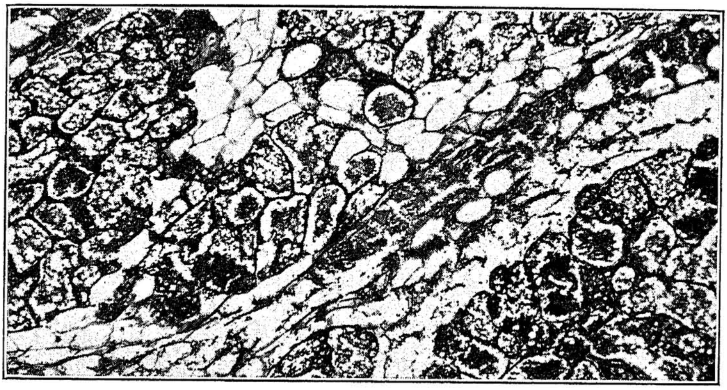

Fig. 10.—Photomicrograph of a section of the mammary gland of a lactating rat (103). The young were not permitted to nurse and the mother rat was sacrificed 6 hours after parturition. The alveoli were filled with milk. X60.

charged into the blood. Rats, in which the young never nursed, were then sacrificed at six hour intervals from the time of delivery until 48 hours after delivery. The alveoli of the mammary glands from rats six hours after delivery were distended with milk, indicating that lactation is established in rats following parturition even in those cases where the young are not nursed (Fig. 10).

DISCUSSION

With the unfolding of the field of endocrinology it becomes more and more apparent that the tiny gland located at the base of the brain, the pituitary, is the integrator of the endocrine system. Since 1928, when it was first established that the anterior lobe contained a principle that would initiate lactation, our knowledge of the endocrine control of the mammary gland has been extended considerably. The central position occupied by the pituitary gland in the endocrine control of the growth and function of the mammary gland is being gradually better understood.

The development of the hypophysectomy technique demonstrated that the pituitary gland is necessary for the initiation and maintenance of lactation, the replacement therapy demonstrated that the lactogenic hormone is one of the pituitary principles required in normal mammary function. Other than the establishment of these two facts concerning the lactogenic hormone, and the species response to this hormone, little has been added to our knowledge since Grueter's pioneer work of 1928.

The lack of a technique for the assay of pituitary glands from small laboratory animals for the lactogenic hormone has been a serious handicap to investigators in furthering their knowledge of this hormone. The development of an assay procedure whereby minute quantities of lactogen contained in a rat pituitary gland could be determined has made possible the assay of pituitary glands when the donors were in various physiological states. This study has elucidated a number of points and has opened the field to a point where it is believed that additional work will add considerably to our knowledge of the lactogenic hormone.

After the development of this technique it was possible to study the factors influencing the secretion and discharge of the lactogenic hormone. One of the most puzzling questions in the endocrine control of the mammary gland has been what stimulates the pituitary gland to the secretion of the lactogenic hormone following parturition? This assay work has demonstrated several factors which play a part in controlling the secretion and discharge of the lactogenic hormone.

We know that as pregnancy continues, the organism comes under the influence of increasing quantities of estrogen. Since estrogen injections into ovariectomized rats stimulates the secretion of the lactogenic hormone it is believed that estrogen acting upon the pituitary gland during pregnancy prepares the cells of the anterior lobe for the secretion of large quantities of lactogen following parturition. However, during pregnancy some factor seemingly is inhibiting the secretion of the lactogenic hormone because the lactogen content of the rat pituitary gland is not increased during pregnancy. It is suggested that this inhibitory factor might be progesterone or a factor associated with progesterone in progestin. It usually has been thought that secretory activity of the mammary gland begins during the last part of pregnancy, but when one examines a histological section of the mammary gland of a rat on the 21st day of pregnancy there certainly is very little evidence of secretory activity as compared with that which follows parturition. Moreover, the alveolar activity during pregnancy is not identical with that during lactation for there is a considerable difference in the composition of colostrum and milk, a fact which suggests that during pregnancy all of the conditions necessary for milk secretion have not been established. Immediately following parturition some mechanism, perhaps a nervous one, causes the discharge of the lactogenic hormone from the pituitary gland for as soon as six hours following parturition, even in the absence of nursing, the alveoli of the rat mammary gland are distended with secretory products. This mechanism at parturition that causes the discharge of lactogen from the pituitary gland is transitory inasmuch as in the absence of nursing the secretory activity of the mammary epithelium has begun to subside as early as 48 hours after parturition. The stimulus of nursing not only brings about a discharge of the lactogenic hormone from the pituitary gland but also stimulates the secretion of a greater amount of this hormone and perhaps that of the other anterior lobe hormones required for the maintenance of normal lactation. Thus, while a part of the controlling mechanism working during pregnancy and at the time of parturition remains unsolved, the necessity of a stimulus to the pituitary gland to bring about the discharge of the lactogenic hormone in order to maintain lactation seems to be established, at least in the rat.

The observation that pituitaries from females contain more of the lactogenic hormone than those from males invites one to conjecture upon this point. The superiority of the female rat pituitary in lactogen content over that of the male is established already in rats weighing 70 to 80 grams, and this sex difference in lactogen content of pituitary glands becomes greater as the animals mature. This sex difference appears to be due to the ovarian influence upon the pituitary gland. In support of this thought is the following information: the pituitaries from ten female rats, which were ovariectomized when their average body weight was 42 grams and sacrificed 60 days later, averaged 7.3 mg. in weight and contained 0.86 of a bird unit of lactogen; pituitaries from ten male rats, which were castrated when their average body weight was 45 gm. and sacrificed 60 days later, weighed 9.9 mg., on the average, and contained 0.99 of a bird unit per pituitary gland. Therefore, gonadectomy of the rat tends to neutralize the lactogen content of the pituitary gland in the male and female. Along with these observations it is worthy of note that the injection of estrogen into normal males for a relatively short time does not increase the lactogen content of their pituitary glands to a height where it is comparable to that found in female rat pituitaries. This suggests that the presence of the ovary during the growing period influences the pituitary of the female so that it has the ability to secrete greater quantities of the lactogenic hormone under estrogen stimulation. Ovarian grafts in males and estrogen injections into mature females which have been ovariectomized when sexually immature would elucidate this point.

The pituitary glands from one species were not assayed against those of another species yet there was sufficient differences in separate assays to make one believe that a species variation in the lactogen content of pituitary glands does exist. Following is a tabulation of the lactogen unitage per milligram of fresh pituitary tissue and per unit of body weight:

Animal	*Sex*	*Bird units per mg. of fresh Pituitary tissue*	*Bird units per 100 gm. body weight*
Mouse	F	0.250	1.32
Rat	F	0.471	2.16
Rat	M	0.143	0.36
Guinea pig	F	0.520	1.40
Guinea pig	M	0.348	0.91
Rabbit	F	0.420	0.32
Rabbit	M	0.074	0.05
Cat (anestrus)	F	0.065	0.07
Cat (estrus)	F	0.224	0.38
Cat	M	0.037	0.03
Beef cows	F	0.482	0.90
Dairy Cows	F	0.804	1.49

Of most interest in these species variations are the facts that the hormone concentration in the pituitary gland of the female rat, guinea pigs, and rabbit is about the same, the greater potency of male guinea pig pituitaries in comparison with male rat pituitaries, the low hormone concentration in glands from anestrum cats, and the superiority of dairy cows' pituitaries over that of pituitaries from beef cows.

Various conditions influence the hormone content of cattle pituitaries, but the difference in lactogen and thyrotropic content of pituitaries from dairy and beef cows has been of most concern. The abilities of dairy cattle to secrete milk and beef cattle to produce meat have constituted the main differences between these two cattle types. When someone asked what was responsible for this difference he was told that dairy cattle inherited the ability to produce greater quantities of milk, and that was about all one could say. Inheritance studies have shown that milk production is not inherited by one single factor but by many factors. The way the genes expressed themselves was unknown and as a result inheritance studies of milk production have not taken very rapid strides.

With the ever increasing knowledge in the field of endocrinology and more specifically, the part that the endocrine glands play in the control of the growth and the function of the mammary gland, the dairy student is given a new mode of attack in attempting to determine why some cows produce large quantities of milk and others only small quantities of milk. This study already has indicated that the pituitary of the dairy cow is secreting more of the lactogenic and the thyrotropic hormones than the pituitary gland of the beef cow.

At the present time, as far as the hormones are concerned, the qualitative phase of milk secretion has been solved. The next logical step is to determine how the hormones control the quantity of milk secreted. It has been proved that the lactogenic hormone is essential for the initiation and maintenance of lactation. Furthermore, there is some evidence that this hormone can raise the level of milk production. The mode of its action is unknown, but it is thought that it acts, in some manner, directly upon the secretory epithelium of the gland parenchyma. Increasing amounts of the lactogenic hormone will increase milk production only until the point where some other hormone becomes the limiting factor.

If granted that the lactogenic hormone can increase milk flow up to a certain point, how can one determine the physiological difference of dairy cows? With the females two possibilities exist. After the heifers come into lactation they could be injected with the purified lactogenic hormone. If they failed to increase in milk production this would indicate that their ability to secrete the lactogenic hormone was not the limiting factor in their capacity to secrete milk. However, if the heifers did increase in milk production one would then determine how much their milk production could be increased by injecting this hormone, and this amount would represent the deficiency of their pituitary glands to secrete the lactogenic hormone. On the other hand, the possibility exists of determining the rate at which this hormone is excreted in the urine. If this can be done one could then measure the excretory rate of the lactogenic hormone. As far as bulls are concerned, there would be the possibility of determining the rate of excretion of this hormone in the urine. Otherwise, the ability of the bull's daughters to secrete the lactogenic hormone would be determined.

After ratings were determined on bulls and heifers, matings would then be made and by a study of their progeny one could determine the mode of inheritance of the pituitary gland to secrete the lactogenic hormone. With the development of tests for determining the rate of secretion of the other hormones concerned in milk production one could then rate dairy cattle for all of these hormones. At least, for the first time it seems possible to break down the inheritance of the milk production complex into its component parts.

The observation of a greater concentration of the thyrotropic hormone in pituitaries from dairy cows than in pituitary glands from beef cows suggests that the difference in the pituitary glands of these two cattle types to secrete the thyrotropic hormone may constitute one of their major differences. It is known that the thyrotropic hormone activates the thyroid gland and in turn the thyroid gland discharges more thyroxine into the blood. The profound influence of thyroxine upon milk production and more especially upon milk fat production has been demonstrated. Therefore, it seems probable that the difference in the ability of the pituitary glands of dairy and beef cows to secrete the thyrotropic hormone physiologically explains one reason why these two cattle types vary in their capacity to secrete milk.

Occasionally a high producing dairy cow suddenly drops in milk production, the dairyman commonly speaks of the cow "as going to pieces." In such cases it is thought that the stimulus for milk production in the animal is so great that the catabolic processes outweigh the anabolic processes. It is very probable that such stimulus has its origin in the pituitary gland. Along this same train of thought one cannot help but suggest that the expression "dairy temperament" may be expresed ultimately in physiological terms.

SUMMARY

1. The average weights of six groups of cattle pituitaries were as follows: 51 fetal pituitaries, 0.0431 gm.; 22 calf pituitaries, 0.6485 gm.; 234 animals between four and ten months of age, 0.9025

gm.; 163 animals between 11 and 23 months of age, 1.1590 gm.; 50 dairy cows two years old or over, 1.7521 gm.; and 22 beef cows two years old or over, 1.8655 gm.

2. An assay technique has been developed whereby one can determine the minute differences in the lactogen content of pituitary glands from laboratory animals under various physiological conditions. A bird unit of the lactogenic hormone is defined as that amount of the hormone which will cause the proliferation of an area of the crop gland about the size of a nickel when injected intradermally over the crop gland of a mature pigeon for four days, the pigeon being sacrificed upon the fifth day.

3. Pituitaries from female animals contained more lactogen than pituitary glands from males. As the female matures there is not only an increase in the lactogen content per pituitary gland but also an increase in the hormone concentration within the gland. Maturity in the male is accompanied by an increase only in the lactogen content per pituitary gland.

4. A species difference was found in the lactogen content of pituitary glands per milligram of fresh pituitary tissue. Pituitary glands from females ranked in the following descending order: dairy cow; guinea pig; beef cow; rat; rabbit; mouse; estrus cat; and anestrus cat. Pituitary glands from males ranked in the following descending order: guinea pig; rat; rabbit; and cat.

5. Pituitary glands from rats which were in diestrum contained 3.8 bird units per pituitary gland, those from rats in proestrum 4.4 units, those from rats in estrum 4.8 units, and those from rats in metaestrum 3.3 units.

6. Pituitary glands from rats which were pregnant for 12 days contained 2.9 bird units of lactogen and those pregnant for 21 days contained 3.5 bird units per gland. Glands from normal female rats contained 4.29 bird units of lactogen.

7. The lactogen content of pituitary glands from rats and guinea pigs was more than doubled following parturition as compared with glands from normal animals.

8. Ovariectomy of the female rat markedly decreased the lactogen content of the pituitary gland, while in the male rat the lactogen content was unaltered by castration.

9. The abdominal anchorage of the testes in male rats did not alter the lactogen content of the pituitary gland.

10. The injection of estrogens (theelin-in-oil and progynon-B) into ovariectomized rats, normal male and castrated rats, and male guinea pigs increased the lactogen content of the pituitary glands.

11. Progestin injections into ovariectomized rats had no influence upon the lactogen content of the pituitary glands.

12. The simultaneous administration of 200 i. u. of estrogen (progynon-B) and 0.5 of a Clauberg unit of progestin into ovariectomized rats produced the same increase in pituitary lactogen content as the injection of 200 i. u. of estrogen into ovariectomized rats.

13. Thyroxine injections into male rats, when sufficient quantities were injected, decreased pituitary size and lactogen content.

14. The daily injection of 1,000 i. u. of estrogen into lactating rats decreased the rate of milk secretion as judged by the daily weights of the young. However, these injections neither inhibited lactogen secretion nor its discharge into the blood.

15. The daily injection of 1,000 i. u. of estrogen into ovariectomized lactating rats caused a slight decrease in milk secretion. These injections neither inhibited lactogen secretion nor its discharge into the blood.

16. Progestin injections into ovariectomized lactating rats produced inconsistent results. In those cases where milk secretion was decreased the lactogen content of the pituitary gland was also decreased.

17. The stimulus of suckling, either with or without the removal of milk, caused a reduction in the lactogen content of the pituitary gland of the rat.

18. The increase in the lactogen content of the rat pituitary gland following parturition was independent of suckling influence.

19. Of the groups of cattle pituitaries assayed, fetal pituitaries had the lowest lactogen content.

20. Pituitaries from bulls and heifers contained more lactogen than pituitaries from calves. In pituitaries from bulls and heifers between the ages of four and ten months, the heifer pituitaries contained 15 per cent more lactogen than the bull pituitaries.

21. Per unit weight of fresh anterior lobe tissue, pituitary glands from 11 to 23 month old heifers contained 59 per cent more lactogen than glands from four to ten month old heifers.

22. Pituitary glands from 11 to 23 month old bulls contained more lactogen per pituitary gland than pituitaries from four to ten month old bulls. However, the concentration of the hormone in the pituitaries in the two groups was practically the same.

23. The removal of the testes from bulls decreased the lactogen content of the pituitary gland and the longer the castration period the greater the decrease in hormone concentration.

24. Per unit weight of fresh anterior lobe tissue, pituitary glands from dry and open dairy cows contained 72 per cent more

lactogen than pituitaries from dry and open beef cows, dry and pregnant dairy cows 37 per cent more than dry and pregnant beef cows, lactating and open dairy cows 69 per cent more than lactating and open beef cows and lactating and pregnant dairy cows 73 per cent more lactogen than lactating and pregnant beef cows.

25. The thyrotropic assay results have been expressed in guinea pig units. The guinea pig unit is defined as that amount of hormone which will elicit a 50 per cent increase in the weight of the thyroid glands of a group of four male guinea pigs weighing between 140 and 170 grams, when injected subcutaneously daily for four days and the animals sacrificed on the fifth day.

26. The following number of guinea pig units of the thyrotropic hormone were contained in one gram of fresh anterior lobe tissue from various groups of cattle: calves, 26.40; four to ten month old heifers, 32.13; four to ten month old bulls, 38.36; four to ten month old steers, 25.59; 11 to 23 month old open heifers, 24.59; 11 to 23 month old heifers pregnant 1 to 140 days, 42.99; 11 to 23 month old heifers pregnant 141 to 283 days, 31.98; 11 to 23 month old bulls, 35.02; 11 to 23 month old steers, 16.30; dry and open beef cows, 22.53; dry and open dairy cows, 36.03; dry and pregnant beef cows, 20.01; dry and pregnant dairy cows, 23.66; lactating and open beef cows, 24.79; lactating and open dairy cows, 32.45; lactating and pregnant beef cows, 29.94; and lactating and pregnant dairy cows, 35.20.

BIBLIOGRAPHY

Allanson, M. 1932 *The growth of the pituitary body in the female rabbit.* J. Exper. Biol., vol. 9, p. 117.

Andersen, D. H. 1933 *Weight of pituitary and thyroid of the rat at various stages of the oestrus cycle.* Proc. Soc. Exper. Biol. and Med., vol. 30, p. 657.

Anderson, E., and Collip, J. B. 1933 *Thyreotropic hormone of anterior pituitary.* Proc. Soc. Exper. Biol. and Med., vol. 30, p. 680.

Anderson, E. M., and Collip, J. B. 1934 *Studies on the physiology of the thyreotropic hormone of the anterior pituitary.* J. Physiol., vol. 82, p. 11.

Anselmino, K. J., Herold, L., Hoffmann, F., und Pencharz, R. I. 1936 *Studien zur Physiologie der Milchbildung. III. Mitteilung. Ueber den Hemmungsstoff der Milchbildung.* Zentralbl. f. Gynäk., Bd. 60, S. 7.

Anselmino, K. J., und Hoffmann, F. 1936 *Studien zur Physiologie der Milchbildung. IV. Mitteilung. Ueber die Laktionshemmung durch Follikelhormon.* Zentralbl. f. Gynäk., Bd. 60, S. 501.

Aron, M. 1929 *Action de la prehypophyse sur la thyroide chez le cobaye.* Compt. rend. Soc. de Biol., t. 102, p. 682.

Aschheim, S. 1926 *Hormon und Schwengerschaft.* Med. Klin., Bd. 22, S. 2023.

Aschheim, S., und Zondek, B. 1927 *Hypophysenvorderlappenhormon und Ovarialhormon im Harn von Schwangeren.* Klin. Wchnschr., Bd. 6, S. 1322.

Asdell, S. A. 1932 *The effect of the injection of hypophyseal extract in advanced lactation.* Am. J. Physiol., vol. 100, p. 137.

Asdell, S. A., Brooks, H. J., Salisbury, G. W., and Seidenstein, H. R. 1936 *Experiments in the physiology of mammary development and lactation.* Cornell Agr. Exper. Sta. Memoir 198.

Asimoff G. J., and Krouze, N. K. 1937 *The lactogenic preparations from the anterior pituitary and the increase of milk yield in cows.* J. Dairy Science, vol. 20, p. 289.

Asimoff, G.,Pchelina, W., Pariskaya, N., and Chvatow, B. 1935 *The influence of anterior pituitary hormones upon lactation in cows.* Prob. Zoo. and Exper. Endocrinology, vol. 2, p, 261 (with English summary).

Asimoff, G., Skarginskaya, M., and Ptchelina, V. 1934 *Effect of alkaline extracts of the anterior lobe of the pituitary gland on lactation in cows.* Prob. Zoo. and Exper. Endocrinology, vol. 1, p. 108 (with English summary).

Atwell, W. J. 1930 *The relative volumes of the several lobes of the hypophysis cerebri in pregnancy.* Anat. Rec., vol. 45, p. 206.

Bacon, A. R. 1930 *A comparative study of the anterior hypophyses in pregnant and non-pregnant states.* Am. J. Obst. and Gynec., vol. 19, p. 352.

Bates, R. W., Riddle, O., and Lahr, E. L. 1935 *An assay of three hormones present in anterior pituitaries of seven types of cattle classified for age, sex, and stage of reproduction.* Am. J. Physiol., vol. 113, p. 259.

Blickenstaff, P. H. 1934 *Some studies on the hypophysis cerebri of cattle.* Vet. Alumni Quart. (Ohio State Univ.), vol. 21, p. 132.

Burch, J. C., and Cunningham, R. S. 1930 *Effect of placental extracts on ovarian stimulating properties of the anterior hypophysis.* Proc. Soc. Exper. Biol. and Med., vol. 27, p. 331.

Catchpole, H. R., and Lyons, W. R. 1934 *The gonad-stimulating hormone of pregnant mares.* Am. J. Anat., vol. 55, p. 167.

Chen, G., and van Dyke, H. B. 1936 *The amount of thyroid-stimulating hormone in the anterior lobe of the pituitary of the thyroidectomized rabbit.* Chinese J. Physiol., vol. 10, p. 285.

Cimorini, A. 1908 *Sur l'hypertrophie de l'hypophyse cerebrale chez les animaux thyreoidectomises.* Arch. ital. de biol., t. 48, p. 387.

Clark, H. M. 1935a *A prepubertal reversal of the sex difference in the gonadotropic hormone content of the pituitary gland of the rat.* Anat. Rec., vol. 61, p. 175.

Clark, H. M. 1935b *A sex difference in the change in potency of the anterior hypophysis following bilateral castration in newborn rats.* Anat. Rec., vol. 61, p. 193.

Cohen, R. S. 1935 *Effect of experimentally produced hyperthyroidism upon the reproductive and associated organs of the male rat.* Am. J. Anat., vol. 56, p. 143.

Covell, W. P. 1927 *Growth of the human prenatal hypophysis and the hypophyseal fossa.* Am. J. Anat., vol. 38, p. 379.

Domm, L. V. 1931a *Precocious development of sexual characters in the fowl by homeoplastic hypophyseal implants. I. The male.* Proc. Soc. Exper. Biol. and Med., vol. 29, p. 308.

Domm, L. V. 1931b *Precocious development of sexual characters in the fowl by homeoplastic hypophyseal implants. II. The female.* Proc. Soc. Exper. Biol. and Med., vol. 29, p. 310.

Dragstedt, L. R., Sudan, A. C., and Phillips, K. 1924 *Studies on the pathogenesis of tetany. IV. The tetany of oestrus, pregnancy and lactation.* Am. J. Physiol., vol. 69, p. 477.

Eckles, C. H., and Reed, O. E. 1910 *A study of the cause of wide variation in milk production by dairy cows.* Mo. Agr. Exper. Sta. Res. Bul. 2.

Ehrhardt, K., und Mayes, B. T. 1930 *Beitrag zum Hormongehalt des menschlichen und tierischen Hypophysenvorderlappens.* Zentralbl. f. Gynäk., Bd. 54, S. 2949.

Ellison, E. T., Campbell, M., and Wolfe, J. M. 1932 *Comparison of the capacity of the anterior lobe tissue of male and female rats to induce ovulation.* Anat. Rec., vol. 52 (suppl.), p. 54.

Emanuel, S. 1931 *Effet de l'implantation intraperitoneale d'hypophyse de rats castres avant la puberte.* Compt. rend. Soc. de biol., t. 106, p. 571.

Emery, F. E. 1932 *The anterior pituitary sex hormone in the blood and urine of rats.* Am. J. Physiol., vol. 101, p. 246.

Emery, F. E., and Winter, C. A. 1934 *The adrenotropic substance of the hypophysis as influenced by age, castration, sex and thyroparathyroidectomy.* Anat. Rec., vol. 60, p. 381.

Engle, E. T. 1929 *The effect of daily transplants of the anterior lobe from gonadectomized rats on immature test animals.* Am. J. Physiol., vol. 88, p. 101.

Enzmann, E. V., and Pincus, G. 1933 *The effect on lactating mice of injecting an extract of the urine of pregnancy.* Am. J. Physiol., vol. 103, p. 30.

Evans, E. I. 1934 *Virgin animals secrete milk after injections of pituitary hormone.* U. S. D. A. Yearbook of Agr., p. 360.

Evans, H. M., and Simpson, M. E. 1929a *A sex difference in the hormone content of the anterior hypophysis of the rat.* Am. J. Physiol., vol. 89, p. 375.

Evans, H. M., and Simpson, M. E. 1929b *Comparison of anterior-hypophyseal implants from the male and female, with reference to their capacity to stimulate the immature ovary.* Anat. Rec., vol. 42, p. 48 (abstract No. 10).

Evans, H. M., and Simpson, M. E. 1929c *The effect of pregnancy on the anterior hypophysis of the rat and cow as judged by the capacity of implants to produce precocious maturity.* Am. J. Physiol., vol. 89, p. 379.

Evans, H. M., and Simpson, M. E. 1929d *Comparison of anterior-hypophyseal implants from normal and gonadectomized animals, with reference to their capacity to stimulate the immature ovary.* Anat. Rec., vol. 42, p. 48 (Abstract No. 11).

Evans, H. M., and Simpson, M. E. 1929e *A comparison of anterior-hypophyseal implants from normal and gonadectomized animals with reference to their capacity to stimulate the immature ovary.* Am. J. Physiol., vol. 89, p. 371.

Evans, H. M., and Simpson, M. E. 1930 *Some effects on the hypophysis of hyper-and hypothyroidism.* Anat. Rec., vol. 45, p. 215.

Fellenberg, T. von, und Grueter, F. 1932 *Beitrag zur Kenntnis des Einflusses der Schilddrusenexstirpation fur sich allein, bei Nachbehandlung mit Hypophysenvorderlappen-Gesamextrak und bei Vorbehandlung mit Placentaextrakt und Corpus luteum-Brei auf die Milchsekretion von Ziegen.* Biochem. Ztschr., Bd., 253, S. 42.

Fellner, O. O. 1931 *Zur Theorie der Milchsekretion.* Klin. Wchnschr., Bd. 10, S. 1164.

Fels, E. 1926 *Untersuchungen ueber das Ovarialhormon im Blute Gravider und Nichtgravider.* Klin. Wchnschr., Bd. 5, S. 2349.

Fevold, H. L., Hisaw, F. L., and Greep, R. 1935 *Effect of oestrin on the activity of the anterior lobe of the pituitary.* Am. J. Physiol., vol. 114, p. 508.

Fichera, G. 1905 *Sur l'hypertrophie de la glande pituitare consecutive a la castration.* Arch. ital. de biol., t. 43, p. 405.

Folley, S. J. 1936a *The effect of oestrogenic hormones on lactation in the cow.* Chem. and Ind., vol. 55, p. 509.

Folley, S. J. 1936b *The effect of oestrogenic hormones on lactation and on the phosphatase of the blood and milk of the lactating cow.* Biochem. J., vol. 30, p. 2262.

Folley, S. J., and White, P. 1936 *The effect of thyroixine on milk secretion and on the phosphatase of the blood and milk of the lactating cow.* Proc. Roy. Soc., A, vol. 120, p. 346.

Folley, S. J., and Young, F. G. 1937 *Stimulation of milk production by prolactin in the cow.* Chem. and Ind., vol. 56, p. 96.

Frankl, O. 1923 *Relation between placenta and the secretion of milk.* Am. J. Obst. and Gynec., vol. 6, p. 399.

Frazier, C. N., and Mu, J. W. 1935 *Development of female characteristics in adult male rabbits following prolonged administration of estrogenic substance.* Proc. Soc. Exper. Biol. and Med., vol. 32, p. 997.

Fremery, P. de. 1934 *Het de Melkafscheiding Prikkelend Hormon uit de Voorkwab van de Hypophysis.* Vlaamsch Geneesk. Tijdschr., Nr. 37.

Fuller, J. M. 1928 *Some physical and physiological activities of dairy cows under conditions of modern herd management.* N. H. Agr. Exper. Sta. Tech. Bul. 35.

Gomez, E. T., and Turner, C. W. 1937 *Hypophysectomy and replacement therapy in relation to the growth and secretory activity of the mammary gland.* Mo. Agr. Exper. Sta. Res. Bul. 259.

Graham, Jr., W. R. 1934a *The effect of thyroidectomy and thyroid feeding on the milk secretion and milk fat production of cows.* J. Nutrition, vol. 7, p. 407.

Graham, Jr., W. R. 1934b *The action of thyroxine on the milk and milk fat production of cows.* Biochem. J., vol. 38, p. 1368.

Grimmer, W. 1918 *Beitrage zur Kenntnis der Milch schilddrusenloser Ziegen.* Biochem. Ztschr., Bd. 88, S. 43.

Grimmer, W., und Paul, O. 1930 *Zur Kenntnis der Milch schilddrusenloser Ziegen. II. Milchwirtsch.* Forsch., Bd. 10, S. 336.

Grueter, F. 1928 *Contribution a l'etude du fonctionnement du lobe anterieur de l'hypophyse.* Compt. rend. Soc. de biol., t. 98, p. 1215.

Grueter, F. 1931 *Experimentell-Hormonale Beeinflussung der Milchsekretion unter besonderer Berucksichtigung von Kuhen and Ziegen.* Proc. 2nd. Internat. Cong. Sex Research (1930), p. 443. Edinburgh. Oliver and Boyd.

Grueter, F., und Stricker, P. 1929 *Ueber die Wirkung eines Hypophysenvorderlappenhormons auf die Auslösung der Milchsekretion.* Klin. Wchnschr., Bd. 8, S. 2322.

Halban, J. 1905 *Die innere Secretion von Ovarium und Placenta und ihre Bedeutung für die Function der Milchdruse.* Arch. f. Gynäk., Bd. 75, S. 353.

Hammond, J. 1927 *The physiology of reproduction in the cow.* p. 157, Cambridge University Press.

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

Hatai, S. 1913a *On the weights of the abdominal and the thoracic viscera, the sex glands, ductless glands, and the eyeballs of the albino rat (Mus norvegicus albinus) according to body weight.* Am. J. Anat., vol. 15, p. 87.

Hatai, S. 1913b *The effect of castration, spaying or semi-spaying on the weight of the central nervous system and of the hypophysis of the albino rat; also the effect of semi-spaying on the remaining ovary.* J. Exper. Zool., vol. 15, p. 297.

Hatai, S. 1914 *On the weight of some of the ductless glands of the Norway and of the albino rat according to sex and variety.* Anat. Rec., vol. 8, p. 511.

Hatai, S. 1915 *The growth of organs in the albino rat as affected by gonadectomy.* J. Exper. Zool., vol. 18, p. 1.

Hellbaum, A. A. 1935 *The gonad-stimulating activity of pituitary glands from horses of different ages and sex types.* Anat. Rec., vol. 63, p. 147.

Herring, P. T. 1920 *The effect of pregnancy upon the size and weight of some of the organs of the body.* Brit. Med. J., vol. 2, p. 886.

Hesselberg, C., and Loeb, L. 1937 *The structure of the secreting and retrogressing mammary gland in the guinea pig.* Anat. Rec., vol. 68, p. 103.

Hill, R. T. 1934a *Species variation in the gonadotropic activity of the hypophysis.* J. Physiol., vol. 83, p. 137.

Hill, R. T. 1934b *Variation in the activity of the rabbit hypophysis during the reproductive cycle.* J. Physiol., vol. 83, p. 129.

Hisaw, F. L., and Meyer, R. K. 1929 *The oestrus hormone in the urine of pregnant cows.* Proc. Soc. Exper. Biol. and Med., vol. 26, p. 586.

Hohlweg, W., und Dohrn, M. 1932 *Ueber die Beziehungen zwischen Hypophysenvorderlappen und Keimdrusen.* Klin. Wchnschr., Bd. 11, S. 233.

Hohlweg, W. und Junkmann, K. 1933 *Ueber die Beziehungen zwischen Hypophysenvorderlappen und Schilddrüsen.* Arch. f. d. ges. Physiol., Bd. 232, S. 148.

Haussay, B. A. 1935a *Action de l'hypophysectomie sur la grossesse et la secretion lactee, chez la chinne.* Compt. rend. Soc. de biol., t. 120, p. 496.

Haussay, B. A. 1935b *Influencia de la hipofisectomia sobre la prenez y la secrecion lactea en la perra.* Rev. Soc. argent. de biol., t. 11, p. 196.

Houssay, B. A. 1935c *Secretion lactee provoquee par l'extrait antehypophysaire chez le chien.* Compt. rend. Soc. de biol, t 120, p 502

Ingelbrecht, P 1935 *Influence du systeme nerveux central sur la mamelle lactante chez le rat blanc.* Compt. rend. Soc. de biol., t. 120, p. 1369.

Jack, E. L., and Bechdel, S. I. 1935 *A study of the influence of thyroxin on milk secretion.* J. Dairy Science, vol. 18, p. 195.

Jongh, S. E. de 1933 *Weitere Untersuchungen ueber Lactationshemmung.* Acta brev. Neerland., Bd. 3, S. 88.

Jongh, S. E., de et Dingemanse, E. 1931 *Menformone et lactation.* Arch. neerl. de Physiol., t. 16, p. 288.

Jongh, S. E. de und Laqueur, E. 1930 *Milchsekretion und Menformon.* Klin. Wchnschr., Bd. 9, S. 2344.

Junkmann, K., und Schoeller, W. 1932 *Ueber das thyreotrope Hormon des Hypophysenvorderlappens.* Klin. Wchnschr., Bd. 11, S. 1176.

Kabak, J., und Kisilstein, M. 1934 *Der Einfluss endogriner Faktoren auf die Laktation bei Schafen.* Z. Zuchtg.: B. Tierzuchtg. u. Zuchtgsbiol., Bd. 29, S. 301.

Kabak, J. M., and Margulis, Z. S. 1935 *The influence of endocrine preparations on lactation in sheep.* Trans. Dynamics of Dev., vol. 9, p. 167 (with English summary).

Kolde, W. 1912 *Untersuchungen von Hypophysen bei Schwangerschaft und nach Kastration.* Arch. f. Gynäk., Bd. 98, S. 505.

Kraul, L. 1932 *Die Beeinflussung der Hypophysenvorderlappenfunktion durch hormonale Substanzen und deren praktische Bedeutung.* Arch. f. Gynäk., Bd. 148, S. 65.

Kunkel, P., and Loeb, L. 1935 *Effects of human anterior pituitary gland on sex organs and thyroid of the guinea pig.* Proc. Soc. Exper. Biol. and Med., vol. 32, p. 1413.

Kuramitsu, C., and Loeb, L. 1921 *The effect of suckling and castration on the lactating mammary gland in rat and guinea pig.* Am. J. Physiol., vol. 56, p. 40.

Kurzrok, R., Bates, R. W., Riddle, O., and Miller, Jr., E. G. 1934 *The clinical use of prolactin.* Endocrinology, vol. 18, p. 18.

Kuschinsky G. 1933 *Ueber die Bedingungen der Sekretion des thyreotropen Hormons der Hypophyse.* Arch. f. exper. Path. u. Pharmakol., Bd. 170, S. 510.

Lane, C. E. 1935 *Some influences of oestrin on the hypophyseal-gonad complex of the immature female rat.* Am. J. Physiol., vol. 110, p. 681.

Laqueur, E. 1927 *Ueber weibliches Sexualhormon im bes. das Menformon.* Klin. Wchnschr., Bd. 6, S. 390.
Laqueur, E., Brochardt, E., Dingemanse, E., und Jongh, S. E. de 1928 *Ueber die weibliches (Sexual-) Hormon, Menformon.* Deutsche Med. Wchnschr., Bd. 54, S. 465.
Leonard, S. L. 1936 *Effect of castration and oestrin treatment on relative amounts of F. S. H. and L. H. in the hypophysis.* Anat. Rec., vol. 67, (suppl.), p. 47.
Leonard, S. L., Meyer, R. K., and Hisaw, F. L. 1930 *The effect of oestrin on the growth of the ovary in immature female rats.* Anat. Rec., vol. 45, p. 268.
Lipschütz, A. 1932 *Le coefficient de luteinisation, indice fonctionnel de la prehypophyse.* Compt. rend. Soc. de Biol., t. 111, p. 610.
Lipschütz, A. 1933 *Nouvelles recherches sur le coefficient du luteinisation de la prehypophyse.* Compt. rend. Soc. de biol., t. 112, p. 1145.
Lipschütz, A. 1934 *Changement experimental de l'equilibre prehypophysaire gonadotrope.* Compt. rend. Soc. de biol., t. 116, p. 89.
Lipschütz, A. 1935 *On the influence of oestrin injections on the balance between the prehypophyseal gonadotropic hormones of the male rat.* Quart. J. Exper. Physiol., vol. 25, p. 109.
Lipschütz, A., et Del-Pino, M. 1936 *Differences prehypophysaires specifiques dux sexe, chez l'homme.* Compt. rend. Soc. de biol., t. 121, p. 203.
Lipschütz, A., Kallas, H., und Paez, R. 1929 *Hypophyse und Gesetz der Pubertat.* Arch. f. d. ges. Physiol., Bd. 221, S. 695.
Lipschütz, A., Palacios, L., et Akel, J. 1936 *"Feminisation" de la prehypophyse du rat male par injection de folliculine.* Compt. rend. Soc. de biol., t. 121, p. 205.
Lipschütz, A., et Reyes, G. 1932a *Differences prehypophysaires specifiques du sexe chez le rat.* Compt. rend. Soc. de biol., t. 109, p. 1330.
Lipschütz, A., et Reyes, G. 1932b *Sur la capacite luteinisante de l'hypophyse de la rate infantile et adulte.* Compt. rend. Soc. de biol., t. 111, p. 608.
Lipschütz, A. et Villagran, G. 1936 *Neutralisation de la prehypophyse par castration, chez le rat.* Compt. rend. Soc. de biol., t. 121, p. 203.
Litt, S. 1933 *Autotransplantation of placenta to the anterior chamber of the eye and its effect on lactation.* Am. J. Obst. and Gynec., vol. 26, p. 37.
Livingston, A. E. 1916 *The effect of castration on the weight of the pituitary body and the other glands of internal secretion in the rabbit.* Am. J. Physiol., vol. 40, p. 153.
Loeb, L. 1932 *The specificity in the action of the anterior pituitary of different mammals as well as of urine of pregnant women on the sex organs and thyroid glands of immature female guinea pigs.* Endocrinology, vol. 16, p. 129.
Loeb, L., and Bassett, R. B. 1929 *Effect of hormones of anterior pituitary on thyroid gland in the guinea pig.* Proc. Soc. Exper. Biol. and Med., vol. 26, p. 860.
Loeb, L., and Bassett, R. B. 1930 *Comparison of effects of various preparations of anterior pituitary gland on thyroid of guinea pig.* Proc. Soc. Exper. Biol. and Med., vol. 27, p. 490.
Loeb, L., and Friedman, H. 1933 *The two main types of anterior pituitary gland present in different species of animals.* Proc. Soc. Exper. Biol. and Med., vol. 30, p. 741.
Loeb, L., Saxton, J., and Hayward, S. J. 1936 *The relationship of the anterior pituitary gland to thyroid and ovary.* Endocrinology, vol. 20, p. 511.
Lyons, W. R., and Catchpole, H. R. 1933 *Assay with the guinea pig of the lactogenic hypophyseal hormone.* Proc. Soc. Exper. Biol. and Med., vol. 31, p. 299.
Lyons, W. R., and Page, E. 1935 *Detection of mammotropin in the urine of lactating women.* Proc. Soc. Exper. Biol. and Med., vol. 32, p. 1049.
Magistris, H. 1932 *Quantitative Untersuchungen ueber den Gehalt des Hypoyhpsenvorderlappens an Follikelreifungs—und Luteinisierungshormon bei verschiedenen Tieren.* Arch. f. d. ges. Physiol., Bd., 230, S. 835.

Margulis, Z. S., Skvortzov, V. A., and Poliakov, A. N. 1935 *The influence of endocrine factors on lactation and live weight in cows.* Trans. Dynamics of Dev., vol. 9, p. 177 (with English summary).

Marrassini, A. 1910 *Sur les modifications que la castration peut determiner dan les organes glandulaires de quelques animaux, et specialement sur celles que l'on recontre dan l'hypophyse.* Arch. ital. de biol., t. 53, p. 419.

Marrassini, A., et Luciani, L. 1911 *Effets de la castration sur l'hypophyse et sur d'autres organes glandulaires.* Arch. ital. de biol., t. 56, p. 395.

McQueen-Williams, M. 1935a *Decreased mammotropin in pituitaries of thyroidectomized (maternalized) male rats.* Proc. Soc. Exper. Biol. and Med., vol. 33, p. 406.

McQueen-Williams, M. 1935b Sex comparison of gonadotropic content of anterior hypophyses from rats before and after puberty. Proc. Soc. Exper. Biol. and Med., vol. 32, p. 1051.

McQueen-Williams, M. 1935c *Is thyrotropic hormone of beef anterior pituitaries identical with indirect interrenotropic factor?* Proc. Soc. Exper. Biol. and Med., vol. 32, p. 1050.

McShan, W. H., and Turner, C. W. 1936 *Bioassay of galactin, the lactogenic hormone.* Proc. Soc. Exper. Biol. and Med., vol. 34, p. 50.

Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J. 1930 *Effect of oestrin on gonad stimulating power of the hypophysis.* Proc. Soc. Exper. Biol. and Med., vol. 27, p. 702.

Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J. 1932 *The influence of oestrin on the gonad-stimulating complex of the anterior pituitary of castrated male and female rats.* Endocrinology, vol. 16, p. 655.

Müller, R., Eitel, H., und Loeser, A. 1935 *Der thyreotrope Wirkstoffgehalt der menschlichen Hypophyse.* Arch. f. exper. Path. u. Pharmakol., Bd. 179, S. 427.

Nelson, W. O. 1933 *Studies on the anterior hypophysis. I. The development of the hypophysis in the pig (Sus scrofa). II. The cytological differentiation in the anterior hypophysis of the foetal pig.* Am. J. Anat., vol. 52, p. 307.

Nelson, W. O. 1934 *Studies on the physiology of lactation. III. The reciprocal hypophyseal-ovarian relationship as a factor in the control of lactation.* Endocrinology, vol. 18, p. 33.

Nelson, W. O. 1935 *Changes in the hypophyses of castrate and cryptorchid guinea pigs.* Proc. Soc. Exper. Biol. and Med., vol. 32, p. 1605.

Nelson, W. O. 1936 *Endocrine control of the mammary gland.* Physiol. Rev., vol. 16, p. 488.

Nelson, W. O. 1937 *Studies on the physiology of lactation. VI. The endocrine influences concerned in the development and function of the mammary gland in the guinea pig.* Am. J. Anat., vol. 60, p. 341.

Nelson, W. O., and Tobin, C. E. 1936 *The effect of thyroidectomy upon lactation in the rat and guinea pig.* Anat. Rec., vol. 67, (suppl.), p. 110.

Novelli, A. 1932 *Role de la castration sur l'action sexuelle de l'hypophyse du crapaud.* Compt. rend. Soc. de biol., t. 111, p. 476.

Ott, I., and Scott, J. C. 1910 *The action of infundibulin upon the mammary secretion.* Proc. Soc. Exper. Biol. and Med., vol. 8, p. 48.

Philipp, E. 1930 *Hypophysenvorderlappen und Placenta.* Zentralbl. f. Gynäk., Bd. 54, S. 450.

Rasmussen, A. T. 1921 *The hypophysis cerebri of the woodchuck (marmota monax) with special reference to hibernation and inanition. Endocrinology*, vol. 5, p. 33.

Rasmhssen, A. T. 1928 *The weight of the principal components of the normal male adult human hypophysis cerebri.* Am. J. Anat., vol. 42, p. 1.

Rasmussen, A. T. 1934 *The weight of the principal components of the normal hypophysis cerebri of the adult human female.* Am. J. Anat., vol. 55, p. 253.

Rathke, H. 1838 *Ueber die Entstehung der Glandula pituitaria.* Arch. f. Anat., Physiol. u. wissensch. Med., Bd. 5, S. 482. (cited by Cushing, H. 1912 The pituitary body and its disorders. p. 2. Philadelphia and London. J. B. Lippincott Company).

Riddle, O., Bates, R. W., and Dykshorn, S. W. 1932a *Prolactin, a new and third hormone of the anterior pituitary*. Anat. Rec., vol. 54, p. 25.

Riddle O., Bates, R. W., and Dykshorn, S. W. 1932b *A new hormone of the anterior pituitary*. Proc. Soc. Exper. Biol. and Med., vol. 29, p. 1211.

Riddle, O., Bates, R. W., and Dykshorn, S. W. 1933 *The preparation, identification and assay of prolactin—a hormone of the anterior pituitary*. Am. J. Physiol., vol. 105, p. 191.

Riddle, O., and Braucher, P. F. 1931 *Studies on the physiology of reproduction in birds. XXX. Control of the special secretion of the crop-glands in pigeons by an anterior lobe hormone*. Am. J. Physiol., vol. 97, p. 617.

Robb, R. C. 1929 *On the nature of hereditary size and limitation. II. The growth of parts in relation to the whole*. Brit. J. Exper. Biol., vol. 6, p. 311.

Robson, J. M. 1935 *Action of oestrin on the mammary secretion*. Quart. J. Exper. Physiol., vol. 24, p. 337.

Rogowitch, N. 1888 *Sur les effets de l'ablation du corps thyroide chez les animaux*. Arch. de physiol. norm. et patht., t. 20, p. 419.

Rowlands, I. W. 1936 *Species variation in thyrotropic activity of the pituitary gland*. J. Physiol., vol. 88, p. 298.

Rowlands, I. W., and Parkes, A. S. 1934 *Quantitative study of the thyrotropic activity of anterior pituitary extracts*. Biochem. J., vol. 28, p. 1829.

Saito, Y. 1923 *Untersuchungen ueber die Hypophysengewichte von Pferden*. Biochem. Ztschr., Bd. 142, S. 308.

Sardi, J. L. 1935 *Secretion lactee sous l'action de l'extrait ante-hypophysaire chez les cobayes males*. Compt. rend. Soc. de biol., t. 120, p. 503.

Schmidt, I. G. 1937 *The effects of hypophyseal implants from normal mature guinea pigs on the sex organs of immature guinea pigs*. Endocrinology, vol. 21, p. 461.

Schultze-Rhonhof, F., und Niedenthal, R. 1929 *Untersuchungen ueber die hormonale Wirksamkeit des Hypophysenvorderlappens des Fetus im Tierversuch*. Zentralbl. f. Gynäk., Bd. 53, S. 902.

Selye, H. 1934 *On the nervous control of lactation*. Am. J. Physiol., vol. 107, p. 535.

Selye, H., Collip, J. B., and Thomson, D. L. 1934 *Nervous and hormonal factors in lactation*. Endocrinology, vol. 18, p. 237.

Severinghaus, A. E. 1932a *The effect of castration in the guinea pig upon the sex-maturing potency of the anterior pituitary*. Am. J. Physiol., vol. 101, p. 309.

Severinghaus, A. E. 1932b *The effect of castration in the guinea pig upon the sex-maturing potency of the anterior pituitary*. Am. J. Anat., vol. 50, p. 351.

Siegert, F. 1933a *Der Implantationseffekt der Hypophyse unter verschiedenen Bedingungen und Voraussetzungen*. Klin. Wchschr., Bd. 12, S. 145.

Siegert, F. 1933b *Der Implantationseffekt der Hypophyse auf den Genitalapparat des infantilen Tieres unter dem Einfluss der Ovarialfunktion und der Graviditat sowie nach Kastration*. Arch. f. Gynäk., Bd. 152, S. 25.

Smith, G. van S., and Smith, W. O. 1933 *The inhibition of lactation in rabbits with large amounts of oestrin*. Am. J. Physiol., vol. 103, p. 356.

Smith, P. E. 1932 *The non-essentiality of the posterior hypophysis in parturition*. Am. J. Physiol., vol. 99, p. 345.

Smith, P. E., and Dortzbach, C. 1929 *The first appearance in the anterior pituitary of the developing pig foetus of detectable amounts of the hormones stimulating ovarian maturity and general body growth*. Anat. Rec., vol. 43, p. 277.

Smith, P. E., and Engle, E. T. 1927 *Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system*. Am. J. Anat., vol. 40, p. 159.

Smith, P. E., and Engle, E. T. 1929 *Evidence of a correlation between the amount of gonadal-stimulating hormone present in the pituitary of the guinea pig and the stage of the reproductive cycle*. Anat. Rec., vol. 42, p. 38.

Smith, P. E., and MacDowell, E. C. 1931 *The differential effect of hereditary mouse dwarfism on the anterior pituitary hormones.* Anat. Rec., vol. 50, p. 85.

Smith, P. E., Severinghaus, A. E., and Leonard, S. L. 1933 *The effect of castration upon the sex-stimulating potency and the structure of the anterior pituitary in rabbits.* Anat. Rec., vol. 57, p. 177.

Stein, K. F. 1934 *Effects of avian pituitary glands in salamanders.* Proc. Soc. Exper. Biol. and Med., vol. 32, p. 157.

Stein, K. F. 1935 *A sex difference in gonad-stimulating potency of young gonadectomized rats.* Proc. Soc. Exper. Biol. and Med., vol. 33, p. 95.

Stein, S. 1931 *Volume of the various lobes of the hypophysis during pregnancy in the rat.* Proc. Soc. Exper. Biol. and Med., vol. 29, p. 282.

Stein, S. I. 1933a *Experimental studies on the hypophysis cerebri. I. The effect of single pregnancy in the albino rat.* Endocrinology, vol. 17, p. 187.

Stein, S. I. 1933b *Experimental studies on the hypophysis cerebri. II. The effect of castration in the male albino rat.* Anat. Rec., vol. 56, p. 15.

Stein, S. I. 1934 *Experimental studies on the hypophysis cerebri. III. The effect of several pregnancies in the albino rat.* Endocrinology, vol. 18, p. 721.

Steinach, E., Dohrn, M., Schoeller, W., und Faure, W. 1928 *Ueber die biologischen Wirkungen des weiblichen Sexual hormons.* Arch. f .d. ges. Physiol., Bd. 219, S. 306.

Stimmel, B. F., McCullagh, D. R., and Picha, V. 1936 *The thyrotropic hormone of the pituitary gland and iodine metabolism.* J. Pharm. and Exper. Therap., vol. 57, p. 49.

Stocklausner, D., und Daum, D. 1932 *Ueber den Einfluss der Hypophysenvorderlappenhormon auf die Milchskeretion der Kühe.* Milchwrtsch. Forsch., Bd. 13, S. 448.

Swett, W. W., Matthews, C. A., Miller, F. W., and Graves, R. R. 1937 *Variations recorded in the study of the conformation and anatomy of 593 dairy cows having records of production.* Bureau Dairy Industry, Mimegraph Form N. 589 Revised.

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

Turner, C. W., and Gomez, E. T. 1934 *The experimental development of the mammary gland. I. The male and female albino mouse. II. The male and female guinea pig.* Mo. Agr. Exper. Sta. Res. Bul. 206.

Turner, C. W., and Reineke, E. P. 1936 *A study of the involution of the mammary gland of the goat.* Mo. Agr. Exper. Sta. Res. Bul. 235.

Ueno, A., and Murata, M. 1931 *Hormone content of the anterior lobe of the human hypophysis.* Trans. Japan. Path. Soc., vol. 21, p. 149.

Van Horn, H. N. 1933 *The relation of the thyroid to the hypophysis and ovary.* Endocrinology, vol. 17, p. 152.

Waterman, L., Freud, J., and Jongh, N. vos-de. 1936 *Influence of oestradiol-benzoate and prolactin on the milk production of the cow.* Acta brev. Neerland, vol. 6, p. 1.

Wittek, J. 1913 *Ueber das Verhalten der Rinderhypophyses bei den verschiedenen Geschlechtern, in der Graviditat und nach der Kastration.* Arch. f. Anat. u. Phys.; Anat. Abth.; Suppl. Band, S. 127.

Wolfe, J. M. 1930 *A quantitative study of ovulation in the rabbit.* Proc. Exper. Biol. and Med., vol. 28, p. 318.

Wolfe, J. M. 1931 *Observations on a cyclic variation in the capacity of the anterior hypophysis to induce ovulation in the rabbit.* Am. J. Anat., vol. 48, p. 391.

Wolfe, J. M. 1932 *The effect of castration on the capacity of the hypophysis to induce ovulation.* Am. J. Anat., vol. 50, p. 351.

Wolfe, J. M., and Cleveland, R. 1931 *Comparison of the capacity of anterior-hypophyseal tissue of mature and immature female rabbits to induce ovulation.* Anat. Rec., vol. 51, p. 213.

Zondek, B. 1931 *Hypophysenvorderlappen, H V H, und Placenta. Vergleichende quantitative Untersuchungen bei Mensch und Tier.* Zentralbl. f. Gynäk., Bd. 55, S. 1.