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The Relation of the Anterior Pituitary Hormones to the Development and Secretion of the Mammary Gland

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PREFACE

An exceedingly important problem in dairy cattle management is the maintenance of a high rate of milk secretion during the lactation period. It has been shown by previous work at this Station, however, that there is a regular rate of decline in milk secretion in spite of the presence of an abundant food supply. By good feeding and frequent milking the rate of decline in secretion may be reduced to a minimum, but it is only by the recurrence of pregnancy that the secretory cells of the udder may be brought back to activity when once they cease to function.

Large economic losses frequently result due to the frequent delay in successfully breeding dairy cattle. Unless animals conceive within three or four months after parturition there is usually an extended period before the next parturition when many animals produce little or no milk.

Many fine dairy cattle become permanently sterile for one cause or another. Frequently this occurs just at the prime of life. These animals are no longer of value to the dairyman with present knowledge of the situation.

Near our large cities, it has often been found not economical to raise dairy cattle. It is a frequent practice to purchase pregnant cattle as replacements in the herd and dispose of them when their milk flow reaches a low level.

These losses to dairy farmers could be reduced or eliminated if it were possible to stimulate the cells of the udder to renewed activity. The experimental work now in progress at this Station, of which the present paper is a preliminary report, forecasts the possibility that at an early date by appropriate hormone treatment, it will be possible to rejuvenate the secretory cells of the udder and greatly increase the rate of secretion.

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ABSTRACT

Alkaline extracts of the anterior pituitary of sheep have been prepared which will induce lactation in mature castrated rabbits whose glands had been developed previously. Even in extensively involuted glands lactation resulted following the administration of the extracts. The active principle appears to cause only the activation of the secreting cells.

The Relation of the Anterior Pituitary Hormones to the Development and Secretion of the Mammary Gland

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Studies of the hormonal control of the physiological activities of the mammary gland may be divided into two distinct divisions. The first phase of this problem involves the hormones promoting the growth of the glands. As mammary gland growth begins to take place following puberty and then continues during pregnancy at a still more rapid rate, it may be assumed that the ovaries and corpora lutea furnish the substances stimulating the growth of the female glands.

The second phase of this problem, namely the, stimulation of the mammary glands to secretory activity, apparently does not fit into the same category as does the growth phase. Normally milk secretion does not start until shortly prior to, or following parturition. Castrated females may secrete milk. In women lactation commonly results following castration. In milk cows castration has been assumed to lengthen the period of lactation.

Recently the anterior pituitary has been shown to be the source of a secretion producing profound ovarian changes. It has been described as the "motor of sexual functions." It has also been considered as the source of a hormone stimulating the mammary glands to secretory activity.

The object of this investigation has been to study experimentally the possible activity of the hormones of the anterior pituitary in the stimulation of the mammary gland—particularly the stimulation of the gland secretory activity.

Before proceeding with this investigation it seemed desirable to become somewhat acquainted with the general structure and the histology of the pituitary. Increased familiarity with the functions of the pituitary has further increased the desirability of some knowledge of the structure of this endocrine gland. For this reason a brief review on the anatomy of the pituitary is given preceding the review of its function. The functions in this case, however, are limited to those which may directly concern the development or secretory activity of the mammary glands.

*The data presented in this paper formed part of a thesis presented by W. U. Gardner in partial fulfillment of the requirements for the Degree of Master of Arts in the Graduate School of the University of Missouri, 1931.

REVIEW OF LITERATURE

The Location and Structure of the Mammalian Pituitary

In mammals the pituitary is located beneath the brain in a saddle-shaped excavation in the basisphenoid bone called the sella turcica. The location of the pituitary in man is shown in Figure 1. It is seen that it is located in one of the most protected positions in the body.

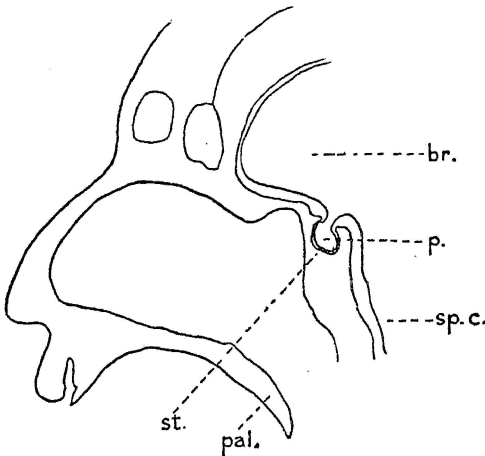


Fig. 1.—Longitudinal section through the head of man showing the location of: br, brain; p, pituitary; pal, palate; sp.c, spinal cord; st, sella turcica. (De Beer)

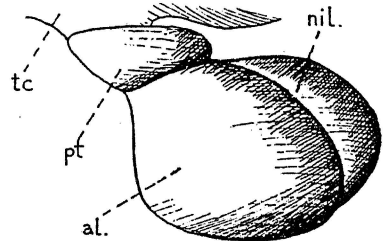


Fig. 2.—Pituitary of the cat from the left side x 14. Al, anterior lobe; nil, neurointermediate lobe; pt, pars tuberalis; tc, tuber cinereum. (De Beer)

The degree of separation of the pituitary from the brain varies with the species. In large animals such as the cow, the mouth of the sella turcica is covered with a thick cartilage sheet through which the infundibular process passes. In smaller animals as for example the rat and the cat, it is separated from the brain by a thin membrane.

The Gross Anatomy of the Pituitary.—Viewed intact, the pituitary can be seen to be divided into two more or less distinct parts, an anterior part and a posterior part.

The pituitary is connected to the brain in the region of the tuber cinereum by a cord-like process, the infundibulum, which is continuous with the neuro-intermediate or posterior lobe. A collar-like process, the pars tuberalis, fits around the infundibulum. The distinctness with which this part may be seen varies with the species. In the cat it is easily seen (Fig. 2, De Beer).

Table 1.—Weight of Bovine Pituitaries.

No. of Cow	Weight of Pituitary	Age Yrs.	Mon.	Weight lbs.	Breed	Stage of Gestation
546	2.27	3	5	1040	Holstein	
166	2.19	3	8	910	Jersey	
538	2.90	4	5	1275	Holstein	
520	2.90	6	9	1285	Holstein	108 days preg.
503	3.70	8	7	1093	Holstein	
288	3.90	8	8	1425	Holstein	
328	2.60	9	10	1200	Ayrshire	
120	5.89	9	11	830	Jersey	20 days preg.
327	2.80	10	10	960	Ayrshire	
121	2.80	11	11	860	Jersey	
110	3.85	11	11	750	Jersey	
270	2.73	11	11	940	Holstein	
Average	3.21					

The weights of the pituitaries of 12 cows have been obtained at the Missouri Station (unpublished) and are given in Table 1. Weights were accurately made only to the second place. As the numbers are small and as it is possible that the pituitaries were not all equally well trimmed before weighing, their significance is questionable. However, a great variation in size, from 2190 mgm. to 5890 mgm. existed. Age, body weight and breed appear to have no effect on the weight of the pituitary. In one instance of gestation of twenty days' duration, a remarkably large pituitary was observed; however in another animal killed at the 108th day of pregnancy, a pituitary smaller than the average was observed.

The entire pituitary is surrounded by a membrane or capsule of thin connective tissue which also extends up and around the infundibulum and is continuous with the meninges of the brain.

The pituitary is one of the smallest endocrine glands. In man it normally weighs but little more than 0.5 grams (Rasmussen 1924, 1928). For the pituitaries of meat producing animals Moulton (1929) gives the following weights: cattle, 3 grams; sheep, 0.37-0.55 grams; and hogs 0.33-0.78 grams. Various factors may influence its weight and size.

Sex and Other Factors Affecting the Size of the Pituitary.—Rasmussen (1924, 1928) noted that in man the pituitaries of females were larger than those of males, ranging in size from slightly larger in nullipara to considerably larger in pregnant females and in multipara. Rasmussen also states that earlier investigators (Comte 1898, Erdheim and Stumme 1909, and Kolde 1912) observed a similar increase in the size of the pituitary during pregnancy and in multipara.

Gentili (1920) found a progressive increase in the weight of the anterior pituitaries of cows from the beginning to the end of

pregnancy. The anterior pituitaries of nulliparous cows weighed but 135-140 mgm. The anterior pituitaries of cows at the end of the first pregnancy weighed about 350 mgm. and in multipara about 460 mgm.

Hill (1931) in an extensive review of the literature on the subject, cites several instances of the increase in size of the pituitary during pregnancy and also following castration, though in the latter case, a decrease in the size of the pituitary has been reported by some investigators.

Sexual activity also causes an increase in the size of the pituitary. Rasmussen (1921) noted a considerable increase in the size of the pituitary of woodchucks when the animals came out of hibernation and the rutting season began.

The Microscopic Structure and Cytology of the Pituitary.—Cobb (1921) states that the body of the pituitary consists of three parts, the pars glandularis, pars intermedia, and pars nervosa. These parts may all be readily distinguished by microscopical examination. A fourth part, the pars tuberalis, is located at the upper part of the pituitary around the infundibular stalk. Figures 2 and 3 diagrammatically show the location of the various parts.

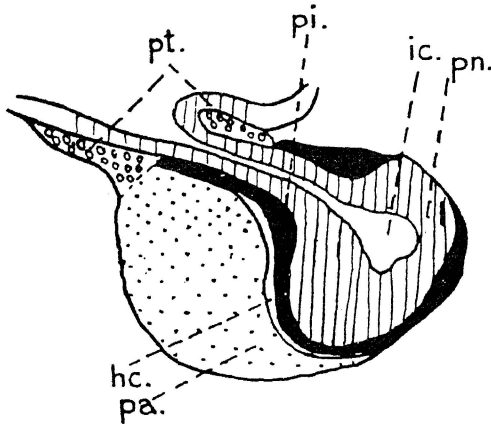


Fig. 3.—Cat, longitudinal section, head end to left. Hc, hypophyseal cavity; ic, infundibulum cavity; pa, pars anterior, pi, pars intermedia; pn, pars nervosa; pt, pars tuberalis. (De Beer)

The pars glandularis is separated from the pars intermedia by the hypophyseal cleft or cavity. Through this cavity the pituitary may be more or less readily split, thus dividing it into two parts. The infundibular cavity is a diverticulum from the brain. In the cat it extends well down into the pars nervosa while in the cow it terminates well up in the infundibular stalk.

The pars intermedia is thin and intimately connected with the pars nervosa, in the cat nearly surrounding it, while in cattle and the pig it lies largely only between the posterior and anterior lobes.

The vascularization of the different parts of the pituitary varies greatly with the glandular activity of these parts, the pars glandularis being the most vascular and the pars nervosa the least vascular (Fig. 4).

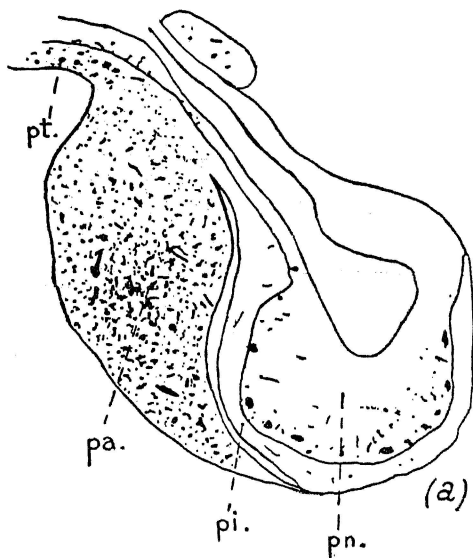


Fig. 4.—The vascularization of the pituitary. Pt. pars tuberalis; pa, pars glandularis, pi, pars intermedia, pn, pars nervosa. (De Beer)

The following review on the cytology of the pituitary was taken largely from De Beer (1920):

(a) *Pars glandularis*. The anterior lobe of the pituitary or pars glandularis is distinctly glandular in character, being composed of a network of epithelial cells and numerous wide thin walled blood vessels. The cell structure thus resembles somewhat that of other actively secreting endocrine glands as the adrenal cortex, thyroid and Isles of Langerhans in the pancreas.

Three distinct types of cells may be distinguished in the anterior lobe of the pituitary. They are as follows:

1. Chromophobes
2. Chromophiles
 - (a) Eosinophils
 - (b) Basophils

De Beer has observed eosinophilic cells that have ruptured and

are emptying their granular cell contents into the the sinusoids. The sinusoids are small cavities around which the secreting cells group in an acinous-like arrangement.

It thus appears that the eosinophilic cells are the actively secreting cells of the anterior lobe. As there are no characteristic nuclear differences in the basophilic and eosinophylic cells it is thought that they are of the same type but that they are at different stages in the secretory cycle. In this case, however, transitional cells should be observed, but with the exception of the rabbit such stages have not been observed.

Smith (1923) noted a region in the anterior lobe of the bovine pituitary containing relatively few eosinophilic cells. He also found that this area contained less growth promoting potency than the rest of the gland.

The work of Bailey and Cushing (1928) indicates that apparently a physiologically different secretion is produced in these different types of cells. The acidophilic cells produce the growth promoting hormones and when hyperfunctional, cause acromegaly. The basophylic cells are associated with the production of the gonad-stimulating hormones. At present this later explanation of the function of the various types of cells in the anterior lobe appears to be better grounded than the explanation suggested by De Beer.

(b) *Pars intermedia*. The pars intermedia contains only basophilic cells. In some mammals, however, such as cattle and swine, there is a projection from this region called the cone of Wulzen which extends across the hypophyseal cavity into the pars glandularis. This projection contains eosinophilic cells.

The pars intermedia is poorly supplied with blood with the exception of the cone of Wulzen and for this reason is thought to have comparatively little secretory activity.

(c) *Pars nervosa*. This part of the gland consists largely of neuroglia fibers and ependyma cells, however, a few cellular elements that have moved in from the pars intermedia are usually found.

This area is more highly vasculated than the pars intermedia and is also apparently richer in hormones than the latter. As neuroglia are not known to secrete, it is thought that the active extracts derived from the pars nervosa have their origin in the pars intermedia cells that have migrated into or lie next to the pars nervosa.

(d) *Pars tuberalis*. The pars tuberalis contains cells arranged in nests surrounded by connective tissues. A distinguishing characteristic of this part of the gland is the presence of vesicles. These vesicles apparently arise from the degeneration of nests of cells and contain coagulated fluid and cellular elements.

This region is thought to be capable of secretory activity, although little is known concerning its specific function.

The Gonad-Stimulating Hormones

Gonad-stimulating hormones may seem rather removed from the subject of this paper; yet, as will be mentioned in more detail later, the gonads have a marked influence on the mammary glands, particularly their development, as has been found particularly in the case of the rabbit. The gonad-stimulating hormones are capable of producing ovarian changes. The two above facts thus indicate at the outset that these may have an indirect effect on the mammary glands.

The gonad-stimulating hormones (there now appears to be two) are present in the pituitary and are generally considered to be produced there, though they are found in other tissues and body fluids under certain conditions in certain species. In these latter cases, investigations have not definitely proven the pituitary as the only source of these hormones. They appear to be biologically identical irrespective of their immediate source but it cannot safely be stated that they are identical in all respects. That the removal of the pituitary invariably results in genital atrophy strongly indicates that in the normal animal it is the source of the gonad-stimulants.

It thus seems possible that the gonad-stimulating hormones obtained from the blood, urine, and perhaps, even the placenta, may have been produced in the pituitary.* For this reason the investigations with gonad-stimulating hormones from these other sources as well as the pituitary are considered.

Early Investigations.—Beck (1922) in an outline of the early literature on the pituitary states that Fröhlich (1901) was the first to conclude that a relationship existed between the gonads and the pituitary. Previous to this time gonadal disorders had been known to be associated with acromegaly, a disease caused by abnormal pituitary functioning. A pituitary tumor, without acromegaly, was observed by Fröhlich to produce the aberration of sexual function; hence his conclusions.

For twenty years but little more was learned concerning pituitary gonadal relationship. Evans and Long (1921 and 1922) and Evans (1923-24), while experimenting with the growth promoting hormone of the anterior pituitary noted irregularities in or complete absence of estrus cycles in rats into which aqueous and alka-

*Since this bulletin went to press evidence has been presented by Evans, Myers, and Simpson (1931) (Proc. Soc. Exp. Biol. and Med. 28, p. 845) which indicates that the substances in urine (Prolan) stimulate the anterior pituitary to activity rather than having a direct gonadal effect. Thus it appears that the prolans of urine is not identical with the gonad stimulating substances of the anterior pituitary.

line extracts of anterior pituitary were intraperitoneally administered. Examinations of these animals showed that this was due to the large amounts of lutein tissue contained in the ovaries. It was concluded that the corpora lutea inhibited ovulation and estrus changes in the accessory organs.

Ovarian Changes Produced by Gonad-Stimulating Hormones.

—For several years after the establishment of a definite pituitary gonad relationship but one hormone affecting the ovary was considered to be produced by the anterior pituitary. As has been stated Evans and Long (1922) and Evans (1923 and 1924) noted the luteinizing effect on the ovary produced by anterior lobe extracts and a marked delay in sexual maturity or activity.

Later investigations, namely, those by Smith and Engle (1927) and Zondek and Aschheim (1927, 1927a), were difficult to explain on the basis of the existence of but one pituitary hormone affecting the ovaries. There then was assumed to be two different hormones present, one causing premature sexual activity, or follicle maturation and ovulation and the other luteinization as observed by Evans and Long. The existence of two gonad-stimulating hormones was debated by Parks (1929), but is apparently well established at the present time by the fact that two different extracts, some of which have been highly purified and concentrated (Claus 1931) (Collip 1930) and obtained from the same source have separately produced but one effect, that is, either follicle maturation or luteinization.

Zondek (1930) states that chemically the two hormones (Prolan A and B as he calls them) are very closely related. At the present time, they have not been chemically differentiated. They are precipitated by ethyl alcohol. Prolan A (sex maturing or follicle maturing) has been obtained separate from Prolan B (luteinizing) only when it exists alone as in the urine of non-pregnant women. It is insoluble in ether and chloroform, destroyed by strong acids, temperatures of 70 degrees Centigrade or over and dialyses rapidly. With the exception that these hormones are thermolabile it appears to the writer that there is considerable disagreement as to the chemical characteristics though the varying sources of these hormones (urine, placenta or anterior lobe of pituitary) may largely account for the difference. That there is some difference in the chemical nature of the two gonad-stimulating hormones is indicated by the separation obtained by Claus (1931). This will be considered again in a later section.

(a) *Luteinizing hormone (Prolan B)*. Luteinization of a Graafian follicle normally results only following ovulation, whether, ovulation occurs spontaneously, as in most animals, or only fol-

lowing coitus, as in the case of the rabbit. The luteinizing hormone appears to cause luteinization without previous ovulation, the follicular granulosa being converted into lutein cells which enclose the egg. These atretic corpora lutea have been observed by Fels (1927), Smith and Engle (1927), Parkes (1928-29), and Johnson and Sayles (1929). The ovaries following continued injection are greatly enlarged and contain largely lutein tissues (Evans 1923 and 1924).

Walker (1924), Brouha (1926 and 1930), Claus (1929), Bellerby (1929), Friedman (1929), Hewitt (1929) Zondek (1929) and Dickens (1930), have all noted the excessive luteinization of follicles in fowls, rats, mice, or rabbits, following injection of extracts of anterior pituitary. The earlier investigations of Evans and Long have been frequently confirmed.

(b) *Ovulation producing or sex maturing hormone (Prolan A).* Smith and Engle (1927) and Zondek and Aschheim (1927) noted that precocious sexual maturity was induced in infantile rats and mice by pituitary transplants. The follicles matured as early as twenty days in some of their animals. An excessive number of follicles matured and superovulation usually resulted (Smith and Engle), as many as 48 apparently normal ova being found in the Fallopian tubes of one 20-day-old rat. The observations of Smith (1930) in hypophysectomized rats further show the hormonal influence of the pituitary on follicle maturation and the entire genital system. Gonadal atrophy and the accompanying atrophy of the accessory genitals followed hypophysectomy in all cases where mature animals were used. Replacement by pituitary implants re-established gonadal activity.

The part played by anterior pituitary hormones as the mechanism inducing ovulation has been studied by Bellerby (1929-1929a) and Fee and Parkes (1929). Bellerby observed the act of ovulation occurring in anesthetized rabbits, ovulation having been induced by the intravenous injection of an extract of the anterior pituitary. He found that ovulation occurred within 11 hours after the injection or at approximately after the same time interval that it normally occurs following coitus (Hammond and Marshall 1925).

Fee and Parkes (1929) found that the removal of the pituitary in the rabbit within one hour following coitus prevented ovulation for a period of sixteen hours and probably indefinitely. When the pituitary was removed more than one hour after coitus ovulation occurred after the normal interval of under eleven hours.

Friedman (1929-1929a) and Jares (1930) found that ovulation in the rabbit was induced by injection of urine from a pregnant woman which contains gonad-stimulating hormones.

Recently Hill and Parkes (1931-1931a) found that ovulation was produced in hypophysectomized rabbits following the injection of the anterior pituitary and of urine of pregnant women though it was somewhat delayed and in the latter case occurred irregularly.

Effects on the Accessory Genital Organs.—Changes occur, not only in the ovaries, but also in the uterus and vagina of normal animals as a result of the injection of the gonad-stimulating hormones (Smith and Engle 1927). Implants of anterior pituitary tissue or the injection of extracts containing the gonad-stimulating hormones into castrated mature or castrated immature female rats are without effect on the remaining reproductive apparatus. Zondek and Aschheim (1927) similarly noted the absence of any changes in the uterus and vagina of gonadectomized animals following the administration of gonad-stimulating hormones.

It was concluded by the above investigators that the ovary alone was affected by the anterior pituitary. The observed estrus changes in the vagina and proliferation of the uterus in immature animals were considered to be due to ovarian hormones produced in the activated ovaries.

Fellner (1927) and Brouha and Simonnet (1927) reported changes in the uterus and vagina of castrate animals as well as normal animals following the injection of anterior pituitary extracts. The latter investigators suggested that the substance active in castrate animals was distinct from that producing changes in normal animals. The substance they describe as producing the changes of the uterus and vagina of castrate animals appears to resemble the estrus-producing hormone. The above results have not been verified by other investigators. It, therefore, seems safe to conclude that the pituitary hormones produce uterine and vaginal changes only indirectly, the ovary being the necessary and active intermediary.

It will be mentioned here that at the time this paper was started the ovary was thought to be a necessary intermediary between the pituitary and mammary glands. That the above assumption, is at most, but partially true is quite obvious at the present time. This relationship will be discussed later.

Variations in the Hormone Content of the Anterior Pituitary.

It has been found that the size of the pituitary varies with the sex and the stage of sexual development and perhaps changes following gonadectomy. Variations in the amount of active principal in the pituitary during these stages have been determined by the relative activity of implants at these stages in immature test animals, the rat or mouse.

Engle (1929), and Evans and Simpson (1929b) noted that the anterior pituitary of the normal male rat was more active than the anterior pituitary of the normal female rat. A relatively smaller amount of anterior pituitary of the male was, therefore, able to produce similar changes in the immature animals produced only by larger amounts of female anterior pituitary. Evans and Simpson state that the male pituitary was more than twice as active as the female pituitary. Castration, however, resulted in producing an increase of two and one-half times in the potency of male anterior pituitary and six times in the potency of the female anterior pituitary (Evans and Simpson, 1929c). Engle (1929) and Smith and Engle (1929) similarly noted an increase in the potency of the anterior pituitary of rats and guinea pigs, respectively, following gonadectomy. The increase in the potency was reported to take place comparatively rapidly, reaching 70 per cent of its maximum in two weeks.

Pregnancy in the rat appeared to have but little effect on the potency of the pituitaries (Evans and Simpson 1929d). Bacon (1930), however, stated that the hormone content of the anterior pituitary of the non-pregnant cow was greater than that of the pregnant cow.

Meyer et al. (1930) found that the injection of estrus-producing hormone caused a decrease in the gonad-stimulating potency of the pituitary of rats. The above observations are in close accord with those of Bacon when it is considered that there is a larger amount of estrus-producing hormone produced by pregnancy, at least in the cow (Turner et al. 1930) and especially during the latter stages. Baniecki (1930) noted that the injection of estrus-producing hormone caused a pregnancy change in the hypophysis.

Smith and Engle (1929) observed a variation in the amount of gonad-stimulating principal in the pituitary, as judged by implant potency, during the estrus cycle of guinea pigs. The implants obtained from animals in diestrus was most active.

The age at which the pituitary gonad-stimulating substance first made its appearance in the pituitary of pigs was found to be in the late fetal stage (Smith and Dortzbach 1929). Bacon (1930) noted the presence of the gonad-stimulating substance in the pituitaries of bovine foetuses.

Distribution of Gonad-Stimulating Hormones.—That gonad-stimulating hormones are found in the pituitary is obvious from the previous work to which reference has been made. The possibilities of other glands furnishing such a hormone has been investigated by Smith and Engle (1927) and Zondek and Aschheim (1927). Thyroid, thymus, adrenal, spleen, suprarenal, pineal, testic-

ular and posterior pituitary tissue all produced no ovarian changes in immature test animals. Anterior pituitary tissue obtained from mice, rats, cats, rabbits, guinea pigs, man, and cattle, however, all produced precocious sexual maturity in the infantile mouse.

Smith (1930) observed extreme atrophy of the ovaries and entire reproductive system of the rat following hypophysectomy; This atrophy was prevented however, by replacement with pituitary implants. Hypophysectomized animals were also restored to normal by pituitary implants after extensive atrophy of the genitals had already taken place. This adds further proof that the pituitary is the source of the hormone regulating ovarian function.

In addition to the pituitary, the placenta, urine, blood and perhaps even milk contain substances biologically identical to the gonad-stimulating hormones of the anterior pituitary. The hormones are present in substance, other than pituitary, however, only in animals in certain definite physiological conditions.

Zondek and Aschheim (1927) and Aschheim and Zondek (1927) noted the presence of gonad-stimulating hormones in the blood of women during pregnancy. Cole and Hart (1930) observed the presence of gonad-stimulating hormone in the blood serum of the pregnant mare. They state that the secretion began on about the thirty-seventh to forty-third day. From the eightieth to the hundred and eightieth day of pregnancy there was a gradual decrease in the amount of hormone present in the blood serum. The delay of the appearance of the hormone following conception is thought to be correlated with the time of implantation of the embryo. Zondek (1930a) also observed the gonad-stimulating hormone in the blood of pregnant mares. The amount found is greatest during the first one-third of pregnancy at which time it is about equal in mouse units per liter to the blood serum of women. On the average, however, 2000 rat units of Prolan A and 1000 mouse units of Prolan B are found per liter. Table 2 gives the comparative amounts of the two gonad-stimulating hormones and of the estrus-producing hormone found in the blood serum and urine of the pregnant woman and mare.

Fels (1927) and Fluhman (1929 and 1930) have also noted the presence of the gonad-stimulating hormones in the blood of pregnant women.

As the hormone had been determined in the blood it was thought that it might be eliminated in the urine. Aschheim and Zondek (1928 and 1928a) definitely determined the presence of a gonad-stimulating hormone in the urine of pregnant women. The amounts varied in concentration, being greatest during the first month and gradually decreasing until parturition, thereafter de-

Table 2—The Amounts of Follicular and Gonad-Stimulating Hormones Found in Blood and Urine of Humans and Horses.

(Zondek 1930a)

	Blood			Urine		
	Folliculin M. U.	Prolan A R. U.	Prolan B M. U.	Folliculin M. U.	Prolan A R. U.	Prolan B M. U.
Human	600	15,000	10,000	12,000	20,000	10,000
Horse	800	2,000	1,000	100,000	800	

creasing rapidly. They have now put this to practical application in diagnosing pregnancy during its early stages.

In their earlier work the above investigators failed to observe the presence of gonad-stimulating hormones in the urine of the rodent, elephant, cow and hog, and generally concluded that man and ape were the only two animals in which the hormones were found in the urine in amounts that could be determined. Zondek (1930a), however, determined the presence of the hormone in the urine of the pregnant mare.

Fels (1927), Friedman (1929a), Engle (1929a and 1929b), and Jares (1930), have verified the presence of gonad-stimulating hormones in the urine of pregnant women. Engle (1929b) states that a small amount of the hormone probably exists in the urine of males.

Zondek (1930) observed that the gonad-stimulating hormone was also present in the urine of women with genital tumors after the menopause. Castration of the rat, mouse, horse and woman has also been observed to be followed by the presence of gonad-stimulating hormones in the urine.

Various investigators have revealed the presence of gonad-stimulating hormones in the placenta, particularly the human placenta. Zondek and Aschheim (1928) observed the effect of the hormone by the implantation of fragments of placenta. Collip (1930-1930a) prepared extracts containing the gonad-stimulating hormones from the human placenta. Collip has obtained a highly potent crystalline substance by further purification of his extracts. Gutman (1930) states that no gonad-stimulating hormone could be determined in cow, sheep or sow placenta.

Recently Heim (1931) has reported that the hormone has been determined in the human milk.

Methods of Extract Preparation and Purification.—Many different methods of extraction for obtaining the gonad-stimulating hormones from the fresh pituitary, desiccated pituitary, urine, and

placenta have been described in the literature, however, with but one or two exceptions, all of the methods employed might well be grouped as follows: aqueous or saline, aqueous alkaline, dilute acid, or alcohol.

As has been previously stated, Evans and Long (1921) and Evans (1923-24) were using aqueous and dilute alkaline extracts of the pituitary when they first observed the gonad-stimulating effect. Teel (1926 and 1929) and Hewitt (1929) report obtaining active gonad-stimulating hormones from the fresh anterior pituitary by alkaline extraction of the fresh glands. Cushing and Teel (1929) noted that alkaline extracts of the anterior lobe of the fresh bovine pituitary contained a growth-promoting hormone, but observed no marked gonadal changes. Acid extracts were noted to contain a hormone causing premature estrus. Putman (1929) also observed growth promotion with his alkaline extracts but it was associated with a hormone causing luteinization. The residue remaining after the alkaline extraction was capable of producing premature estrus in infantile animals.

Hewitt (1929) obtained only growth-promoting hormone from fresh pituitary after filtering an alkaline extract through a Seitz filter. A similar extract, but unfiltered, produced in addition, an estrus-inhibiting effect.

Collip (1930 and 1930a) prepared two gonad-stimulating hormones by alcoholic extraction of human placentas and later purified the original extract. A concentrated (Prolan A) crystalline substance was obtained causing premature sexual maturity and follicular growth. The luteinizing hormone, though concentrated, was not so highly purified.

In previous work on the stimulation of lactation, Corner (1930), and Nelson and Pfiffner (1930) used alkaline extracts prepared by Parke, Davis, and Company.

Bellerby (1929) prepared an aqueous acetic acid extract which he observed to produce both luteinization and maturation of follicles.

Claus (1929 and 1931) obtained two active fractions from desiccated pituitary by acid alcohol extraction (Armour's Desiccated Pituitary). One of these fractions produced premature sex development and the other luteinization. Washing the alcoholic extract with glacial acetic acid removed the soluble luteinizing hormone. The sex maturing hormone was thus obtained in a highly concentrated crystalline form.

Fevold et al. (1930) have reported that a very potent water soluble substance capable of causing precocious sexual maturity has been obtained by extracting desiccated anterior pituitary with an aqueous pyridine solution followed by further purification.

Brouha (1930) states that acid and neutral extracts of the anterior pituitary act as implants in both males and females while alkaline extracts act only in the female producing luteinization.

Loeser (1930 and 1930a) prepared a saline suspension of desiccated anterior pituitary capable of producing ovulation in guinea pigs and rabbits.

Gonad-stimulating hormones have been obtained from the urine of pregnant women by Evans and Simpson (1929g), Zondek (1928 and 1930) and Veshjakov and Lipschutz (1929) by alcoholic extraction. Zondek precipitated the hormone from the neutralized urine by the addition of several volumes of alcohol and further purified the precipitate obtained. Evans and Simpson and Vesnjakov and Lipschutz removed the hormone by adding a small amount of alcohol, filtered the mixture to remove the insoluble material, evaporated the filtrate and purified the residue.

There are marked differences in methods of obtaining the gonad-stimulating extracts as may be seen from the above, particularly from varying sources. With the exception of highly purified extracts obtained from desiccated pituitary by Claus (1931), from the placenta by Collip (1930) and from the urine by Zondek (1930) the extracts are probably all rather crude and contain large amounts of impurities. Though the gonad-stimulating hormones show considerable physiological resemblance they appear to show varying chemical properties as indicated by the above variations in methods of extraction.

Methods of Administration.—The hormones of the anterior lobe as a group are all relatively easily destroyed. Heat, 70 degrees Centigrade or above, digestive enzymes and small concentrations of acid and alkali have been found to cause them to lose their physiological activity (Zondek 1930). It would thus be assumed that oral administration would be largely unsatisfactory. Such results were obtained by Schafer (1912), Wulzen (1914), Pearl (1916), Evans and Long (1921), Sisson and Broyles (1921), Smith, C. S. (1923), and Smith, P. E. (1927). Marinus (1929), however, noted an increase in growth in young rats following pituitary feeding.

It may be concluded that the gonad-stimulating hormones are either largely destroyed during digestion or are but slightly absorbed, and, hence, oral administration is generally ineffective. Large doses, however, have proven effective (Dickens 1930).

At the time of the discovery of the relationship of the pituitary to the ovary by Evans and Long (1921) as was previously stated, alkaline and saline extracts were being injected intraperitoneally.

Intraperitoneal and subcutaneous injection of extracts are both largely used at the present time and implants are made either

subcutaneously or intramuscularly. Evans and Simpson (1930) are the only investigators who have compared the results of intraperitoneal and subcutaneous injection and they note a distinct difference in the responses of immature female rats, apparently attributable only to differences in mode of injection. Anterior pituitary hormones prepared from placenta by the sulphosalicylic method of Weisner failed to produce the opening of the vagina of immature rats when intraperitoneally injected; however, both methods produced luteinization in the ovary. They concluded therefore, that the extracts injected intraperitoneally were more rapidly absorbed and removed from the body, thus being effective over a much shorter time interval.

The Ovarian and Gonad-Stimulating Hormones and the Mammary Glands

Experimental Growth of the Mammary Glands.—Natural observations indicate a very close relationship between the growth of the mammary glands and the stage of sexual development. Puberty and pregnancy are two sexual stages which normally result in marked growth changes in the mammary glands.

That the growth of the mammary gland is stimulated by hormones or chemical agents as postulated by Lane-Clayton and Starling (1906) has since definitely been proven.

The ovary, fetus and placenta have all been considered as sources of the hormones causing the growth of the mammary glands. Turner and Frank (1930) extensively reviewed the early literature on the hormonal growth of the mammary gland and it will not be repeated here.

Turner and Frank (1930) found that the estrus-producing hormone obtained from the urine of pregnant cows caused in castrated male and in immaturely castrated female rabbits a definite growth of the mammary glands. This growth involved the duct system and is similar to that produced in the normal estrus rabbit. The growth of the mammary gland of castrated male rabbits and rats, however, were further increased by the administration of a corpora lutea extract in addition to the estrus-producing hormone (Turner and Frank 1931). A development similar to that observed at the end of pregnancy was produced in the latter case. Lactation did not result, however, and it was suggested that some other hormone might be necessary in order to establish the secretory activity of the mammary glands. Parkes (1930) obtained similar results. He observed only duct growth following the injection of the estrus-producing hormone and concluded that an active luteal phase was necessary for the lobular growth.

Parkes (1929a) was able to produce a development of the mammary glands of the normal mature rabbit equal to that occurring at parturition by maintaining active corpora lutea in the ovaries for a period equal to the gestation period. The injection of an anterior pituitary extract was used to cause luteinization and hence continuous active corpora lutea.

Corner (1930) reported that corpora lutea extracts produced no growth of the mammary glands of castrated rabbits. Similar results were obtained by Turner and Frank (1931) when corpora lutea extracts were alone used. Growth of the mammary gland beyond the puberty stage, resulted only when the estrus-producing hormone and corpora lutea extracts were simultaneously administered.

A proliferation of the mammary glands of the rabbit was observed by Corner (1930) to take place following the injection of an alkaline extract of fresh sheep pituitary. If actual growth is implied by the term proliferation it would appear that both ovary and pituitary would be involved directly in mammary gland growth. However, as Corner's experiments were carried out on rabbits sexually mature before castration it is as yet scarcely proven that such is the case.

Experimental Secretory Activation of the Mammary Glands.—

It seems quite obvious that the factors causing the growth of the mammary gland and then its secretory activity are not identical, as a gland may be experimentally developed in castrate animals and yet show no evidence of secretory activity. Two distinct sets of stimulants, thus, appear to be required.

Numerous early investigators have reported lactation, but in view of the fact that their experimental animals were not castrated and their extracts were very crude, the significance of their experiments is greatly reduced.

Secretory Activity Induced by the Estrus-Producing Hormone.

—Secretory activity of the mammary gland has been observed in castrated guinea pigs following the injection of placental extracts rich in the estrus-producing hormone by Steinach, Dohrn, Schoeller, Hohlweg and Faure (1928). Laqueur, Borchardt, Dingemans, and DeJough (1928) also obtained similar results with the guinea pig. The doses measured in "mouse units" in the above cases were very large as highly purified and concentrated extracts were used.

Whether the estrus-producing hormone has more effect on certain species than others is not definitely known, but it has been shown that lactation has not occurred following the administration of the estrus-producing hormone alone, in the rabbit (Turner and Frank, 1930 and Parks 1929 and 1930).

Secretary Activity Induced by Gonad-Stimulating Hormones.—Evans and Simpson (1929e) observed a hyperplasia of the mammary gland of adult virginal rats after a month of daily subcutaneous injections of an alkaline extract particularly potent in growth-promoting hormone and comparatively poor in the gonad-stimulating hormones. Hyperplasia took place more rapidly following treatment with the gonad-stimulating hormones present in extracts prepared from bovine or rat pituitaries, human placentas, or urine. They reported lactation, however, only following the administration of the growth-promoting alkaline extract formerly mentioned.

A similar hyperplasia was observed in the mammary glands of immature female rats (Evans and Simpson 1929f). A brief treatment with bovine and rat pituitary implants and with folliculin and growth-hormone free extracts of human placenta and urine resulted in noticeable hyperplasia in four days. Corpora lutea secretion, they state, could not have been responsible for the mammary changes produced though these changes were always more pronounced where corpora lutea were coincident with the hypertrophied follicular apparatus.

Putman, Benedict and Teel (1929) observed a considerable increase in the growth of the mammary glands of a dog and a slight secretion of milk following an extended injection of an alkaline extract of bovine anterior pituitary.

As previously stated, Parkes (1929a) was able to produce complete mammary gland development in rabbits by the injection of alkaline anterior pituitary extracts. He explained this extended development as due to an extended period of luteal activity, similar to that occurring in normal pseudo-pregnancy, over a period equal to that of normal pregnancy. After the injections were stopped lactation took place.

Corner (1930) concludes that the proliferation of the glands observed by Parkes was not due to the corpora lutea, but to the injected anterior pituitary extract as he was unable to obtain growth of the mammary glands by luteal extracts.

Evidence of a Lactation Stimulating Hormone in the Anterior Pituitary.—Stricker and Grüter (1929) were the first to report lactation as a result of the administration of extracts of anterior pituitary. An aqueous extract of bovine anterior pituitaries was injected into immature female, virgin adult, spayed adult, pregnant and pseudo-pregnant rabbits. Rabbits injected with such an extract for a period of four or five days after a previous ten-day period of pseudo-pregnancy showed abundant milk secretion. Similarly, rabbits ovariectomized after ten days pseudo-pregnancy and then injected with the pituitary extract were stimulated to milk pro-

duction. A rabbit ovariectomized after sterile coitus was not injected until three months later. During this time marked regression of the mammary glands had taken place. Upon the injection of the pituitary extract the gland was again activated and milk was secreted.

Immature animals (6-8 weeks old) did not produce milk following the injection of such extracts, though marked ovarian changes were produced.

Grüter and Stricker (1929) state that they consider their results to indicate that the presence of corpora lutea in the ovaries was necessary previous to castration if anterior pituitary extracts were later to be effective in producing lactation. They were unable to produce lactation in immature females or male rabbits. The development of the mammary gland equal to that obtained near the end of pseudo-pregnancy was necessary if lactation was to result. Once the gland had developed, however, regression extending over an interval of several months did not prevent the appearance of lactation following the injection of the pituitary extracts.

The extract when injected into non-lactating multiparous dogs, hogs and cows, caused the reappearance of lactation. An increase in the amount of milk produced was observed when lactating animals were injected. This increase was much more pronounced in low producing than in high producing animals.

Grüter and Stricker suggest that the active principle of their extracts is distinct from the gonad-stimulating hormones obtained from the pituitary. That it is active in the absence of the ovary indicates that such may be the case.

Corner (1930) reported obtaining lactation in rabbits following the injection of alkaline extracts of the anterior pituitary. Virgin castrated mature females were used in his experiments. The extracts used were prepared by Parke, Davis and Company after a method similar to that used by Teel (1926).

Corner states that the substance causing the secretory activity of the mammary gland appears to be distinct from the gonad-stimulating fraction. It is much less stable than the gonad-stimulating hormones and acts in absence of the gonads.

A similar growth and lactation was observed in the mammary glands of virgin castrated mature guinea pigs by Nelson and Pffner (1930) following injection of extracts similar to those used by Corner.

In view of the work of Stricker and Grüter and Corner previously mentioned, it seems highly probable that a separate hormone, one producing secretory activity of the mammary glands, is present in the anterior pituitary. As it has thus far always been found present in alkaline extracts it would seem to indicate a

rather close resemblance to the growth-promoting hormones. Whether this "lactation-producing" hormone is present in the blood and urine, and the relative amounts present in the pituitaries in the different stages of sexual development is entirely unknown. A comparative test has as yet not been suggested by which it may be quantitatively tested.

EXPERIMENTAL PROCEDURE

Source of Extracts and Methods of Extraction.—As has been stated, at the time the work on this problem was started, it was considered that the gonad-stimulating hormones were possibly the active agents in stimulating secretory activity in the mammary gland. For this reason the possibility of obtaining these hormones from various sources was investigated. The pituitaries of mature rats and rabbits from the laboratory stock showed that they contained considerable quantities of the hormones as determined by ovarian changes produced in infantile rats, but this supply was too limited for extended experiments.

Full term placentas were obtained from a horse, a cow, and several sheep and were extracted according to a method described by Collip (1930). This method consisted of removing the hormone by alcoholic extraction. Purification was effected by absolute alcohol and acetone precipitation of inert materials from the residue of the first alcoholic extraction and repeated extractions with ether to remove the estrus-producing hormone. With the exception of the horse placenta extract no gonad-stimulating hormones were observed. As but one extraction was run in each case it is not stated that gonad-stimulating hormones are not present in cattle or sheep placentas. For our purpose the results indicated that they were present either in very insignificant amounts or were not removed by the methods employed.

Desiccated anterior pituitary was obtained from three biological supply houses (Armour and Company, Wilson and Company, and Parke, Davis and Company). Aqueous suspensions of the desiccated material indicated the presence of a gonad-stimulating hormone. The injections were very toxic, however, causing large subcutaneous lesions and resulting in a high death rate in the immature rats.

Other extracts prepared from the desiccated anterior pituitary following the method of Teel (1926) or of Claus (1931) gave either negative results or inconsistent results.

An extract of the anterior pituitary of sheep similar to that used by Corner (1930) was furnished by Parke, Davis and Com-

pany. In the limited number of immature rats injected no ovarian responses were observed and sexual maturity was not induced in the eight days these animals were observed following the injections.

Fresh sheep pituitaries obtained from Armour and Company were extracted by a method similar to that used by Teel (1926). The whole pituitaries were shipped on ice and arrived at the laboratory in good condition. The posterior and anterior lobes were both used in all cases. As the lactation stimulating portion of the extract was stated to be unstable by Corner (1930) small amounts of the pituitary were received at weekly intervals so that fresh extracts would be available over an extended period of time.

The process of extraction consisted of the following steps:

1. The pituitaries were ground to a pulp with sterile sand in a sterile mortar.
2. To the macerated tissue an equal volume of distilled water was added.
3. Ten per cent NaOH was added until acid thymol blue paper turned to a distinct blue after the alkali had been well stirred up with the tissue. (The macerated tissue becomes very gelatinous at this time. When the consistency of the mass was too great, a small amount of water was added).
4. The mixture was placed in an ice box at temperatures near freezing for a period of 24 to 36 hours.
5. After removal from the ice box, one per cent acetic acid was added until the mixture became neutral to litmus.
6. The neutralized mixture was then centrifuged.
7. The clear slightly reddish fluid was decanted off and placed in small sterile bottles. The number of cubic centimeters being determined as it was desired to keep the relative strength of the different extractions as constant as possible. (1 c.c. = .5 gm. of fresh tissue).
8. A few drops of tricresol were added and the extract kept in an ice box until used. After standing a few hours, a fine white precipitate invariably formed. This was not removed and its potency was not determined. The bottles were always shaken before the extract was used so that in all cases the precipitate was injected as well as the fluid portion.

The estrus-producing hormone and the corpus luteum extract were prepared in the laboratory by Mr. A. H. Frank. The estrus-producing hormone was prepared from the urine of pregnant cows (Turner and Frank 1930). The corpus luteum hormone was prepared from sows corpora lutea by a method obtained from Hisaw (by letter).

Method of Extract Injection.—All extracts were subcutaneously administered. One injection was made each day in most cases. In mice and rats, however, injections occasionally have been made more frequently.

When pituitaries were injected without extraction they were crushed in a very small amount of Ringer's solution, drawn into a large hypodermic needle for injection.

Methods of Determining Developmental Changes in the Mammary Glands.—In order to determine the changes induced in the mammary glands following the injection of the extracts, parts of the glands were removed to serve as controls at the time the rabbits were selected for the experiments. For the determination of slight changes in the glands a microscopic examination of stained sections would no doubt be the only definite test. As far greater changes,—changes that could be macroscopically determined, were being sought, microscopic examinations were made in but few cases.

The sections of gland removed were considered typical of the entire gland system. For their removal the rabbits were fastened to an operating board and a local anesthetic was injected around the gland area. A cut through the skin was made on the inside of the teat. The gland was cut free from the skin and then from the underlying muscle. A section of the gland from the teat extending inward to the midline, or as far as the gland area extended, was thus obtained.

These cuts healed rapidly and in three or four days the stitches were removed. The rabbits were watched closely for several days as they occasionally pulled out the stitches and opened the wound to infection.

The pieces of gland removed were stretched out on and fastened to cork blocks and fixed in Bouin's fluid. After fixation they were trimmed, all muscle or tissue layers over the gland layer being removed. The glands were then stained in Mayor's hemalum, destained to the proper degree in acid alcohol and cleared in xylol. The glands to be preserved and photographed were mounted in balsam between glass plates. Closing the edges of the plates with sealing wax greatly inconvenienced the handling of the mounts, particularly when thick glands were mounted.

It was found that the glands thus mounted could be studied readily under low magnification, thus affording a comparative study of the degree of development and the activity of the gland.

Where microscopic examinations were made the sections of gland were fixed in Bouin's or Flemming's osmic fixative. The latter fixative was particularly desirable where active glands were being studied as the fat was stained and made insoluble in alcohol and xylol. The glands were sectioned in paraffin blocks at a thick-

ness of five microns and stained in Mallory's triple connective tissue stain.

No control rabbits were run on any experiment. The original gland mounts shown by Turner and Frank (1930), however, were available and the different stages of development could thus be readily checked with them when necessary.

Time of Renewal of Experimental Glands.—It was assumed that the water soluble extracts that were used were absorbed and were possibly removed from the body of the rabbits at most within one day after injection. For this reason all experimental glands were removed on the day following the last injection.

In Experiments I and II in which the estrus-producing hormone in oil was used (Turner and Frank 1930), the glands were also removed on the day injections ceased. This extract was undoubtedly more slowly absorbed than the aqueous extracts of the anterior pituitary and is was possibly active after the last glands were removed.

Presentation of Experimental Data

Experiment I.—The estrus-producing hormone had been previously shown by several investigators to have a marked effect in producing the growth of the mammary glands of immature ovariectomized female and male rabbits. The effects of this hormone, however, on the mature glands had never been determined. The object of this experiment was to determine whether or not the mammary glands of the rabbits that had previously been pregnant or pseudo-pregnant would respond in any way to moderate and continuous injections of estrus-producing hormone obtained from the urine of pregnant cows.

Three rabbits were selected from the laboratory stock for this purpose. Two of them had produced young and one had been pseudo-pregnant. Each was subcutaneously injected with 25 rat units of the estrus-producing hormone daily for a period of twenty-one days. Control glands were removed on the day injections started. Other sections of the gland were removed at seven day intervals. In all cases the glands were sectioned and stained for microscopical examination.

No significant changes were observed in the mammary glands of any of the rabbits. There is an indication that the glands of two of the rabbits underwent a very slight regression and some indication of a slight proliferation of the glands of the third rabbit. These changes were very slight, however, and therefore questionable. It may therefore, be assumed that when administered in small amounts the estrus-producing hormone obtained from the urine of pregnant cows does not produce changes that

can be microscopically determined in the mammary gland of mature female rabbits.

Experiment II.—The object of this experiment was to determine the effect of large amounts of estrus-producing hormone and the corpus luteum extract when injected simultaneously, upon the mammary glands of mature castrated rabbits.

Two rabbits from the laboratory stock were selected for this experiment.

R68 was a mature female rabbit. She was ovariectomized on February 14 after 14 days of pseudo-pregnancy produced by sterile coitus. A control gland showed that normal pseudo-pregnant development had been produced. Beginning February 15, one-half c.c. of the corpus luteum extract and 400 rat units of estrus-producing hormone were injected daily until March 6th. On February 24 the glands were examined and a section removed. The lobules and ducts were filled with milk which flowed freely from the gland when sections were removed. A section removed March 6 showed that little if any change had occurred in the gland.

R54 was a mature female rabbit ovariectomized December 6, 1930. A control gland was removed February 6, 1931. The gland was involuted and apparently had never had much lobule development. Injections were started February 24, the dosage being the same as received by R68. A second gland was removed March 6. The ducts were filled with milk though the lobules did not appear to be extended.

The results obtained in this experiment seem to indicate that lactation may be induced by the simultaneous injection of large amounts of estrus-producing and the corporea lutea extract. As but two animals were included in this experiment it is scarcely safe to make the statement that lactation will always result if such a treatment is followed.

There is a possibility that the estrus-producing hormone may have contained some gonad-stimulating or lactation producing hormone. As neither of these, however, have as yet been determined in the urine of pregnant cattle and also as it is unlikely that the lactation producing factor, if present in the pregnant cow's urine, would be present in the extract as it has been considered to be quite unstable, it is hardly possible that the extracts contained these hormones.

Meyer et al. (1930) observed changes in the anterior pituitary of the rat following the injection of estrus-producing hormone. These changes greatly resembled those occurring during pregnancy. If such a change is also produced in the anterior pituitary of the rabbit the assumption might be made that the lactation in the above rabbits was due to the hormones produced in the rabbit's own pituitary under the stimulation of the estrus-producing hormone. As this experiment has not as yet been repeated on control hypophysectomized rabbits, the above statement is purely an assumption.

Hartman, Firor and Geiling (1930) were unable to obtain menstruation in the hypophysectomized female monkey following the injection of estrus-producing hormone. Menstruation resulted, however, when the pituitary was present. This further indicates that the estrus-producing hormone may act on the pituitary.

This experiment indicates that the simultaneous injection of the corpus luteum extract and large amounts of estrus-producing hormone results in milk secretion and these results may be taken to suggest that the pituitary is activated to the production of the lactation stimulating principle in the presence of the large amounts of estrus-producing hormone. The extent of the proliferation of the glands, however, did not equal that which was produced following the injections of the anterior pituitary extract to be described later.

Experiment III.—The objects of this experiment were, first, to attempt to stimulate the mammary glands of a series of rabbits to secretory activity and, second, to determine the possibility of growth changes in the mammary glands produced by the anterior pituitary and to compare such changes with those produced during experimental pseudo-pregnancy. An alkaline extract of sheep's anterior pituitary kindly supplied by Parke, Davis and Company was used throughout the experiment. The extracts were kept in a refrigerator at a temperature of about 42 degrees Fahrenheit until they were used.

A variety of rabbits were selected from the laboratory stock and a stock purchased just previous to the time the experiments started. Protocols of the experiment are given below.

R3 and R31 were mature rabbits and had both previously borne young. These rabbits were from old laboratory stock and had previously been used on other experiments. Injections at the rate of 1 c.c. per day started December 8, and continued until December 18. A control section of gland was removed on the day the experiment started and a second gland was removed when the injections were stopped. No changes were noted in the glands. The ovaries were not examined.

R5 was a mature female rabbit and had also previously borne young. She was castrated December 3. The ovaries contained large follicles. A control gland was removed December 7. The gland was involuted to a marked degree. Two c.c. of the extract were administered daily for nine days. A second gland was removed December 18. It greatly resembled the control in appearance, showing that no development had taken place.

R6 was a mature normal female rabbit from the laboratory stock. She had been pseudo-pregnant but never pregnant. She had been previously used on the estrus-producing hormone experiment mentioned earlier and a gland removed November 10 was used as a control. Injections at the rate of two c.c. daily were started December 8 and continued until January 5. The glands were inspected and a section removed on December 18 and January 5. No changes were observed.

R8 was a mature hysterectomized female. She was castrated December 9 and a control gland removed on the same date. Injections were started December 9 and continued until December 18, two c.c. of the extract being injected December 18. No changes had occurred.

R9 was a mature female rabbit recently added to the laboratory stock. A control gland was removed December 9, and injections at the rate of 2 c.c. daily were started and continued until January 5. The glands were inspected and a section was removed December 18 and January 5. No changes were observed.

R81 and R82 were normal female littermate rabbits about five months old. Control glands removed December 8 showed but partially developed glands. Each rabbit was intravenously injected with 2 c.c. of the extract. On the following day, December 9, laparotomy showed corpora lutea forming in the ovaries and follicles near the point of ovulation. On December 18 the glands were inspected and sections removed. Pseudo-pregnant development had taken place. A second intravenous injection was given and a laparotomy performed on the following day showed that ovulation had again taken place. On January 6 the mammary glands were again inspected and a section of gland removed. No further development had taken place. A third intravenous injection was given January 9, but it is doubtful if ovulation occurred. Another gland removed January 19 showed that the mammary glands had regressed.



Fig. 5.—The control section of gland removed from R82. This gland is very similar to the gland of R81 though it is in a slightly more advanced stage of development. (Enlarged $2\frac{1}{2}$ times.)

In all the above cases where subcutaneous injections were made no changes in the mammary glands of the mature normal female or the mature castrated female rabbits were observed. According to Corner (1930) the hormone active in producing lactation is very unstable and it is quite obvious that it was not present in these extracts.

Injections of the gonad-stimulating hormones which produce ovulation or luteinization should result in changes in the mammary

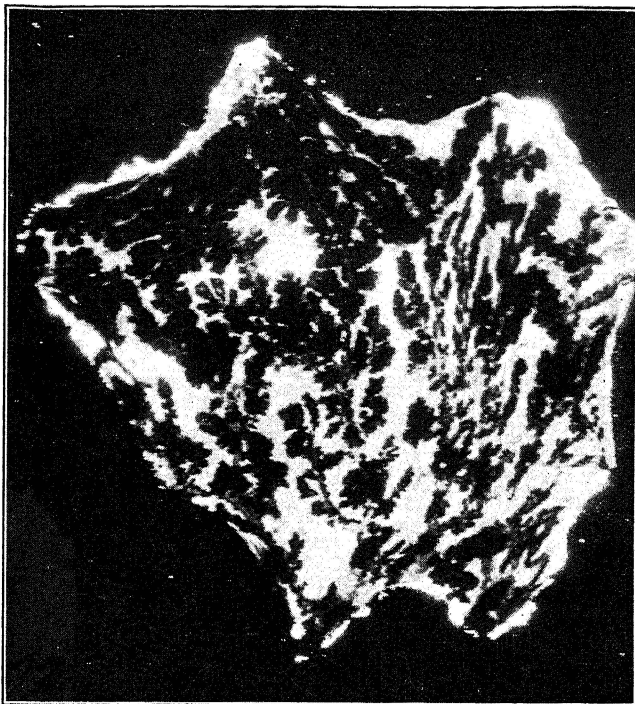


Fig. 6.—A section of mammary gland of R81 ten days after an intravenous injection of 2 c.c. of an alkaline extract of sheep pituitary. The lobules had developed so as to nearly fill up the space between the ducts. As the gland was stretched before fixing so as to enable microscopic examination it appears considerably less compact than normal. This gland is comparable to glands of rabbits ten days pseudo-pregnant. (Enlarged $2\frac{1}{2}$ times.)

glands as in normal pseudo-pregnancy in the normal animals R3, R31, R6, and R9. In no case, however, were such changes observed following the subcutaneous injections. As laparotomy was not performed either before or following the injections the condition of the ovaries was not known. That there were no ovarian changes in the experimental animals is concluded only from the lack of change occurring in the mammary glands.

Intravenous injections of the extract into immature animals indicated the presence of some gonad-stimulating hormones as corpora lutea and follicles near the point of ovulation were observed at laparotomy approximately eighteen hours after the injection. Marked changes of the mammary glands were observed in R81 and R82, (Figures 5, 6, 7, and 8) though this was due no

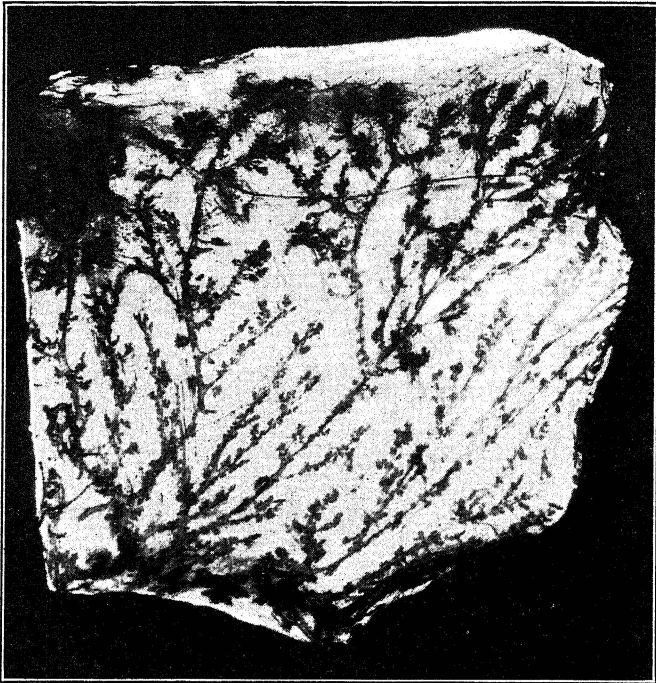


Fig. 7.—Control section of gland removed from RSI on December 8. This is the normal type of gland found in rabbits after puberty has been reached. At the time this section was removed its bearer was about five months of age. The duct system is developed to nearly as great an extent as in a mature gland. The lobules, however, are but slightly developed. (Enlarged $2\frac{1}{2}$ times.)

doubt to the ovarian stimulation. Prolonging the period of pseudo-pregnancy by repeating the intravenous injection after ten days produced no further changes in the glands.

As the extract was injected directly into the blood it was fairly concentrated for a short period and produced ovarian changes where subcutaneous injections failed. As a result of this experiment the conclusion arrived at by Corner (1930) relative to the case of lactation reported by Parkes (1929a) seems to be verified. Lactation is not due to the presence of corpora lutea in the ovaries over an extended period of time and the lactation observed by Parkes was very likely due to the anterior pituitary extracts given.

Further evidence that the extract contained but small amounts of gonad-stimulating hormone was obtained as a result of experiments with immature rats. One-half c.c. of the extract injected

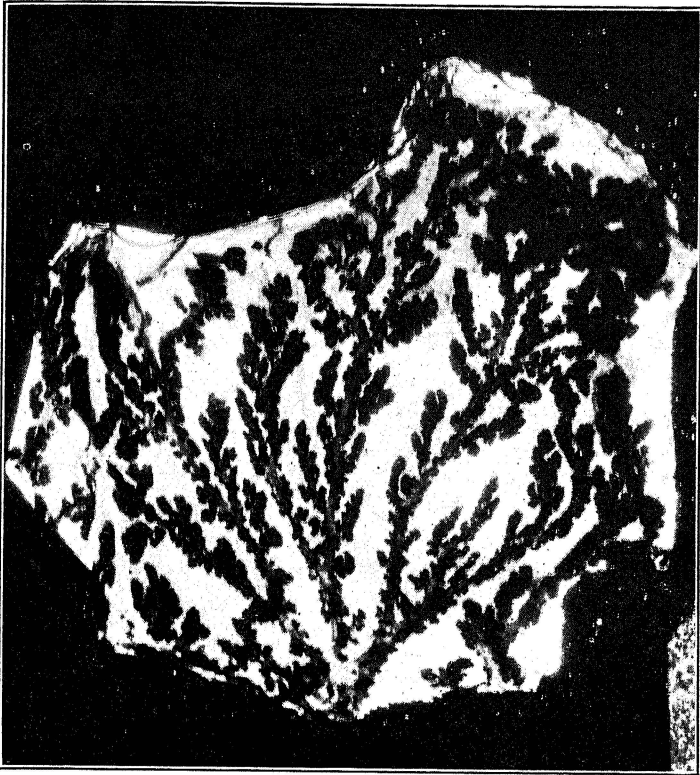


Fig. 8.—A section of the mammary gland of R82 ten days after an intravenous injection of two c.c. of an alkaline extract of sheep's pituitary had been administered. The development of the glands is very similar to that of R81. This gland also appears less compact than normal having been slightly stretched prior to fixation. (Enlarged $2\frac{1}{2}$ times.)

daily into twenty-two-day-old rats for a period of three days did not result in precocious sexual development as determined by the opening of the vagina during the period of eight days that they were observed.

Summing up the results obtained it was readily concluded that the extracts contained no active fraction causing lactation or growth of the glands. The growth of the mammary glands obtained in the two rabbits R81 and R82 might be more plausibly explained as being due to ovarian hormones. As pseudo-pregnancy was not produced in the four non-castrated animals, as was determined to be the case, judging from the appearance of the mammary glands, it was assumed that the extracts contained little

gonad-stimulating hormone.

Experiment IV.—The object of this experiment was to determine whether or not a hormone was present in the pituitary capable of acting directly on the mammary glands stimulating them to secrete milk. The extracts used were those prepared from fresh sheep pituitary by a method described by Teel (1926). As but a small amount of pituitary was received at weekly intervals a large number of rabbits could not be used at one time. As a result the rabbits did not all receive the same extracts and there is a possibility that the potency of the various extracts may have varied considerably, due to variation in the source of the pituitaries and to variations in extraction although the same method of extraction was followed throughout the experiment. The following are the protocols of this experiment.

R68 was the same rabbit previously used in Experiment 3. A small amount of milk was present in the glands at the time she was started on this experiment. On March 6 one c.c. of the fresh sheep's pituitary extract was given daily until March 16. At this time the rabbit was sacrificed. The mammary glands were greatly distended with milk.

R54 was used on an experiment similar to R68. The glands, however, were less proliferated than those of R68. On March 6 one c.c. of the extract was injected daily until March 14. At this time the rabbit was sacrificed. The glands were filled with milk.

R37 was a mature female rabbit ovariectomized December 6, 1930. A control gland was removed February 26, 1931. The glands were greatly involuted. Injections were started March 1, one c.c. of the fresh pituitary extract being given daily until March 21. The glands were inspected March 11 and a section removed. The ducts and lobules were greatly filled with milk and the extent of the gland could readily be determined through the skin. On March 21 the glands showed a condition very similar to that observed ten days earlier.

R77 and R78 were two mature females recently added to the laboratory stock. They were castrated March 3 and control glands were removed. On March 7 a second control gland was removed and injections at the rate of two c.c. daily were started. A gland removed from R77 on March 12 was filled with milk. Inspection of the glands on March 16 showed both rabbits with glands filled with milk. Sections of the glands were removed. Milk flowed from the glands when the sections were cut out.

R75 and R76 were mature females recently added to the laboratory stock. Both rabbits were castrated March 3 and control glands were removed. A second control gland was removed March 9. The glands of R75 were greatly involuted and R76 showed at least more than one month's involution. Injections were started on March 9 at the rate of 2 c.c. daily. On March 12 a gland was removed from R75. Development of the gland had started but no milk was observed. On March 15, R75 died, apparently from the toxic effects of the extracts though no lesions or infections could be observed. A gland removed was greatly thickened and filled with milk. R76 was sacrificed on March 19. Her glands were greatly distended with milk.

R79 and R80 were mature female rabbits. Both rabbits were castrated on March 6 and a control gland was removed. The glands of both were greatly involuted. A second control was removed March 16 and injections were started at the rate of 2 c.c. daily. Both rabbits were in the same cage and were found dead on the morning of March 20 and 21 respectively. The glands of R79 were examined. A trace of milk was observed in the ducts but the gland was yet definitely involuted. R80 was not examined.

R81 and R82 were previously used on Experiment 3. They were ovariectomized on March 14 and control glands were removed. Both rabbits showed slightly involuted pseudo-pregnant development of the mammary glands. Injections of the alkaline pituitary extract at the rate of 2 c.c. daily were started on March 14 and continued until March 24. At this time the glands were inspected and sections removed. The glands of R81 were greatly thickened and filled with a thick colostrum-like milk. The glands of R82, though slightly thickened, showed the presence of only a little milk in the ducts and but a few drops flowed out when the sections of gland were cut out. The two rabbits were again subjected to similar injections from March 26 to April 5. On April 5 inspection of the glands showed that no change had occurred in the glands except that the secretion in the glands of R81 at this time resembled normal milk and flowed freely from the gland when a section was being removed.

A total of 11 mature rabbits were used in this experiment. In all cases where the injections were continued for more than four days the secretion of milk was either initiated or greatly increased. One rabbit, R79, after receiving the extract for 3 days, had milk present in the larger ducts though the entire gland had

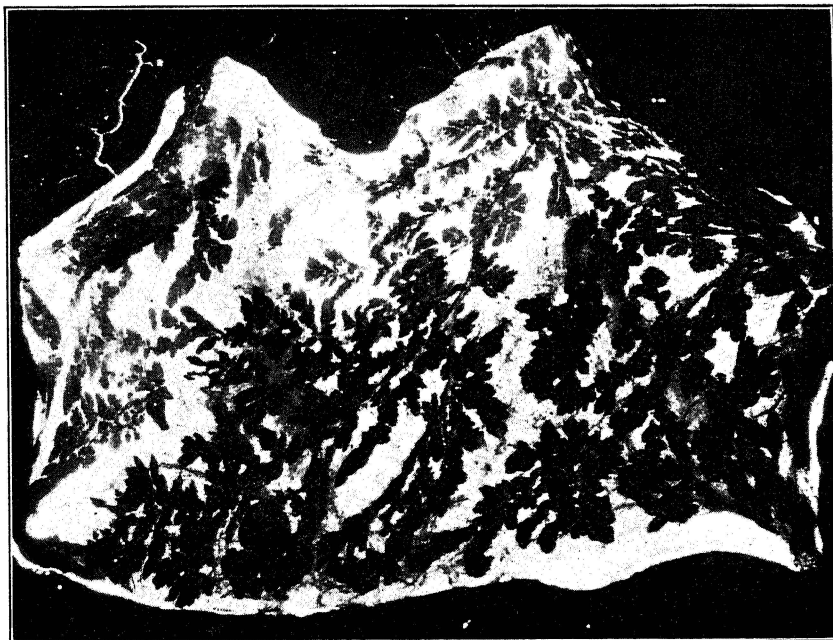


Fig. 9.—Control section of gland removed from R76 on March 3. This gland is in a stage of development very similar to that usually observed at the fourteenth day of pseudo-pregnancy. This gland was removed on the same day the rabbit was castrated. A gland removed six days later is shown in Figure 10. Though both glands were not sufficiently destained so that their structure throughout could be determined, there was no noticeable difference in the two control glands. No milk was present in the above gland. (Enlarged $2\frac{1}{2}$ times.)

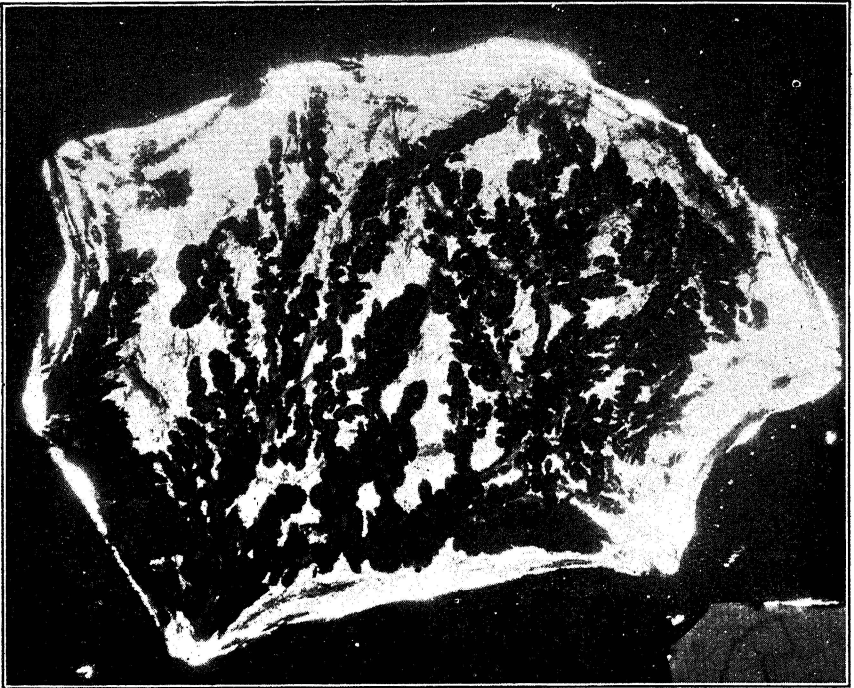


Fig. 10.—Second control gland removed from R76 on March 9. This gland is similar to the first control gland removed March 3. No milk was present in the gland. By comparing the above photograph with Figure 11 the change following a nine-day treatment with pituitary extract may be seen. (Enlarged $2\frac{1}{2}$ times.)

not yet become filled with secretion. A study of a fixed gland under low magnification showed that the lobules had not yet become fully distended.

Another rabbit, R82, did not show as great a secretion as the other rabbits on the experiment. The ducts, though filled with milk, were not as greatly distended as were the ducts in the glands of the other rabbits and the lobules of the distal part of the gland were not filled with secretion. As this rabbit had received the same extracts as its littermate sister (R81) no explanation can be offered for the lack of a more pronounced stimulation of milk secretion.

Without exception, the eight other rabbits in the experiment showed marked proliferation of the mammary glands. The outline of the glands could be seen clearly through the skin and streams of milk could be removed through the teats. Figures 11, 15 and 19 show the proliferate glands of R76, R54, and R68. The ducts were greatly



Fig. 11.—The mammary glands of R76 after the alkaline extract of sheep's pituitary had been injected for nine days. The glands were greatly thickened and filled with milk. The arrows show the place where sections of the glands have been removed to serve as controls. The control glands are shown in Figure 9 and Figure 10. Milk can be seen running down over the skin from an accidental cut made in the left rear thoracic gland when the skin was being removed.

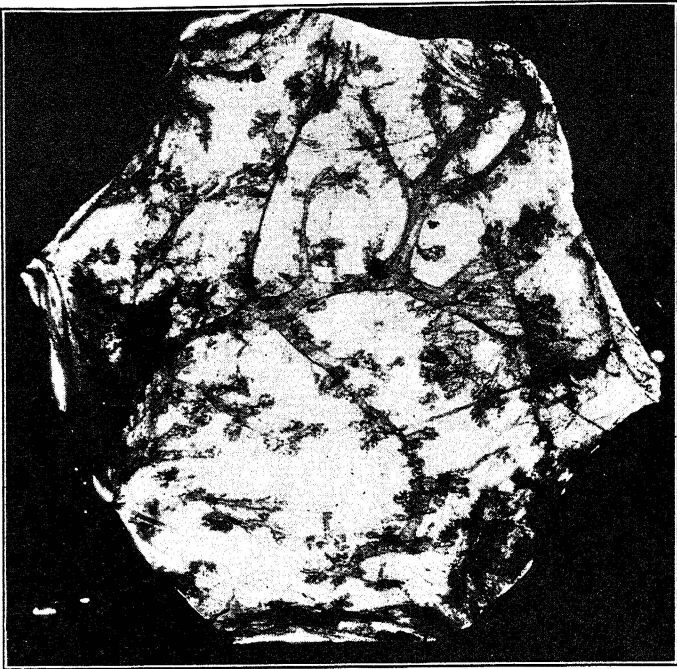


Fig. 12.—Control gland of R54 removed February 6. This gland shows the presence of but a very small amount, if any, lobular tissue. Most of the ducts appear to end in blind branches. The duct system, however, is quite well developed. This rabbit had been castrated for two months. (Enlarged $2\frac{1}{2}$ times).

distended with secretion and the entire gland system was filled with milk as can be seen from the white outline of the glands.

Examination of the fixed and stained sections of the glands at low magnification showed that in all cases where lactation occurred the alveoli were greatly distended. Even when the control glands were greatly involuted (Figure 20) the proliferation of the alveoli was equal to that produced in glands that were but slightly involuted (Figure 9).

The rate of involution of one rabbit had been previously determined by sectioning pieces of gland removed at five-day intervals following parturition. After forty days the alveoli were greatly reduced in size but could be distinctly seen. By comparing the control glands with this involution series it was concluded that, with the exception of R68 and R54, a period of at least forty days or more had elapsed since the glands were last actively secreting milk.

It appears that the hormone stimulating the mammary glands to secretory activity acts largely, if not entirely, on the secretory cells of the alveoli stimulating them to activity. As mature rabbits were used on this experiment, it cannot be said that growth of the glands may not also be produced. All of the evidence that could be obtained from this experiment, however, indicates that no growth changes of the mammary glands are produced by the pituitary, at least by the extracts used. Though the glands of all rabbits were approximately equally proliferated following the injection of the extract, whether or not the control glands showed the presence of numerous alveoli, there appeared to be a similar difference in



Fig. 13.—Control gland of R54 removed March 6. A very small amount of milk was present in the ducts of the glands at the time this section was removed. By comparing this section of gland with Figure 14 and Figure 15 some idea of the extent of the proliferation of the glands produced following injections of the pituitary extract may be obtained. As R54 previously received daily injections of large amounts of estrus-producing hormone and the corpus luteum extract for a period of ten days the above glands should be comparable to those of rabbits ten days pseudo-pregnant. (Enlarged $2\frac{1}{2}$ times).

number of alveoli in the proliferated glands compared with their respective controls. A great difference in the size of the alveoli was observed, however. When the presence of but a small number of alveoli was indicated by the examination of the control



Fig. 14.—A section of the proliferated glands of R54 removed March 14. This photograph was taken at the same magnification as Fig. 21. The extent of the proliferation of the glands following the injection of the pituitary extract may be seen by comparing this photograph with Fig. 13. The extremely milky appearance of the glands in Fig. 15 is no doubt due to the large size of the alveoli which would greatly increase the amount of milk present as compared with the amount of tissue.

glands, the alveoli, after secretory activity had been induced, became greatly enlarged. R54 afforded a particularly good illustration of this.

Figure 13 shows the control section of gland of R54. The proliferated gland is shown in Figure 14. In contrast Figure 20 shows a control section of gland of R75 and Figure 21, the resulting proliferated gland. Examination of the fixed and stained glands of these two rabbits at low magnification show that the average size of the alveoli of R54 are several times larger than those of R75.

In the human, lactation has been reported to frequently follow castration. As it was desired to use some of the rabbits within a few days after castration, two control glands were removed from six of the rabbits in order to check the effect of castration. The first control was removed at the time of castration and the second was removed from two rabbits (R77 and R78) four days later, from R75 and R76, six days later, and from R79 and R80, ten days later. No significant difference between any pair of the control glands could be observed and it was concluded that castration of rabbits did not result in stimulating lactation.

The results of this experiment show that the pituitary contains an active substance which is capable of stimulating the mammary glands to secretory activity. The active substance or hormone is equally effective in stimulating milk secretion irrespective of the degree of involution of the glands within the range covered in this experiment. The lactation experimentally obtained appears to be equally as great as that observed during the first few days after parturition. It also appears that the hormone acts only on the secreting cells stimulating them to secretory activity and does not produce an increase in the number of alveoli.

General Discussion of the Experiments

The knowledge recently obtained on the hormonal control of the growth and the secretory activation of the mammary glands has but opened the field for further investigations. At the present time only the source and the function of extracts of the ovary and the pituitary have been established. The interrelationships and the causes of their activity are largely unknown. Questions such as the following arise: Why do the glands, particularly the ovaries in this case, become active at a certain more or less definite time in the development of the animal? Is the substance in the anterior pituitary that initiates lactation secreted in the pituitary continuously and liberated only near the time of parturition or following parturition or is it secreted continuously becoming functionally active only when certain other conditions are favor-

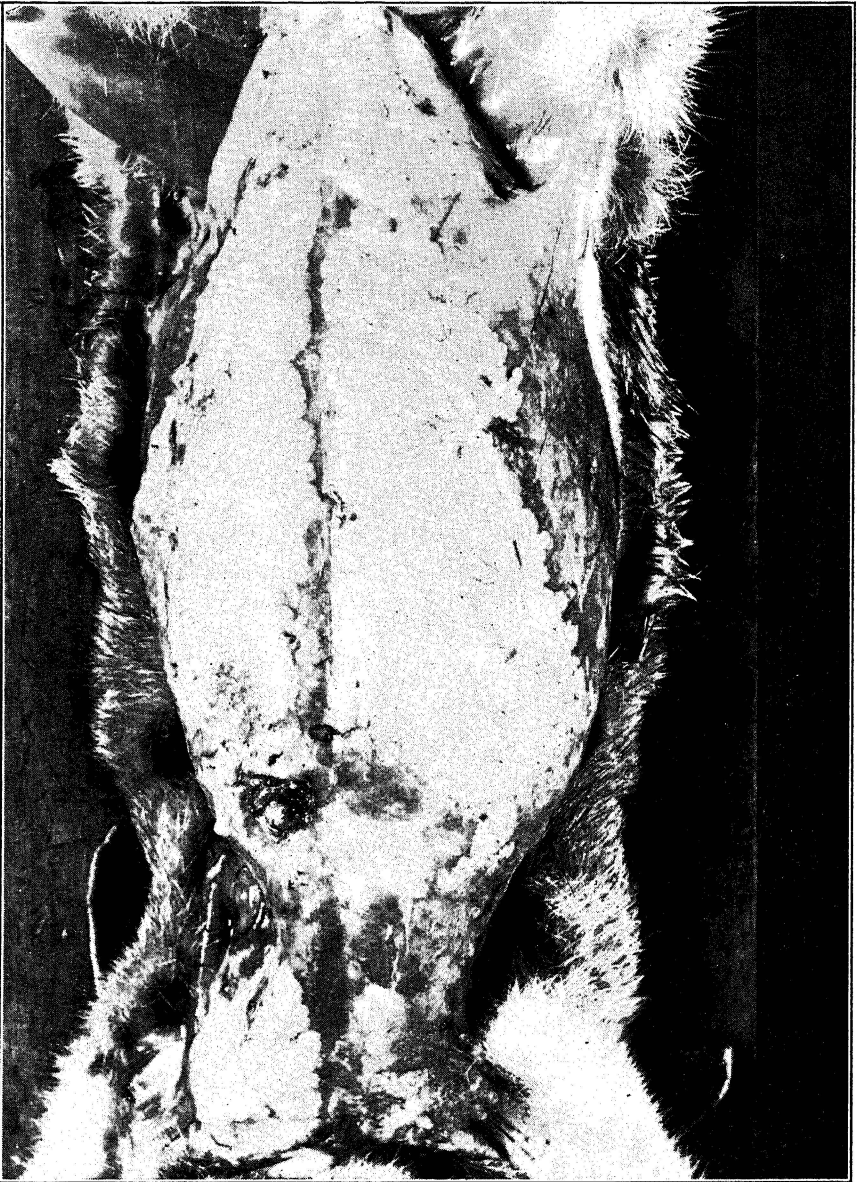


Fig. 15.—The mammary glands of R54 after an alkaline extract of sheep pituitaries had been injected for seven days. The lobules and ducts were greatly distended with milk. The scar near the right mid-thoracic teat and the area directly to the left of it are the places from which control sections of the glands were previously removed.

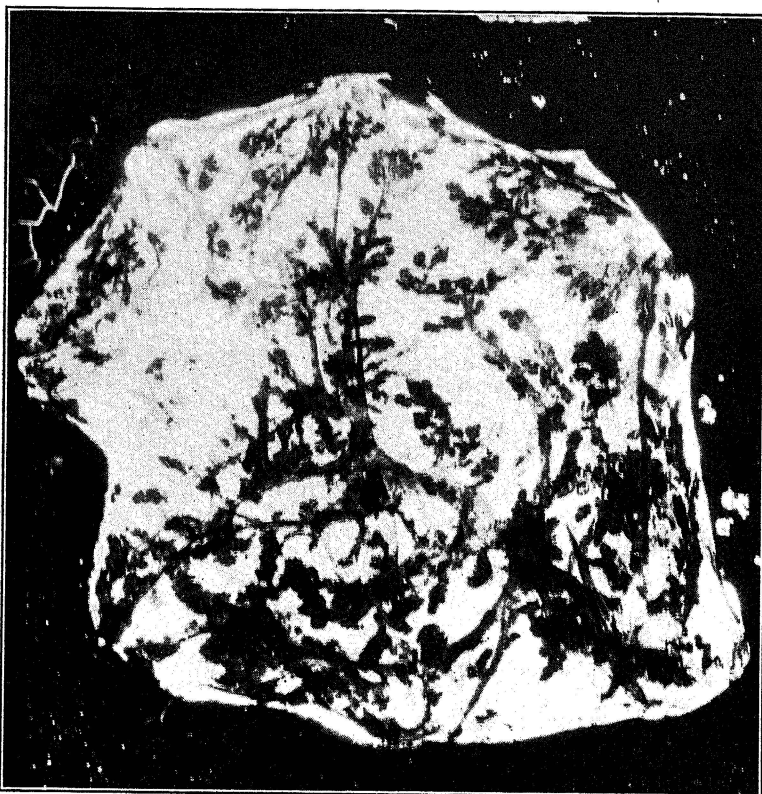


Fig. 16.—A control gland of R68 removed February 14. This gland was removed fourteen days after the rabbit had been mated with a vasectomized buck. This section is not very distinct but it can be seen that the ducts and a considerable amount of lobular tissue are present. There was no indication of the presence of any milk in the glands at this time. As the sections of glands were somewhat stretched before fixation they appear less compact than they do when observed while on the rabbit. (Enlarged $2\frac{1}{2}$ times).

able? After lactation has been initiated what part does the pituitary play in the persistency of lactation, What effect does the activity of the pituitary have in determining the milk production of various individuals? At the same time it may be asked, is there any relationship between the quantity of ovarian hormones produced and the size of the mammary glands?

Many other questions arise in this connection for which no definite answer can, as yet, be offered. At the same time there are many technical problems to overcome. What is to be the test for quantitatively comparing the amounts of the lactation-produc-

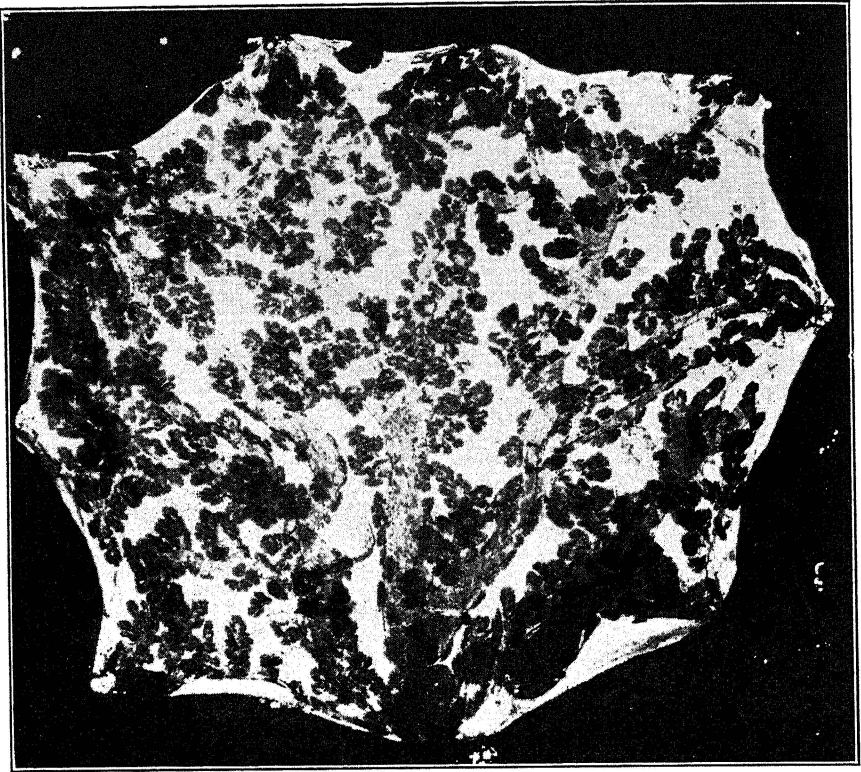


Fig. 17.—Section of gland removed from R68 on February 24. At this time the ducts were distended with milk and some of the lobules appeared to be filled with milk. Four hundred rat units of estrus-producing hormones and 5 c.c. of the corpus luteum extract had been administered daily for a period of ten days. At low magnification the alveoli can be distinctly seen. (Enlarged $2\frac{1}{2}$ times).

ing hormone in various extracts? How can the variations in the amount of the hormone produced in the same animal during different stages of development, reproduction, and lactation be determined?

In Figure 24 the various factors that have been proven to have an effect on the mammary glands, though in most cases only in experimental animals, are diagrammatically presented in the cow. The functions of the various hormones or extracts have not all been demonstrated on the cow. In the following discussion it is necessary, therefore, that certain assumptions be made. The first assumption is that there are no species differences in the function of the different endocrine gland extracts to be discussed. At the present time this is in accord with all observations.

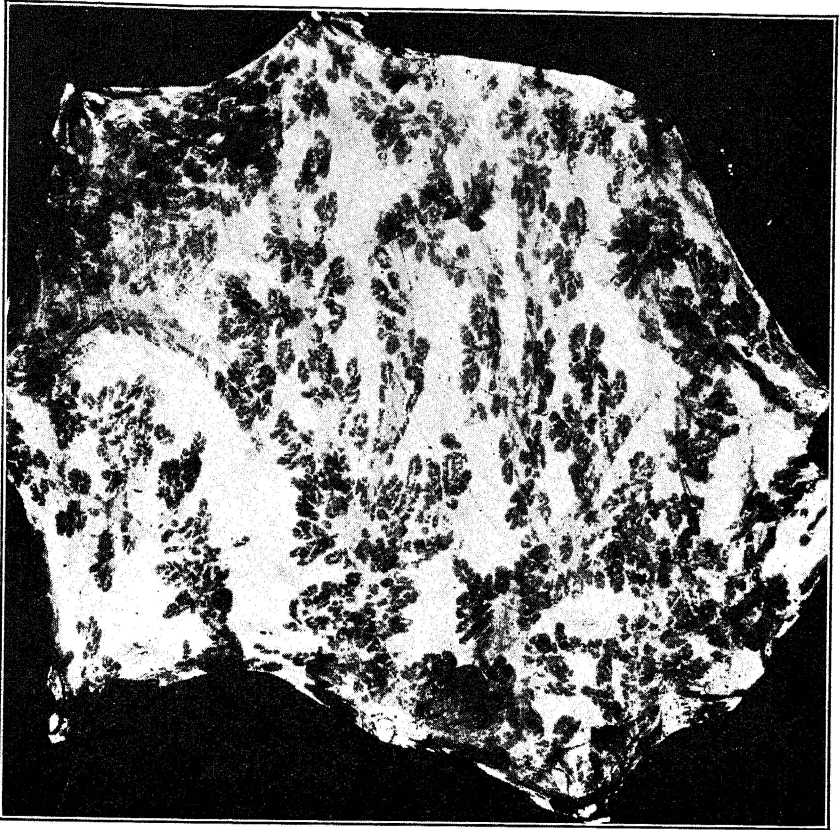


Fig. 18.—A section of mammary gland removed from R6S on March 6 after the estrus-producing hormone and the corpora lutea extract had been administered for a second ten-day period in the attempt to bring the glands into full secretion. See Fig. 17. Though milk was still present in the ducts and the alveoli were still enlarged there is evidence of a slight regression in the glands during this second period. Fig. 19 shows the glands of the same rabbit after the pituitary extract had been subcutaneously administered for a period of nine days. (Enlarged $2\frac{1}{2}$ times).

On the basis of the truth of the above assumption, what are the relationships between the pituitary, the gonads, and the mammary glands, with special emphasis placed on the latter? The following theory is advanced in the light of the present facts.

If the pituitary is the "motor of sexual function" as stated by Zondek and Aschheim (1927a) the changes in the ovaries at puberty must be initiated by the pituitary. It may then be assumed that at puberty the production of the growth-stimulating hormone of the pituitary declines to some extent and the pituitary then be-

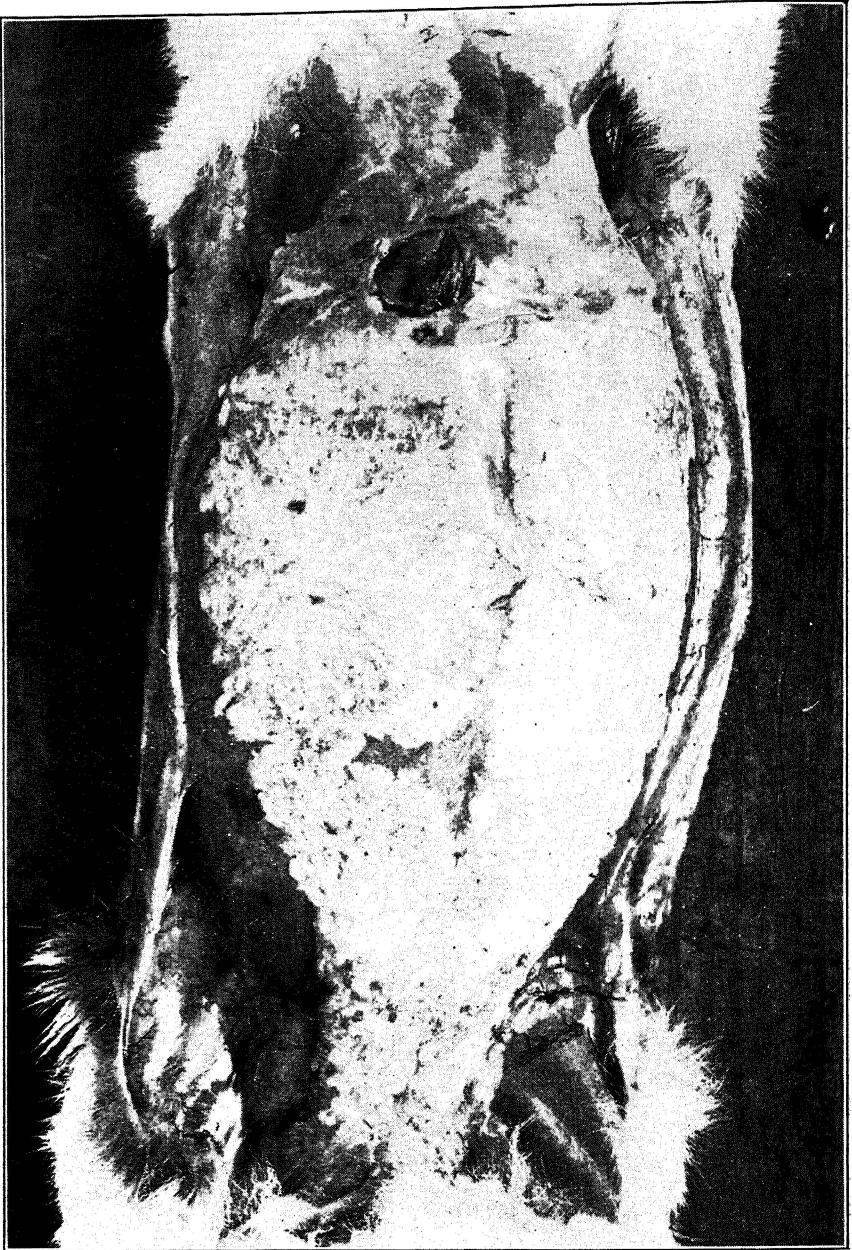


Fig. 19.—The exposed mammary glands of R68 after an alkaline extract of sheep pituitary had been subcutaneously injected for nine days. The ducts are greatly distended with milk and the entire glands greatly thickened. In this rabbit a small amount of milk was present at the time the injection started. Scars marking the area from which these sections of gland have been removed can be seen.

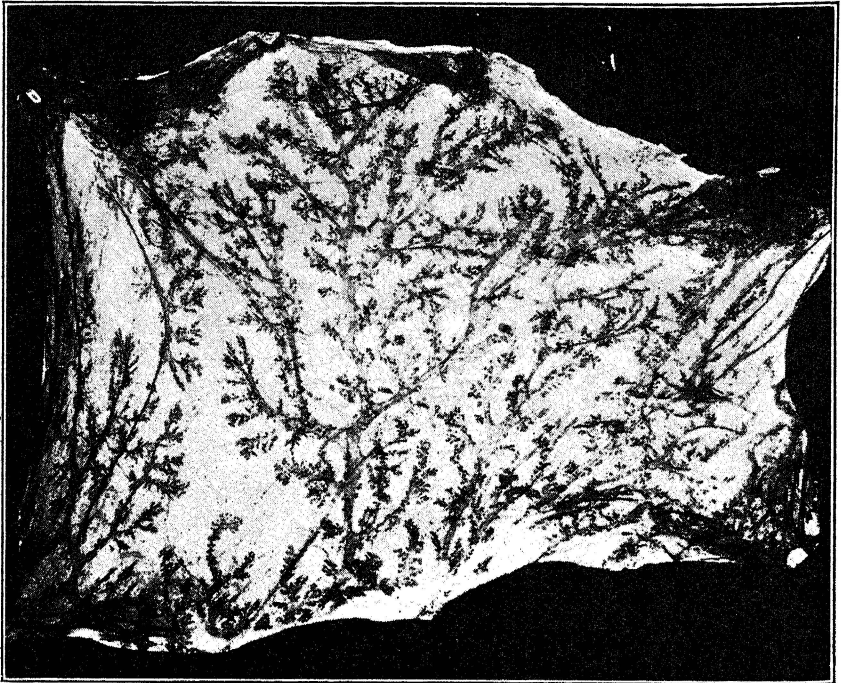


Fig. 20.—The control gland of R75 removed March 9. This gland shows a marked degree of involution, in fact it was so greatly involuted that it is difficult to distinguish it from the glands of virgin rabbits. Examination of the section under magnification, however, shows that the gland was completely developed though the lobules were greatly shrunken. A highly magnified section of a gland of the same rabbit after the pituitary extract had been subcutaneously injected for five days is shown in Figure 21. (Enlarged $2\frac{1}{2}$ times).

gins to secrete a gonad-stimulating hormone in increased amounts.

The ovarian activity produced by the gonad-stimulating hormone results in marked growth changes in the mammary glands. That this growth of the mammary glands is due to an estrus-producing hormone thought to be of ovarian origin, has been indicated by the work of Turner and Frank (1930) and Parkes (1930), who produced a similar growth of the glands in male and immaturely castrated female rabbits by injections of the estrus-producing hormone. This development during puberty is characterized by extensive growth of the duct system.

Further development of the glands apparently requires supplementary stimulation. During pregnancy this further development normally takes place. There are, it appears, two possible sources of hormones stimulating this second active growth period



Fig. 21.—A greatly enlarged section of the mammary glands of R75 removed on March 15. Though the gland was quite thick the alveoli can be quite clearly seen. The control gland removed on March 9 is shown in Fig. 20. By comparing the two photographs some idea of the extent and the rapidity of the changes produced may be obtained. The clearer streaks extending across the photograph are ducts. At the time the gland was removed these ducts were distended with milk.

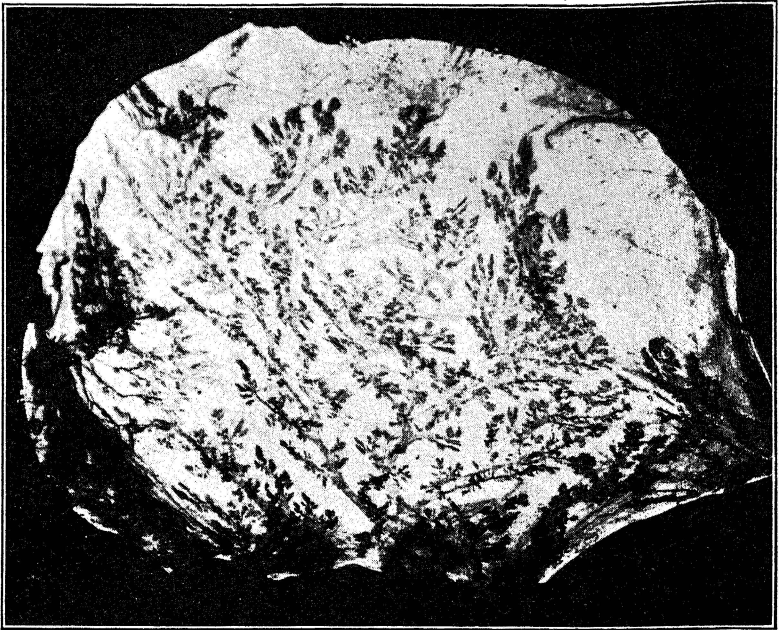


Fig. 22.—A control gland removed from R79 on March 6. This gland shows a marked involution. Though R79 died after three days of injections of the pituitary extract, the glands showed indications of rejuvenation and a thin, milk-like fluid was present in small amounts in the ducts.

of the glands. Corpora lutea are established in the ovaries and are persistent throughout the gestation period. During pregnancy the pituitary has been found to increase in size (Rasmussen 1924-1928 and Gentili 1920), and in some species, man, ape, and horse, an overproduction and excretion of the gonad-stimulating hormone has been observed during pregnancy. At the present time it seems more probable that the corpus luteum produces the secretion which, in conjunction with the estrus-producing hormone, causes this second rapid growth stage of the mammary glands (Turner and Frank 1931) and the assumption is made that the gonad-stimulating hormone is functional in causing the corpora lutea to persist.

It is, thus, concluded that the ovary supplies the stimulants producing the development of the mammary glands. The pituitary is active in the growth phase only indirectly in that it regulates ovarian function.

Lactation, however, is apparently under the direct control of the pituitary. The ovary has no direct function in the initiation of milk secretion. This might be concluded merely from the fact

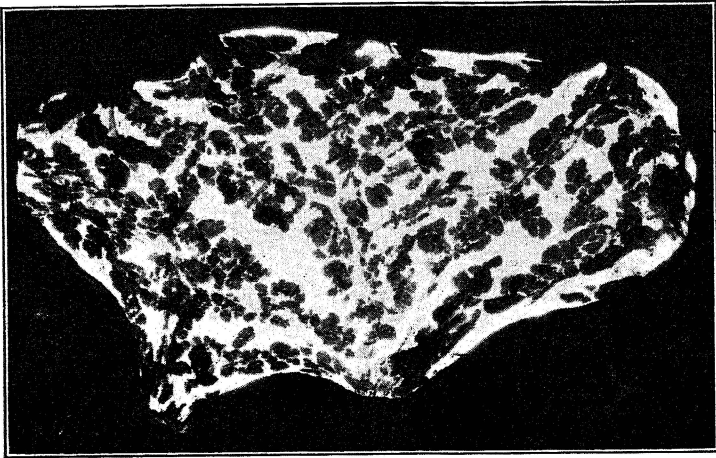


Fig. 23.—Control gland removed from RS1 on March 14. This gland is very similar to the glands resulting during normal pseudo-pregnancy. After the extracts had been used for ten days the above gland was greatly distended with a colostrum-like milk. The section was so thick that it could not be photographed. (Enlarged $2\frac{1}{2}$ times).

that lactation frequently follows castration in women. However, it has been definitely proven that the pituitary directly initiates mammary activity as a result of experiments conducted on castrated female rabbits by Stricker and Grüter (1929), Corner (1930) and Experiment IV reported in this paper.

In the normal animal there must be some factor or factors which cause the sudden release or production of the lactation producing stimulus. It might be assumed that the removal of the fetus would cause the secretion of milk particularly in the human, where milk secretion begins only following parturition. In other animals, as for example, the cow, lactation begins before parturition and the above assumption would be deficient.

As a result of Experiment II reported in this paper and the work reported by other investigators, the following theory for the initiation of lactation in the normal animal is suggested. During pregnancy there is a gradual increase in the production of the estrus-producing hormone which reaches its maximum about the time of parturition. In the cow the excretion of the estrus-producing hormone in the urine has been found to be nearly 2800 rat units per day at the time of parturition (Turner et al. 1930). Meyer et al. (1930) has reported pregnancy-like changes in pituitary activity following the administration of the estrus-producing hormone and Hartman, Firor and Geiling (1930) have ob-

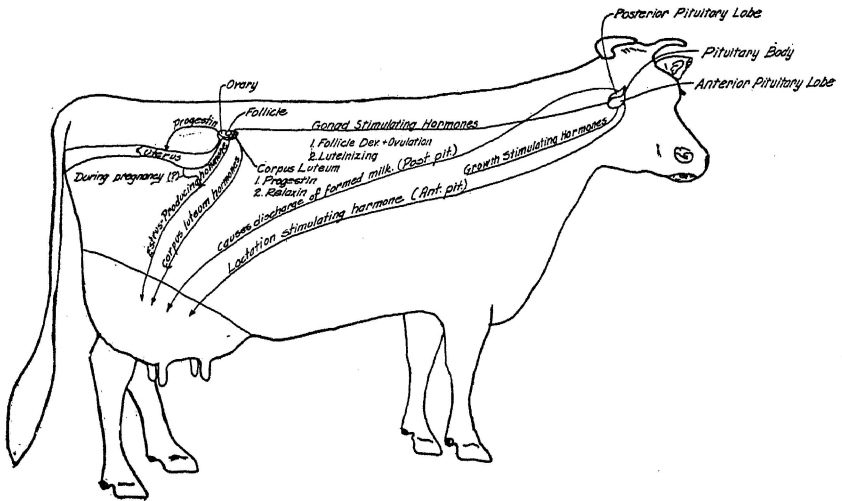


Fig. 24.—Relation of the endocrine glands to the growth and the secretory activity of the mammary gland.

served that the estrus-producing hormone acts on the pituitary of the monkey which in turn produces menstruation. It is also possible that the concentration of the estrus-producing hormone, when sufficiently great, may cause either the production or the liberation of the lactation producing hormone. Such an explanation has been suggested for the two cases of lactation observed in Experiment II, where lactation resulted following the injection of large amounts of estrus-producing hormone. The lactation reported in guinea pigs by Laquer, Borchardt, Dingmanse and De Jough (1928) and Steinach, Dohrn, Schoeller, Hohlweg and Faure (1928) may be explained on the same basis. There are experiments reported where lactation has not resulted following the injection of estrus-producing hormone even in quite large amounts (Turner and Frank 1930, and Parkes 1930), however, immaturely castrated female or male rabbits were used by Turner and Frank. Parkes reports that the administration of the estrus-producing hormone alone in doses up to 1200 mouse units per day resulted largely in the extension of the duct system of mature castrated female rabbits.

The above experiments, however, do not oppose the theory advanced. The corpus luteum extracts appear to be essential in the development of the mammary glands in castrated animals.

It has not yet been proven that lactation will result in immature rabbits even following the injection of the pituitary extracts. Experiments in progress at the present time indicate that there

is no stimulation to lactation in such cases. Thus it would scarcely be expected that lactation would be produced in the immature gland by the indirect stimulation of the estrus-producing hormone. The above theory advanced for the appearance of lactation in the normal animal is briefly as follows. When the estrus-producing hormone increases, a concentration is reached at about the time of parturition, which activates the pituitary to secrete a lactation stimulating hormone in amounts sufficient to cause the initiation of milk secretion.

SUMMARY AND CONCLUSIONS

1. Moderate doses of estrus-producing hormone obtained from the urine of pregnant cattle when administered daily over a period of twenty-one days produced no significant changes on the mammary glands of mature rabbits.
2. Large amounts (400 rat units) of estrus-producing hormone obtained from the urine of pregnant cows and the corpus luteum extract produced a slight secretion of milk when simultaneously administered. The assumption has been made that this lactation was due largely to the estrus-producing hormone which in large amounts may activate the pituitary.
3. Pseudo-pregnancy may be produced in the rabbit by intravenous injections of extracts containing too small an amount of gonad-stimulating hormone to be effective when subcutaneously administered. Pseudo-pregnancy thus induces the characteristic development of the mammary glands.
4. The mammary glands of mature castrated female rabbits have been stimulated to secretory activity by an alkaline extract obtained from sheep's anterior pituitary. The lactation was equal to that occurring following parturition.
5. The degree of involution of the mammary glands of the mature castrated female rabbits did not in any way alter the effect produced by the alkaline extract.
6. A great variation in the size of the alveoli of the glands of the experimental animals was observed. The larger alveoli were found in the proliferated glands of rabbits whose control glands had shown the existence of but a small amount of lobular tissue.
7. From the results of the experiment it appears that the stimulating substance is effective only in the activation of the secretory cells of the alveoli.
8. It appears that the initiation of milk secretion is due to a definite "lactation-producing" hormone secreted in the pituitary as has been suggested by Stricker and Grüter (1929) and Corner (1930).

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