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# The Mammogenic Hormones of the Anterior Pituitary.

## II. The Lobule-Alveolar Growth Factor

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# The Mammogenic Hormones of the Anterior Pituitary.

## II. The Lobule-Alveolar Growth Factor

JOHN P. MIXNER AND C. W. TURNER

During the past decade and a half endocrine research has come to center around the anterior hypophysis as the master gland in hormonal regulation and interrelation, especially in relation to reproduction and associated phenomena. Of the many body processes with which the anterior pituitary has been associated, those of mammary gland growth and secretion have received their due share of attention.

The investigations to be reported in this paper are the continuation of studies resulting in the discovery of a factor in the anterior pituitary, named "mammogen," which directly stimulates the growth of the mammary gland parenchyma. It is not an overstatement to say that it is now generally acknowledged that there is a pituitary factor which is vitally concerned in the direct stimulation of mammary growth. The details of this control are, however, a matter of considerable debate at the present time.

The present studies are concerned primarily with the anterior pituitary factor which directly stimulates mammary lobule-alveolar growth, and with other factors which indirectly affect alveolar growth. It was necessary to develop an assay technic for this anterior pituitary mammogenic lobule-alveolar growth factor to be used as a tool in these investigations.

The points that were given particular study include the following:

1. The determination of the role of estrogen in the stimulation of mammary growth.
2. The demonstration of the relationship of the mammogenic lobule-alveolar growth factor to other anterior pituitary hormones.
3. The determination of the relative ability of progesterone and certain progesterone-like materials to stimulate lobule-alveolar growth.
4. The influence of certain adverse conditions on the growth of the mammary lobule-alveolar system.
5. The effects of thyroxine and thyroidectomy upon mammary lobule-alveolar growth.
6. Investigation of the relative effects of progesterone and diethylstilbestrol upon the mammary glands of virgin female goats.

It cannot be claimed that complete answers have been found to the above problems, but at least a background of research has been developed which can be used as the basis for future research on these and related problems.

## GENERAL REVIEW OF LITERATURE

### A. Normal Development of the Mammary Gland

The literature on the normal growth of the mammary gland has been extensively reviewed by Turner (1939). Briefly the development of the mammary glands of the female from birth to postparturient lactation may be divided into the following periods: (1) birth to puberty; (2) puberty; (3) pregnancy; and (4) lactation.

At birth the mammary glands of the typical mammal consists of the primary duct from which secondary ducts have sprouted. From birth to puberty very little mammary growth occurs, although there may be a slight extension of the secondary ducts. During puberty with recurring estrous cycles considerable development of the mammary duct tree takes place. In certain species such as the dog in which a long luteal phase follows ovulation, mammary gland development similar to that which occurs during pregnancy takes place. Complete growth of the mammary lobule-alveolar system takes place during the first half to two-thirds of pregnancy. During the last third of pregnancy there is considerable swelling of the gland with secretion, but this is not accompanied by much actual proliferation of glandular tissue. Considerable confusion in the literature on mammary growth has arisen because of a lack of appreciation of this fact. Increase in the size of the gland due to the onset of secretion has been mistakenly called growth. Investigations also indicate that in the absence of pregnancy there is very little, if any, new growth during lactation, only a maintenance of existing structures.

This paper is concerned with the factors which are responsible for the growth of the mammary gland during the first two-thirds of pregnancy. Such growth will be spoken of as mammary lobule-alveolar growth. This is in contrast to mammary duct growth which occurs primarily during the estrous cycles of puberty.

### B. Experimental Development of the Mammary Gland with Anterior Pituitary Materials

1. **Positive Results.**—The early experiments on the growth of the mammary gland with pituitary materials are complicated by the fact that lactation was usually simultaneously initiated. It is very difficult to observe whether growth has been stimulated when lactation is also initiated. It is doubtful in most of these early experiments if true hyperplasia of the gland was caused rather than a hypertrophy due to secretion, especially since lactogenic extracts were usually employed.

The pioneer experiment on the growth of the mammary gland with pituitary materials was reported by Corner (1930).\* Young sexually mature castrate rabbits were injected for fourteen days

\*For list of references, see page 56.

with an alkaline extract of sheep anterior pituitary. He stated that a growth of the mammary gland was secured which was scarcely distinguishable from that seen at full pregnancy.

Nelson and Pffner (1931) similarly claimed that they secured full mammary development in castrate rats given an extract of the anterior pituitary plus an extract of the corpus luteum, the corpus luteum extract being ineffective alone.

Asdell (1931) also reported that spayed virgin female rabbits were brought into full mammary development with alkaline extracts of the anterior pituitary.

Using the ovariectomized mouse as an experimental animal, Bradbury (1932) reported that he secured alveolar development of their mammary glands with pituitary extracts.

Catchpole and Lyons (1933) also reported securing extensive duct and alveolar development in spayed female rabbits given daily injections of anterior pituitary extracts.

Later, Lyons (1936) claimed that he secured alveolar proliferation of mammary glands of a male rabbit pretreated with estrogen and then injected with a lactogenic extract. Similar results were reported with mature non-ovulated ovariectomized rabbits.

Nelson and Tobin (1936) were not able to secure any evidence of mammary growth in hypophysectomized rats with a purified lactogenic extract plus estrogen, but using a crude pituitary extract a well developed gland was reported as being obtained.

Gomez, Turner and Reece (1937) secured the first evidence that estrogen may cause the increased secretion by the pituitary of a factor which directly stimulates the mammary gland. They reported that the mammary glands of hypophysectomized guinea pigs could be stimulated to growth by the implantation of pituitaries from estrogen-injected donor rats. If the donor rats were not injected with estrogen, their pituitaries were ineffective in causing mammary growth.

Of course it is well established that in most species of mammals estrogen will cause the development of a mammary duct system in the normal or castrate animal while progesterone plus estrogen is necessary to secure complete growth of the mammary gland. As to the necessity of a pituitary factor to explain mammary growth, the classical experiment has involved the injection of estrogen or a combination of estrogen and progesterone into hypophysectomized animals. If positive results were secured, it was claimed that it was unnecessary to postulate the mammary stimulating action of the pituitary. Conversely, if negative results were secured, the possible activity of the pituitary in stimulating mammary growth was considered. The literature on this subject is both long and controversial, and it has been adequately reviewed by Turner (1939), Lewis and Turner (1939), and Riddle (1940).

However, the first of a series of careful studies on the necessity of the intact pituitary to secure mammary growth with estrogen or estrogen and progesterone was reported by Reece, Turner and Hill (1936). Other reports soon followed (Gomez and Turner, 1936) and Gomez, Turner, Gardner and Hill (1937). These studies showed uniformly that the ovarian hormones were ineffective in causing mammary growth in the hypophysectomized animal. Selye, Collip and Thompson (1935) had concluded that because estrogen failed to maintain the mammary glands of six-day lactating hypophysectomized rats, a pituitary factor must be involved in mammary growth. This can hardly be considered a critical experiment because estrogen has not been shown to maintain the lactating mammary glands of non-suckled normal animals.

These hypophysectomy studies plus the work demonstrating the effectiveness of pituitary material in the castrate or hypophysectomized-castrate animal led Gomez and Turner (1937, 1938a) to postulate that estrogen stimulates the pituitary to secrete an increased amount of a mammogenic factor which causes duct growth. Estrogen plus progesterone was likewise believed to stimulate the pituitary to secrete an increased amount of a factor which causes lobule-alveolar growth. These pituitary factors were named mammogen duct growth factor and mammogen lobule-alveolar growth factor.

Further evidence for the presence of mammogenic hormones in the anterior pituitary rapidly accrued.

Fresh anterior pituitary tissue from pregnant cattle caused the development of both ducts and alveoli in castrate rats. Anterior pituitary tissue from non-pregnant cattle was ineffective. Also fresh or acetone-dried anterior pituitary tissue from pregnant cattle caused complete development of mammary glands of ovariectomized rabbits while rabbits receiving injections of non-pregnant pituitary tissue showed no growth (Gomez and Turner, 1938a).

Using the male mouse as an assay animal Gomez and Turner (1938b) also showed that estrogens in the form of anol, estradiol or a combination of the two greatly increased the duct growth promoting potency of donor rabbit pituitaries, indicating again that estrogen stimulates the pituitary to the increased production of a mammogenic factor.

Nelson (1938a) was also able to confirm the presence of a mammogenic factor in the pituitary, securing duct and alveolar growth in hypophysectomized rats given an unfractionated pituitary extract plus estrogen. When pituitaries from estrogen-injected and normal rats were injected into hypophysectomized female rats, he observed no superiority of the estrogen-injected over the control pituitaries (1938b, 1939) thus failing to confirm the observation of Gomez and Turner (1938b) that estrogen will increase the pituitary mammogen content of donor animals.

In a similar series of experiments Reece and Leonard (1939) also were unable to detect any superiority of estrogen-injected pituitaries over control pituitaries when injected into hypophysectomized rats. The failure of Nelson, and Reece and Leonard to secure superior results with estrogen-injected donor rat pituitaries over controls is probably due to the insensitiveness of the hypophysectomized rat as compared for instance to the male mouse.

Using the male mouse mammogenic duct growth factor assay (Fig. 1), Lewis and Turner (1939) reported an extensive series of studies on the mammogen content of various types of cattle pituitaries. With adequate amounts of pituitary material available for assay and using a sensitive assay animal, it was found that varying amounts of mammogen were present in pituitaries of all types of animals, but that the concentration varied with the physiological state of the animal. The studies indicate the close correlation between normal mammary growth and pituitary content of mammogen. They also found that by injecting male rabbits with estrone, the mammogen content of their pituitaries could be raised to double that found in pregnant rabbits.

More recently Astwood (1941) has reported that whole pituitary powder caused lobule-alveolar proliferation of the mammary glands of pseudopregnant hypophysectomized rats.

Reece and Leonard (1941) stated that an anterior pituitary preparation containing growth hormone caused slight development of the mammary glands of hypophysectomized male rats while the extract plus estrogen was more effective.

Using a purified lactogenic extract (35 I. U. per mg.) plus estrogen, Gardner and White (1941) secured slight duct growth in hypophysectomized male mice.

Mammary gland growth in hypophysectomized rats was also secured by Greep and Stavely (1941) with anterior pituitary tissue desiccated *in vacuo*.

Gardner and White (1942) obtained rather poor mammary growth in hypophysectomized male mice with estrogen plus a commercial or a purified lactogenic extract and rather good mammary growth with estrogen plus either a saline extract of pituitary or an adrenotropic hormone fraction.

Gomez (1942) reported that duct growth was obtained in hypophysectomized male and female guinea pigs by implanting or injecting macerated fresh anterior pituitary tissue of adult male or female guinea pigs. The minimal effective dosage was 20 mg. Slight mammary growth was also obtained with a lactogenic extract. Growth was improved by using estrogen plus the lactogenic extract.

The daily injection of 300 micrograms of testosterone propionate plus 0.5 cc. growth hormone extract for fifteen days caused lobule-

alveolar growth in spayed hypophysectomized rats. The testosterone propionate alone was ineffective (Reece and Leonard, 1942).

Lyons, Simpson and Evans (1942) secured minimal lobule-alveolar growth in hypophysectomized rats with 60 I. U. of lactogen plus 10 I. U. of estrone (or F. S. H.) daily for ten days. Excellent lobule-alveolar growth was secured in hypophysectomized rats given daily injection of 10 I. U. of estrone plus an anterior pituitary extract containing adrenotropic, growth as well as lactogenic hormones.

These reports of mammary duct and alveolar growth in castrate, hypophysectomized and castrate-hypophysectomized animals support the claim of Gomez and Turner (1937, 1938a) that there is a definite pituitary mammogen which directly stimulates mammary growth.

**2. Negative Results.**—There are a considerable number of reports which indicate that mammary growth was not obtained with pituitary materials. In reviewing these reports it was evident that the majority of them were experiments on lactation, the mammary observations being rather incidental. Also in most instances the authors concerned have later reported experiments in which positive results have been secured with pituitary materials. Many negative results have been reported so because of the great difficulty of judging mammary growth occurring simultaneously with mammary secretion.

## EXPERIMENTAL SECTION

As a result of experimental work already reviewed, Gomez and Turner (1937, 1938a) postulated that the ovarian hormone, estrogen, stimulates the pituitary to secrete an increased amount of a mammo-genic duct growth factor, while the ovarian hormones, progesterone and estrogen, stimulate the pituitary to secrete an increased amount of a mammo-genic lobule-alveolar growth factor. These pituitary factors directly stimulate mammary gland growth. Lewis, Turner and Gomez (1939) developed an assay technic for the mammo-genic duct growth factor (Fig. 1). In the following section are related the experiments resulting in the development of an assay method for the mammo-genic lobule-alveolar growth factor.

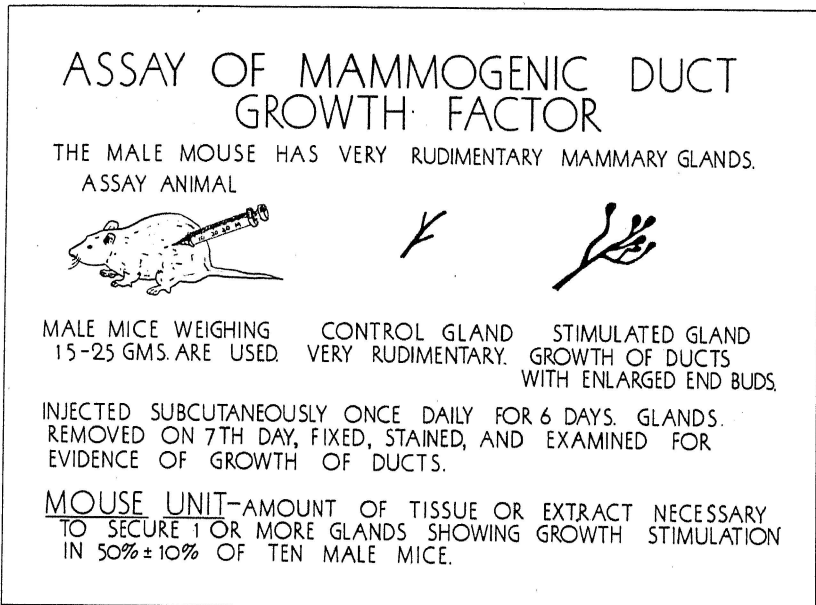


Fig. 1.—The assay of the anterior pituitary mammo-genic duct growth factor.

#### A. Development of an Assay Method for the Mammo-genic Lobule-Alveolar Growth Factor

1. **Assay Animal.**—The ovariectomized, virgin female mouse was selected tentatively as an assay animal because of its uncomplicated type of duct system (Turner and Gomez, 1933), its ability to respond to pituitary preparations with alveolar growth (Bradbury, 1932), and its small size, cheapness and ease of handling in large numbers. A photomicrograph of a mammary gland of a normal virgin female

mouse is shown in Fig. 2. It was observed that the first easily detectable signs of alveolar formation in pregnant mice consisted of the proliferation of numerous side stubs and flowery ends on

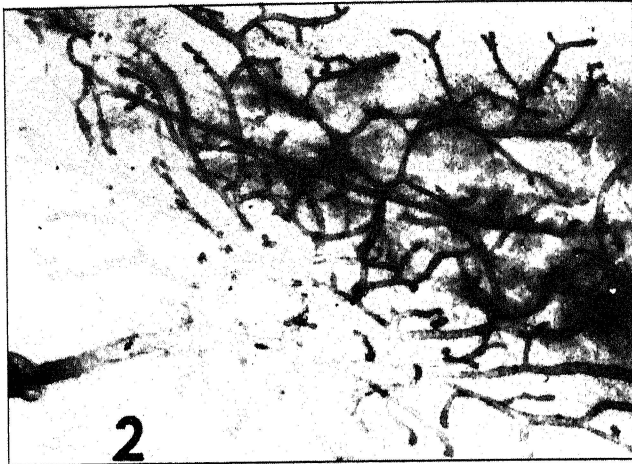


Fig. 2.—Whole mount of a check mammary gland from a normal virgin female mouse (14x).

short interlobular ducts. This stage was taken as the criterion of minimal alveolar development for positive results in the assay method (Fig. 3), and it is similar to the condition present at four

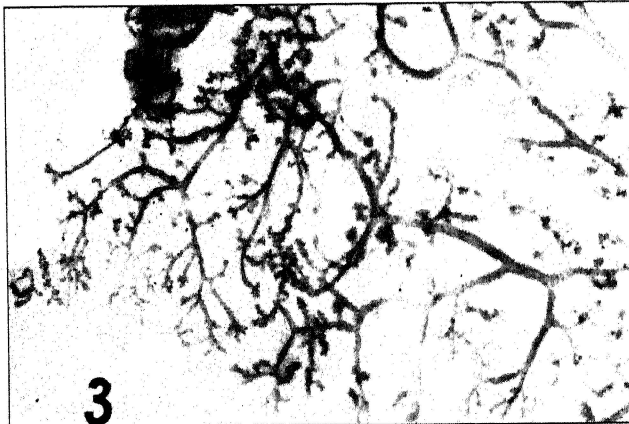


Fig. 3.—Whole mount of a mammary gland of a young spayed virgin female mouse injected with one mg. of progesterone and 133 I.U. of estrone over a ten-day period showing minimal alveolar development required for assay response (14x).



days of pregnancy. It is not present in normal female mice which have not been pregnant or pseudopregnant. Much greater alveolar development, however, is obtained at times (Fig. 4).

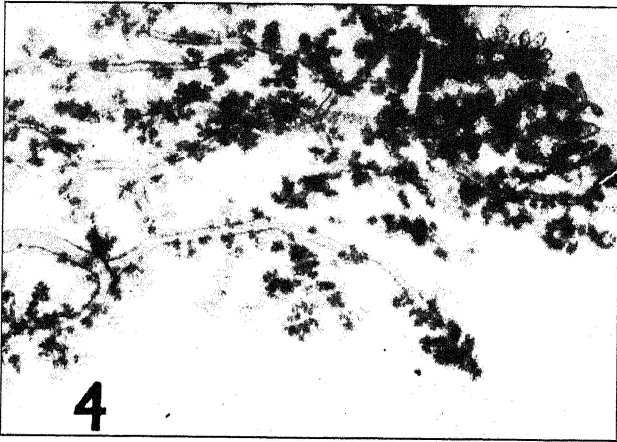


Fig. 4.—Whole mount of a mammary gland of a young spayed virgin female mouse injected for ten days with a total of 400 mg. of fresh anterior pituitary tissue secured from non-pregnant lactating cattle showing a more advanced development of the alveolar system than is required for assay response (14x).

In these studies check mammary glands were removed from all assay mice at the time of ovariectomy. Any development of the gland beyond a smooth duct system was cause for rejection of the mouse. A study of the relation of body weight of over 2000 mice to the rejection percentage was made (Fig. 5). It will be seen that if animals eighteen grams and under are selected, the number which must be discarded due to the presence of alveolar growth will be minimal.

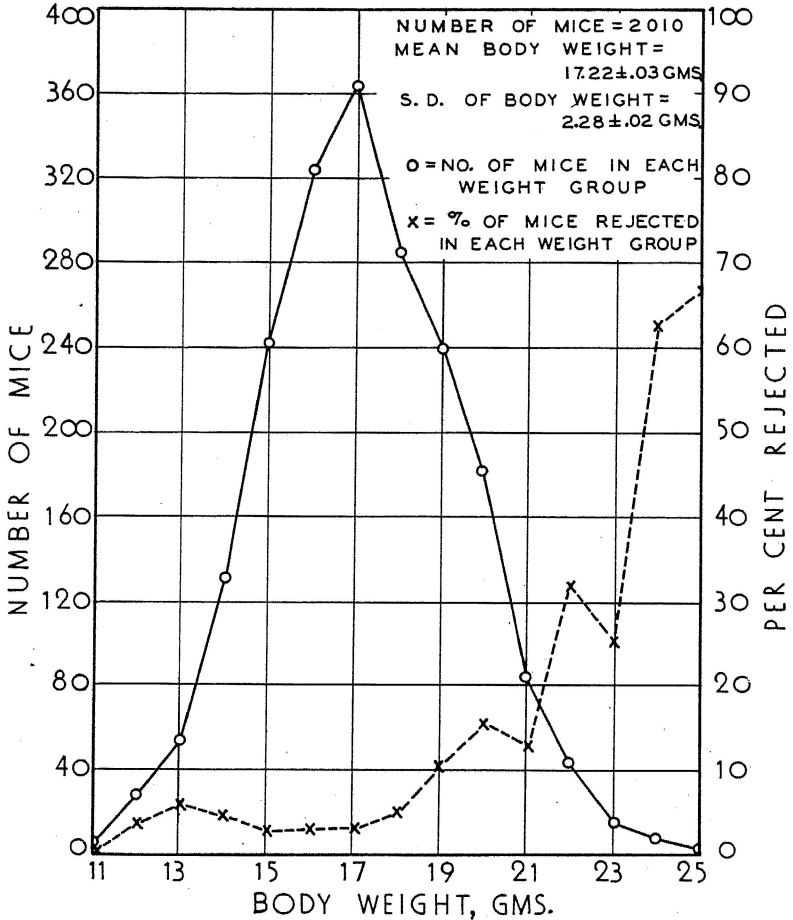


Fig. 5.—Relation of body weight to rejection percentage in virgin female mice. The percentage rejected because of lobule-alveolar development is fairly low (3-6 per cent) up to 18 gm., above which weight the rejection percentage increases sharply. Thus 18 gm. may be set as the upper weight limit for assay mice.

**2. Methods and Materials.**—The fresh cattle pituitaries used in these experiments were secured from Swift and Company of Kansas City. They were shipped in a frozen condition. The meninges and posterior lobes were removed and then the anterior lobes ground four times through a fine meat chopper, refrozen and stored in sealed jars at a low temperature. Tissue for fresh injection was weighed out of the storage jars, mashed through an eighty-mesh screen with the assistance of a little water, diluted to a concen-

tration of about 500 mg. per ml. of water and refrozen until it was ready for use. This stock material was diluted with water for injection purposes such that not over two-tenths ml. of liquid suspension was injected per mouse per day, preferably one-tenth ml.

The mice were ovariectomized through an incision over the right abdominal mammary gland so that a mammary gland could be taken at the same time, as a check on the condition of mammary growth. A triangular piece of the subcutaneous fatty tissue fanning out laterally from the teat contains the mammary gland. This tissue containing the mammary gland was dissected out, pinned on a flat cork and treated as described below for the entire glands.

Unless otherwise stated, all injections were made once daily, and the mice killed twenty-four hours after the last injection. The mice were slit down the center of the back and their skins removed together with the subcutaneous fascia, pinned out on flat sheets of cork and fixed in Bouin's solution. The subcutaneous fascia containing the glands was scraped from the skins, washed in water, stained in Mayer's hematoxylin and the mammary glands examined with a binocular dissecting microscope while differentiating in seventy per cent acid-alcohol. Turner and Gomez (1933) describe in more detail the process of preparing and mounting mouse mammary glands for examination.

**3. Mammary Lobule-Alveolar Growth with Anterior Pituitary Preparations Alone.**—The following series of experiments were designed to determine a suitable assay method for the mammogenic lobule-alveolar growth factor of the anterior pituitary using ovariectomized virgin mice as the assay animal. Minimal development of the lobule-alveolar system was used as the criterion of positive response.

Groups of mice were injected over periods of four, five, six or eight days with graded dosages of fresh pituitary tissue secured from pregnant cattle, designated Lot 5. The data show first that lobule-alveolar responses could be secured with this fresh anterior pituitary tissue over periods of injection ranging from four to eight days, no injection period appearing to have superiority over the others (Table 1). However, there was a poor correspondence between the dosages injected and the per cent positive mammary responses.

As considerable numbers of positive alveolar responses were secured in the six-day injection period, it was decided to try new groups of mice on a standard six-day injection hoping that more uniform results could be secured than was the case in the first experiments. Anterior pituitary preparations used in these trials included an acetone-ether dried fraction of Lot 5, Lot 6 fresh pituitary from non-pregnant cattle, and Lots 7 and 8 fresh pituitary from pregnant cattle. Lobule-alveolar responses were secured with

TABLE 1. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR VARYING NUMBER OF DAYS WITH VARYING DOSAGES OF ANTERIOR PITUITARY PREPARATION

Pituitary Preparation			Lobule-Alveolar Responses			
Name	Total Dosage, Mg.	Days Injected	Number of Mice	+	-	Per Cent Positive
Lot 5, fresh anterior pituitary, pregnant cattle	75	4	4	0	4	0.0
	100	4	12	5	7	41.7
	150	4	4	3	1	75.0
	200	4	4	1	3	25.0
	75	5	2	1	1	50.0
	100	5	4	1	3	25.0
	150	5	4	2	2	50.0
	200	5	4	3	1	75.0
	50	6	4	0	4	0.0
	60	6	4	0	4	0.0
	70	6	4	0	4	0.0
	75	6	4	3	1	75.0
	80	6	6	1	5	16.7
	90	6	6	0	6	0.0
	100	6	22	6	16	27.3
	110	6	12	1	11	8.3
	120	6	18	6	12	33.3
	130	6	9	0	9	0.0
	135	6	5	5	0	100.0
	150	6	9	5	4	55.6
165	6	6	6	0	100.0	
180	6	7	6	1	85.7	
200	6	5	3	2	60.0	
210	6	6	1	5	16.7	
285	6	6	6	0	100.0	
50	8	4	0	4	0.0	
75	8	4	0	4	0.0	
100	8	4	2	2	50.0	

the Lot 5 acetone-ether dried fraction, indicating that the mammo-genic factor is proteinaceous in nature (Table 2). No mammary growth was secured with Lot 6 fresh pituitary from non-pregnant cattle even in doses as high as 600 mg. showing that non-pregnant pituitaries are low in this factor. Positive responses were secured with both Lots 7 and 8 fresh anterior pituitary from pregnant cattle. In these series of experiments, as in the last, the dosages of extract injected corresponded poorly with the per cent positive mammary responses.

In a further attempt to secure better correspondence between the dosage of pituitary preparation injected and the per cent

TABLE 2. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR SIX DAYS WITH ANTERIOR PITUITARY MATERIALS

Pituitary Preparation Name	Total Dosage, Mg.	Days Injected	Number of Mice	Lobule-Alveolar Responses		
				+	-	Per Cent Positive
Lot 5, acetone- ether dried anterior pituitary, pregnant cattle	45	6	4	1	3	25.0
	60	6	4	1	3	25.0
	75	6	4	2	2	50.0
	90	6	4	1	3	25.0
Lot 6, fresh anterior pituitary, non-pregnant cattle	200	6	4	0	4	0.0
	400	6	4	0	4	0.0
	600	6	4	0	4	0.0
Lot 7, fresh anterior pituitary, pregnant cattle	50	6	4	0	4	0.0
	100	6	4	0	4	0.0
	150	6	8	0	8	0.0
	200	6	7	0	7	0.0
	250	6	4	4	0	100.0
	280	6	4	3	1	75.0
Lot 8, fresh anterior pituitary, pregnant cattle	100	6	6	1	5	16.7
	150	6	12	4	8	33.3
	200	6	12	7	5	58.3
	250	6	12	5	7	41.7
	300	6	6	0	6	0.0

positive mammary response, other groups of mice were injected over a ten-day period using Lot 8 acetone-ether dried pituitary from pregnant cattle, Lots 8, 9, 10, and 11 fresh pituitary from pregnant cattle and Lot 12 fresh anterior pituitary from lactating cattle. Again, there was a poor relation between the dosage of material injected and the per cent positive mammary responses (Table 3).

The results of these three series of experiments showed that pituitary materials alone do have the ability to stimulate mammary lobule-alveolar growth. Of 630 mice, 144 or 22.9 per cent responded with mammary growth to pituitary injections. However, the correspondence between the doses of pituitary preparations injected and the per cent positive mammary responses was very poor, showing that some variable was still missing or uncontrolled. These data were secured in ovariectomized mice in which the normal supply of

TABLE 3. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH ANTERIOR PITUITARY MATERIAL

Name	Pituitary Preparation		Number of Mice	Lobule-Alveolar Responses		
	Total Dosage, Mg.	Days Injected		+	-	Per Cent Positive
Lot 8,	100	10	5	0	5	0.0
fresh	150	10	5	0	5	0.0
anterior	200	10	5	0	5	0.0
pituitary,	250	10	5	1	4	20.0
pregnant	300	10	5	1	4	20.0
cattle	333	10	5	5	0	100.0
Lot 8,	25	10	6	0	6	0.0
acetone-	50	10	6	0	6	0.0
ether dried	75	10	5	0	5	0.0
anterior	100	10	6	1	5	16.7
pituitary,	125	10	6	0	6	0.0
pregnant						
cattle						
Lot 9,	150	10	29	5	24	17.2
fresh	200	10	28	5	23	17.9
anterior	250	10	39	9	30	23.1
pituitary,	300	10	36	4	32	11.1
pregnant	350	10	24	8	16	33.3
cattle	400	10	21	5	16	23.8
Lot 10,	100	10	5	0	5	0.0
fresh	200	10	20	5	15	25.0
anterior	300	10	19	3	16	15.8
pituitary,	400	10	6	2	4	33.3
pregnant						
cattle						
Lot 11,	100	10	5	1	4	20.0
fresh	200	10	5	0	5	0.0
anterior	300	10	16	4	12	25.0
pituitary,	400	10	6	2	4	33.3
pregnant						
cattle						
Lot 12,	300	10	8	3	5	37.5
fresh	350	10	6	1	5	16.7
anterior	400	10	8	4	4	50.0
pituitary,						
lactating						
cattle						

endogenous estrogen is very low or entirely lacking. Consequently, it was reasoned that the endogenous estrogen might play some secondary role in mammary growth (other than stimulating a pituitary duct growth factor) which had not been appreciated.

The following section describes a series of experiments concerning a possible secondary role of estrogen in stimulating mammary gland growth.

**4. Mammary Lobule-Alveolar Growth with Anterior Pituitary Preparations Plus Estrogen.**—Several investigators have demonstrated that estrogen enhances the ability of pituitary extracts to cause mammary growth. Nelson (1938b) reported that when hypophysectomized rats received estrogen in addition to rat pituitaries, better mammary growth was secured than when rat pituitaries alone were given.

Mixner and Turner (1941a) suggested in preliminary report that estrogen enhances the activity of pituitary extracts in promoting mammary growth through its hyperemic and permeability-increasing action on the vascular system of stromal tissue surrounding the mammary gland. Further evidence that this is the case was presented in a later report (1942).

Gardner and White (1941) reported that estrogen plus a lactogenic extract was more effective in causing mammary gland growth in hypophysectomized male mice than was lactogen alone. They concluded that estrogen sensitizes the mammary gland to the action of lactogen.

Growth hormone extract alone produced slight growth of the mammary gland in hypophysectomized male rats while estrogen plus growth hormone extract was more effective (Reece and Leonard, 1941).

Gomez (1942) also secured slight mammary growth in hypophysectomized-castrate guinea pigs with a lactogenic extract. This growth was improved when estrogen was given in addition to the lactogenic extract.

Gardner and White (1942) found that estrogen plus either a commercial lactogenic extract, a more purified lactogenic extract, a saline extract of pituitary or a good adrenotropic extract gave mammary growth in hypophysectomized male mice, the pituitary hormones alone in the dosages given being ineffective.

These reports indicate that estrogen has some special enhancing effect on the activity of pituitary preparations in causing mammary growth.

*a. Experimental.* In this series of experiments groups of assay animals were injected with either of two pituitary preparations alone, while other groups received a total of 75 I. U. of estrone in addition to the pituitary preparations (Table 4). Typically poor results were secured with both Lot 13 fresh pituitary from stockyard run of cattle and an initial extract of Lot 13 when they were injected without estrogen. With 50 mg. of Lot 13 fresh pituitary plus estrone, eight out of thirteen (62 per cent) mice gave positive responses, while with 100 mg., 100 per cent of thirteen mice

gave positive responses. This is in contrast to two out of eight (25 per cent) positive responses with 400 mg. of Lot 13 fresh pituitary injected alone. Similar results were secured with the initial extract of Lot 13. With this extract at a dosage of 7.5 mg., three of six mice responded with lobule growth when estrone was also given, a 50 per cent positive response. Another group of eleven mice gave six positive responses with 7.5 mg. of extract plus estrogen while still a third group of eighteen mice gave nine positive responses, a 50 per cent positive response.

These results (Table 4) show that by the use of a small amount of estrone in conjunction with pituitary mammogenic preparations, the dosage of pituitary required for an assay response was greatly reduced as compared to pituitary extract injected without estrone. Also graded mammary responses could be secured that corresponded to the graded pituitary dosages injected. These two factors, sensitiveness of assay animals to relatively small dosages of pituitary extract and the graded positive responses of animal assay groups to graded pituitary dosages, make the use of estrogen with pituitary materials very desirable in an assay method for this mamrogenic factor.

TABLE 4. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH VARYING DOSAGES OF ANTERIOR PITUITARY MATERIAL WITH AND WITHOUT ESTRONE

Pituitary Preparation Name	Total Dosage, Mg.	Estrone, Total Dosage, I. U.	Days Injected	Number of Mice	Lobule-Alveolar Responses		
					+	-	Per Cent Positive
Lot 13, fresh anterior pituitary, stockyard run of cattle	100	-	10	6	1	5	16.7
	200	-	10	5	1	4	20.0
	300	-	10	5	1	4	20.0
	400	-	10	6	2	4	33.3
	50	75	10	13	8	5	61.5
	100	75	10	13	13	0	100.0
Lot 13, initial extract of anterior pituitary from stock- yard run of cattle	5.0	-	10	5	0	5	0.0
	10.0	-	10	5	0	5	0.0
	15.0	-	10	4	0	4	0.0
	20.0	-	10	5	1	4	20.0
	25.0	-	10	4	0	4	0.0
	50.0	-	10	4	0	4	0.0
	7.5	75	10	6	3	3	50.0
	7.5	75	10	11	6	5	54.5
	7.5	75	10	18	9	9	50.0
10.0	75	10	21	12	9	57.1	
12.5	75	10	23	20	3	87.0	
17.5	75	10	34	33	1	97.1	



Estrone, alone, injected into the assay animals in doses over five times as high as those used with the pituitary materials (Table 4) produced no signs of lobule growth, confirming a similar observation of Turner and Gomez (1934).

With the development of the use of estrogen in the assay procedure, it was desirable to reinvestigate the proper length of time for the injection period. Accordingly groups of mice were injected for four, six, eight, ten, fifteen, and twenty days with 17.5 mg. of Lot 15 acetone-ether dried anterior pituitary of pregnant cattle plus 75 I. U. of estrone. With the twenty-day group, the estrone dosage was doubled over that of the other five groups (Table 5).

TABLE 5. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR VARYING NUMBER OF DAYS WITH A CONSTANT DOSAGE OF ANTERIOR PITUITARY MATERIAL PLUS ESTRONE

Pituitary Preparation Name	Estrone			Number of Mice	Lobule-Alveolar Responses		
	Total Dosage, Mg.	Total Dosage, I. U.	Days Injected		+	-	Per Cent Positive
Lot 15, acetone- ether dried anterior pituitary from pregnant cattle	17.5	75	4	13	0	13	0.0
	17.5	75	6	13	4	9	30.8
	17.5	75	8	12	6	6	50.0
	17.5	75	10	13	9	4	69.2
	17.5	75	15	15	9	6	60.0
	17.5	75	20	15	8	7	53.3
	17.5	150	20	15	10	5	66.7

The per cent positive responses increased as the number of days injection was lengthened up to the ten-day injection period where the per cent positive responses were highest. Thus the ten-day injection period appeared to be optimal under the assay conditions.

Still another factor investigated was the effect of the length of time after the mice were ovariectomized before injections were begun, on the lobule-alveolar responses. Groups of mice were injected over a ten-day period with 20 mg. of Lot 15 acetone-ether dried anterior pituitary from pregnant cattle starting one, five, ten, twenty, thirty, forty and fifty-five days respectively after ovariectomy (Table 6). The group ovariectomized fifty-five days received 7.5 I. U. of estrone per day over the last five days of this period. Positive lobule-alveolar responses decreased in an orderly manner from 84.6 per cent for the one-day ovariectomized group to 16.7 per cent for the forty-day ovariectomized group. However, the group ovariectomized for fifty-five days and which received 7.5 I. U. of estrone per day over the last five days gave assay results comparable with the one-day ovariectomized group, showing that the effects of ovariectomy can be overcome by this procedure.

TABLE 6. THE EFFECT OF THE TIME AFTER OVARIECTOMY AND BEFORE INJECTIONS BEGAN ON LOBULE-ALVEOLAR RESPONSES

Pituitary Preparation Name	Total Dosage, Mg.	Estrone, Days			Lobule-Alveolar Responses		
		Total Dosage, I. U.	Ovariect Before Injected	Number of Mice	+	-	Per Cent Positive
Lot 15, acetone- ether dried	20	75	1	13	11	2	84.6
	20	75	5	13	9	4	69.2
anterior pituitary, pregnant cattle	20	75	10	13	7	6	53.8
	20	75	20	12	5	7	41.7
	20	75	30	13	4	9	30.8
	20	75	40	12	2	10	16.7
	20	75	55*	11	10	1	90.9

\*Last five days each mouse received 7.5 I. U. of estrone per day.

Thus from the viewpoint of the accuracy and sensitivity of the assay, the mice should be started on assay-injections immediately after ovariectomy or else be given preliminary estrogen injections.

5. **The Assay Technic.** As a result of the first six series of experiments (Tables 1-6) sufficient information was at hand to formulate an assay technic for the mammogetic lobule-alveolar growth factor. It was found that minimum mammary lobule-alveolar growth responses in ovariectomized virgin female mice (12-18 gm.) were directly proportional to the dosage of anterior pituitary prepara-

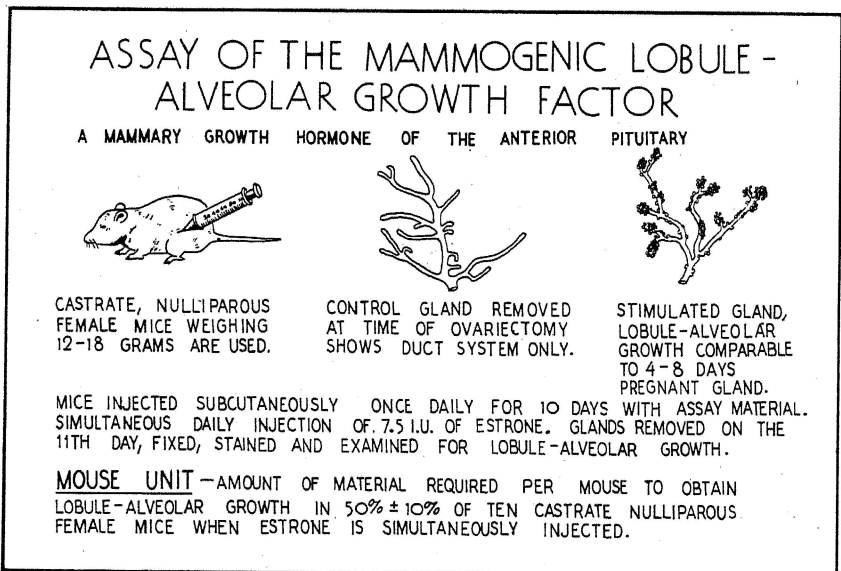


Fig. 6.—The assay of the anterior pituitary mammogetic lobule-alveolar growth factor.

tion injected if a small amount of estrone was simultaneously injected. The highest percentage of positive responses was secured with a given dose of pituitary preparation and estrone in a ten-day injection period. Also it was found that assay mice should be started on injections immediately after ovariectomy in order that accurate and sensitive assay results may be secured.

Accordingly, an assay technic was formulated (Fig. 6). A mouse unit of the mammogenic lobule-alveolar growth factor is defined as the amount of material required per mouse injected over a period of ten days to obtain minimal mammary lobule-alveolar growth in  $50 \pm 10$  per cent of ten or more castrate nulliparous female mice when a total of 75 I. U. of estrone is simultaneously injected.

The role that estrogen apparently played in the stimulation of mammary lobule-alveolar growth in these experiments seemed especially significant. Therefore a study was made in an effort to discover the means by which estrogen exerted this special effect in lobule growth.

## **B. The Role of Estrogen in the Stimulation of Mammary Lobule-Alveolar Growth**

**1. Review of Literature.**—An extensive literature has developed in regard to the synergism of estrogen and progesterone in their actions on the various sexual processes with which they are associated. Corner and Allen (1929), Hisaw and Leonard (1930), Allen (1930, 1932), and Leonard, Hisaw and Fevold (1932), as well as many others, have demonstrated this synergism on the growth of the endometrium.

Weichert (1928) demonstrated that a "1-2" relationship exists between estrogen and progesterone in causing the decidual reaction in the uterus of the rat and guinea pig.

Dempsey, Hertz and Young (1936) and Hertz, Meyer and Spielman (1937) showed that female guinea pigs pretreated with estrogen and injected with progesterone displayed the copulatory reflex when the appropriate stimulus was applied.

Turner (1939) reviewed a voluminous literature showing that in most species of animals a combination of estrogen and progesterone is necessary to secure complete mammary growth, estrogen or progesterone alone being insufficient at the dosage level administered. Lyons and McGinty (1941) and Scharf and Lyons (1941) have more recently demonstrated the quantitative relationships existing between progesterone and estrogen in stimulating mammary gland growth in the male rabbit. On the other hand, Selye (1940a, 1940b), Selye, Borduas and Masson (1942) and Reece and Bivens (1942) have reported that progesterone alone in high dosages will cause complete mammary growth in ovariectomized rats. Hartman and Speert (1941) made a similar observation in ovariectomized monkeys.

The review and experimental data presented in the previous section shows that estrogen also enhances the ability of pituitary preparations to stimulate mammary growth.

In attempting to further determine the quantitative and qualitative relationships existing between estrogen and progesterone in causing alveolar growth, the following experiments were conducted.

**2. Experimental.**—In these experiments progesterone and a progesterone-like compound, pregneninolone, were substituted for the pituitary preparation in the general framework of the assay method as described in the preceding section. Both pituitary preparations and progesterone-like compounds may be assayed for their ability to stimulate lobule-alveolar growth using this assay method.

*Estrone constant, progesterone varied.*—In the first experiment groups of mice were injected with a constant amount of estrone over the ten-day assay period while the amount of progesterone was varied in different groups from 25 to 1500 micrograms (Table 7). It will be seen that an assay dose of progesterone (plus 133 I. U. of estrone) was approximately 875 micrograms, the responses being directly proportional to the dose of progesterone injected.

TABLE 7. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH VARYING AMOUNTS OF PROGESTERONE AND A CONSTANT AMOUNT OF ESTRONE

Progesterone, Total Dosage, Micrograms	Estrone, Total Dosage, I. U.	Number of Mice	Lobule-Alveolar Responses		
			+	-	Per Cent Positive
25	133	4	0	4	0.0
50	133	4	0	4	0.0
125	133	4	0	4	0.0
250	133	4	0	4	0.0
500	133	16	4	12	25.0
750	133	12	5	7	41.6
1000	133	10	6	4	60.0
1250	133	12	10	2	83.3
1500	133	3	3	0	100.0

*Estrone varied, progesterone constant.*—In the second experiment the progesterone was kept constant at one mg., slightly above the assay dose, while the estrone was varied in amount for the ten-day period from 10 I. U. to 2400 I. U. per mouse (Table 8). In the range of estrone injections of 40 I. U. to 133 I. U., optimal synergism was observed. Below 40 I. U. the estrone was insufficient

for optimal synergism with one mg. of progesterone. When the estrone was increased to 1200 and 2400 I. U. respectively, the synergism was again reduced. The estrone appeared to override the progesterone activity in these cases such that little or no mammary growth resulted.

TABLE 8. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH A CONSTANT AMOUNT OF PROGESTERONE, BUT VARYING AMOUNTS OF ESTRONE

Progesterone, Total Dosage, Micrograms	Estrone, Total Dosage, I. U.	Number of Mice	Lobule-Alveolar Responses		
			+	-	Per Cent Positive
1000	10	10	2	8	20.0
1000	40	10	6	4	60.0
1000	70	11	7	4	63.6
1000	100	11	6	5	54.5
1000	133	10	6	4	60.0
1000	1200	9	3	6	33.3
1000	2400	9	0	9	0.0

*Progesterone alone.*—Graded doses of progesterone alone were given to groups of assay animals in the third experiment (Table 9). The results demonstrate quantitatively the greatly increased amount of progesterone required to secure mammary lobule-alveolar growth when estrogen is not given in conjunction with the progesterone. The assay animals were ovariectomized about ten days previous to the start of progesterone injections so that endogenous estrogen was largely eliminated from their system. Approximately six times as much progesterone alone was required to secure a unit response as was required in the first experiment (Table 7) when estrone was injected with the progesterone.

TABLE 9. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH VARYING AMOUNTS OF PROGESTERONE ALONE

Progesterone, Total Dosage, Mg.	Number of Mice	Lobule-Alveolar Responses		
		+	-	Per Cent Positive
3.33	24	2	22	8.3
4.67	24	5	19	20.8
6.00	26	15	11	57.7
7.33	24	17	7	70.8

*Pregneninolone with and without estrone.*—It was interesting to determine whether a compound related to progesterone in its activity had a similar synergism with estrogen. This compound, pregneninolone (anhydro-hydroxy-progesterone or ethinyl testosterone), known to have progesterone-like activity (Emmens and Parkes, 1939), was injected both with and without estrone in groups of mice (Table 10). Pregneninolone with estrone required about two mg. for an assay response while pregneninolone alone required about ten mg. This ratio of one to five of pregneninolone with and without estrone compares very closely to the one to six ratio of progesterone with and without estrone.

TABLE 10. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH VARYING AMOUNTS OF PREGNENINOLONE WITH AND WITHOUT ESTRONE

Pregneninolone, Estrone,			Lobule-Alveolar		
Total	Total	Number	Responses		
Dosage,	Dosage,	of Mice	+	-	Per Cent
Mg,	I. U.				Positive
1.0	133	12	1	11	8.3
1.5	133	12	3	9	25.0
2.0	133	12	7	5	58.3
2.5	133	10	9	1	90.0
5.0-10.0	133	12	12	0	100.0
2.5	-	4	0	4	0.0
5.0	-	6	0	6	0.0
7.5	-	8	2	6	25.0
10.0	-	12	5	7	41.7
12.5-20.0	-	16	16	0	100.0

These experiments are especially interesting and significant when compared to the experiments (Tables 1-6) with pituitary mammary growth preparations with and without estrone. It appears that the pituitary factor which directly stimulates alveolar growth needs the accompanying action of estrogen to the same extent as does progesterone, the indirect stimulator of alveolar growth.

It is interesting to note that 2400 I. U. of estrone was able to completely inhibit the normal unit response of one mg. of progesterone (Table 8). It is probable that the pituitary was adversely affected by the large dose of estrone. In Table 11 is presented a similar experiment with a unit of pituitary material, given in one case with 75 I. U. of estrone and in the other with 2400 I. U. of estrone. Due to the direct action of the injected mammogenic factor, the large dose of estrone was unable to inhibit the normal unit response of the pituitary extract.

TABLE 11. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH A CONSTANT AMOUNT OF ANTERIOR PITUITARY MATERIAL AND A HIGH AND LOW AMOUNT OF ESTRONE

Pituitary Preparation Name	Total Dosage, Mg.	Estrone, Total Dosage, I. U.	Number of Mice	Lobule-Alveolar Responses		
				+	-	Per Cent Positive
Lot 15, acetone- ether dried anterior pituitary, pregnant cattle	15	75	14	8	6	57.1
	15	2400	12	7	5	58.3

*Substitution of other estrogens for estrone.*—Both estradiol benzoate and diethylstilbestrol may replace estrone as an estrogen in conjunction with progesterone to secure mammary lobule-alveolar growth (Table 12). Without doubt other estrogens as well could be substituted satisfactorily for estrone.

TABLE 12. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED FOR TEN DAYS WITH A CONSTANT AMOUNT OF PROGESTERONE BUT WITH DIFFERENT ESTROGENIC COMPOUNDS

Progesterone Total Dosage, Mg.	Estrogenic Compounds		Number of Mice	Lobule-Alveolar Responses		
	Name	Total Dosage, Micrograms		+	-	Per Cent Positive
1	Estrone	7.5	9	5	4	55.5
1	Estradiol Benzoate	5.0	11	6	5	54.5
1	Diethylstilbestrol	25.0	12	7	5	58.3

*Ovariectomy and estrogen on mammary glands.*—From Table 6 it is seen that mammary lobule-alveolar responses decreased as a result of delaying the start of pituitary injections after ovariectomy. It was interesting, therefore, to compare the appearance of mammary glands and the surrounding stromal tissue of a group of mice ovariectomized 62 days with another group also ovariectomized 62 days, but injected for the last five days with a total of 37.5 I. U. of estrone.

The mammary gland fat pads of the two groups of mice were examined after the mice were skinned. The group of animals ovariectomized but not receiving estrogen had white fat pads in

which the mammary glands were located, indicating poor vascularity of the gland. End buds were lacking on the mammary glands and the ducts were thin and regressed to some extent. The uteri of these animals were also atrophic as would be expected. The estrogen-treated group of ovariectomized mice, on the other hand, showed a deep pink coloring of the stromal tissue surrounding the mammary glands, indicating good vascularity. Also the mammary glands showed developing end buds and normal ducts. The uteri of these animals were hypertrophied.

These observations taken in conjunction with the other experiments involving the need of estrogen with pituitary materials or progesterone in promoting mammary lobule-alveolar growth, suggested the means by which estrogen enhances mammary growth.

**3. Discussion.**—Lyons and McGinty (1941) and Scharf and Lyons (1941) conducted experiments on mammary gland growth in male rabbits and determined that one mg. of progesterone synergized best with about 240 I. U. of estrone. These results are in accord generally with those recorded in Tables 7 and 8. Apparently the range of estrogen that will synergize with a given dosage of progesterone is quite wide, but an estrogen dose which is too high or too low will adversely effect mammary gland growth as shown by data recorded in Table 8. Gardner (1941a) was able to inhibit mammary gland growth with large amounts of estrogen in male mice, immature female and male monkeys, and female dogs. As seen in Table 8 of this paper, 2400 I. U. of estrone inhibited completely the activity of one mg. (one mouse unit) of progesterone, but this same estrone dosage was unable to inhibit the activity of fifteen mg. (one mouse unit) of a pituitary extract (Table 11). It would appear from this comparison that the inhibitory action of estrogen on progesterone is mediated through the pituitary, the high estrogen dosage causing the pituitary to be unresponsive to the progesterone stimulation. As shown by the experiment with pituitary plus high estrogen dose (Table 11), estrogen does not inhibit the capacity of the mammary glands as end organs to respond.

Progesterone alone (Table 9) and pituitary materials alone (Tables 1-6) caused mammary lobule-alveolar growth. Much smaller quantities of these materials were necessary if estrogen was supplied (Tables 4, 7). This indicates that estrogen has some common action with both progesterone and pituitary preparations in promoting lobule-alveolar growth. It appears that this action is not mediated through the pituitary, but rather at the site of mammary growth.

A theory that explains this activity of estrogen is that estrogen acts directly on the stromal tissue surrounding the mammary gland producing an increased hyperemia and vascularity associated with an increased permeability of the vascular system. By this action



of estrogen, more rapid growth of the mammary gland is promoted indirectly by increasing the amount of pituitary mammogen in the intercellular spaces in the region of the mammary gland and also by increasing the amount of nutrients available to the growing gland. This theory has been advanced in a preliminary paper by Mixner and Turner (1942).

In evaluating the literature which has a bearing on this subject it must be appreciated that the mammary gland is a sexual organ and changes occurring in it are associated with similar changes occurring in other sexual organs or glands.

Hechter, Lev and Soskin (1940) pointed out that the effects of estrogen may be divided into specific effects which are not dependent on its hyperemic action and those which are non-specific and may be, therefore, associated with the acetylcholine-liberating action of estrogen. Reynolds and Foster (1940) and Riddle (1941) have reviewed these secondary, non-specific effects of estrogen.

MacLeod and Reynolds (1938) and Reynolds (1939a, 1939b, 1941) reported that the initial hyperemia of the uterus following estrogen administration is accompanied by an increase in its acetylcholine content. As blood vessels dilated they also became more permeable and an accumulation of water took place in the tissue. These same authors and also Kerly (1940) observed an increased metabolism of the uterus following estrogen administration. Hechter, Krohn and Harris (1941) found that estrogen caused an increased permeability of uterine blood vessels to trypan blue injected intravenously. Freed and Lindner (1941) reported that estrone increased the capillary permeability of abdominal skin of rabbits while Reynolds and Foster (1940) observed capillary and venule dilation in rabbit ears, caused by estrogen.

Chamberlin, Gardner and Allen (1941) applied estrogen cutaneously to the sexual skin and breasts of male monkeys and secured local vascular responses of edema and hyperemia in each instance. Speert (1941), also studying the monkey, found definite cyclic changes in the mammary gland associated with an ovulatory cycle. There was lobular dilation and enlargement accompanied by an increased vascularity of the gland. Similar observations on the effect of estrogen on the vascularity of the mammary gland stromal tissue were reported in the experimental section.

These effects of estrogen are suggested as the explanation for the enhancing action of estrogen on progesterone and pituitary extracts secured in the experiments already reported and also secured by other investigators. Increased vascularity and permeability of the vascular system would greatly increase the efficiency of action of a circulating pituitary mammogen.

These effects of estrogen may also explain how local growth of the mammary gland has been secured by the percutaneous application of estrogen (Jadassohn et al., 1937; Zondek, 1938; Mac Bryde,

1939; Lyons and Sako, 1940; Speert, 1940a; Gardner, 1941b; Nelson, 1941a; and Lewis and Turner, 1942a). Pituitary mammogen, which was circulating in the blood stream in subminimal or minimal amounts for mammary growth, would find a localized hyperemic and edematous condition at the site of percutaneous application of estrogen. This would allow the circulating mammogen to be maximally effective. That a circulating pituitary mammogen is necessary for local mammary gland growth is suggested by the experiments of Gomez (1941) and Leonard and Reece (1942) who found that doses of estrogen which were effective in normal animals in promoting local growth of the mammary gland were ineffective in causing such growth when the animals were hypophysectomized.

### C. The Relation of the Mammogenic Lobule-Alveolar Growth Factor to Other Anterior Pituitary Hormones.

1. Review of Literature.—Gomez and Turner (1937, 1938a), both on the basis of the normal physiology of mammary gland growth and on the basis of hypophysectomy and replacement therapy experiments, have postulated the secretion by the pituitary of two mammogenic factors, a duct growth factor, stimulated to increased secretion by estrogen, and a lobule-alveolar growth factor, stimulated to increased secretion by progesterone and estrogen.

From the viewpoint of mammary gland growth, it is of considerable importance to know the exact nature of the pituitary factors or factor which are responsible for mammary gland growth. Are there specific pituitary mammogenic factors which are different from the identified or known pituitary factors? Are these mammary growth affects due to several known pituitary factors or to a single known factor? The definite solution of these questions awaits further research on the subject.

*A priori* considerations would indicate that the lactogenic hormone is not responsible for mammary lobule-alveolar growth, as several studies (Reece and Turner, 1937, and Holst and Turner, 1939) have already indicated that the pituitary secretion of the lactogenic hormone during pregnancy does not rise appreciably above the level of the non-pregnant animal until parturition after which, or at this time, it rises very sharply. Thus, lobule-alveolar growth occurs at a time during pregnancy when there is no significant rise in the level of the lactogenic hormone secretion of the anterior pituitary.

Gomez and Turner (1937) have reviewed some of the earlier papers on the effect or non-effect of lactogenic extracts on mammary growth. These reports are rather controversial, but not very significant as regards the question of the possible identity of the lactogenic and mammogenic hormones due to the relative crudeness of the earlier extracts. These authors report that they secured no

mammary growth in hypophysectomized guinea pigs with lactogenic, thyrotropic or adrenotropic extracts, with or without estrogen, indicating that these hormones were not the active pituitary mammary growth hormones.

Nelson (1941b) reported that an adrenotropic extract of the pituitary caused slight to marked mammary growth in immature male and female rats or in castrate and hypophysectomized rats.

Astwood (1941) found that a crude pituitary powder caused marked proliferation of the lobule-alveolar system of hypophysectomized-pseudopregnant rats. Lactogenic-adrenotropic fractions caused little or no proliferation. Although some luteotropic extracts caused lobule formation, there was no correlation between corpus luteum formation and mammary growth.

Gomez (1942) secured slight mammary growth in hypophysectomized castrate guinea pigs with a lactogenic extract, which was improved by the simultaneous injection of estrogen.

Using hypophysectomized male mice Gardner and White (1942) secured mammary growth with estrogen plus a commercial lactogenic extract, a purified lactogenic extract, a saline extract of pituitary or a good adrenotropic extract. Neither gonadotropic or thyrotropic hormone extracts plus estrogen caused any mammary growth.

Lyons, Simpson and Evans (1942) found that whereas a lactogenic extract plus thyroxin plus glucose in hypophysectomized animals did not cause any lobule-alveolar growth, a lactogenic extract plus estrogen was slightly effective. They also secured excellent lobule-alveolar growth in hypophysectomized animals given estrogen (or F. S. H.) plus an extract containing adrenotropic, growth and lactogenic hormones.

Reece and Leonard (1941, 1942) secured slight mammary growth in hypophysectomized-castrate rats with growth hormone extract alone, while growth hormone plus estrogen or testosterone propionate was more effective in causing such growth.

It may be seen from this review of experiments that anterior pituitary extracts which have stimulated mammary growth have been rich in lactogen, adrenotropin and growth hormone. Extracts rich in thyrotropin or gonadotropin have shown little or no mammary growth effects.

The development of sensitive technics for assaying pituitary materials and extracts for their potency in mammogen, lactogen, thyrotropin and gonadotropin has made possible this quantitative study of the relation of the mammogenic lobule-alveolar growth factor to these other anterior pituitary hormones.

The purpose of this section, then, is to present a comparison of the mammogenic lobule-alveolar growth factor content of a group of pituitary extracts with their lactogenic, thyrotropic and gonadotropic hormone contents. The extracts varied widely in their con-

tent of the different hormones. Such a comparison should reveal whether or not the mammogenic fraction is identical with or is primarily associated with any one of these other pituitary hormones.

**2. Procedure.**—Fourteen anterior pituitary materials and extracts were assayed for their lactogenic and mammogenic lobule-alveolar growth hormones. Three of these fourteen were also assayed for their content of the thyrotropic and gonadotropic hormones. One additional extract was assayed for its thyrotropic, gonadotropic and mammogenic hormone content. These extracts varied in nature from fresh anterior pituitary of cattle to a very highly purified lactogenic extract (30 I. U. per mg.), and also to an extract very high in thyrotropic and gonadotropic hormones. Lactogenic assays were made according to the method of McShan and Turner (1936). The thyrotropic and gonadotropic hormone assays were made using the Bergman-Turner chick methods (Bergman and Turner, 1939, and Bergman, Houchin and Turner, 1939).

**3. Experimental.**—The data for the fifteen anterior pituitary extracts which were assayed for the mammogenic lobule-alveolar growth factor are presented in Table 13. Some of these extracts were very high in their content of lactogenic hormone, while one, Thy. 2a-40, was especially high in the thyrotropic and gonadotropic hormones. It will be seen from the data that there was a very good correspondence between the dosage of extracts injected and the per cent positive mammary responses obtained.

Fourteen of the fifteen extracts assayed for the lobule-alveolar growth factor were also assayed for lactogenic hormone (Table 14). As may be seen from the right hand column in this table, the International Units of lactogen per mammogenic unit range from approximately two to 352, a differential of 176 times. Thus these preparations vary widely in their relative content of mammogen and lactogen. The more potent lactogenic preparations required extreme amounts of extract on a lactogen basis, as compared to the less potent extracts, to secure lobule-alveolar growth. Of course it is possible that in the more crude extracts of the pituitary, synergistic relations may exist between the various pituitary factors and the mammogenic factor so that much less of the active principle was required. However, it is not thought that this synergism could be so great as to explain the mammary growth obtained in these experiments on the basis of lactogen as the active mammogenic factor. It is more probable that the mammogenic and lactogenic factors are separate entities, and that even in the purest amorphous lactogenic extract assayed, impurities of a mammogenic factor are present.

It is incumbent upon those who claim that "pure" protein hormones can be prepared (Li, Lyons and Evans, 1940, 1941a, 1941b, and White, Bonsnes and Long, 1942) to show that the physical-chemical methods for determining protein purity, such as have been suggested by

TABLE 13. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED WITH VARIOUS ANTERIOR PITUITARY PREPARATIONS UNDER ASSAY CONDITIONS

Anterior Pituitary Preparation Name	Total Dosage, Mg.	Estrone, Total Dosage, I. U.	Number of Mice	Lobule-Alveolar Responses		
				+	-	Per Cent Positive
Lot 14, fresh anterior pituitary, pregnant cattle	35.0	75	13	7	6	53.8
	50.0	75	15	13	2	86.7
	100.0	75	14	14	0	100.0
	150.0	75	12	12	0	100.0
Lot 15, fresh anterior pituitary, pregnant cattle	35.0	75	13	6	7	46.1
Lot 13, fresh anterior pituitary, stockyard run of cattle	50.0	75	13	8	5	61.5
	100.0	75	13	13	0	100.0
Lot 14, acetone-ether dried anterior pituitary, pregnant cattle	15.0	75	13	6	7	46.1
	30.0	75	12	12	0	100.0
	45.0	75	3	3	0	100.0
Lot 15, acetone-ether dried anterior pituitary, pregnant cattle	15.0	75	14	8	6	57.1
	17.5	75	13	9	4	69.2
Lot 13, acetone-ether dried anterior pituitary, stockyard run of cattle	20.0	75	13	8	5	61.5
	40.0	75	6	6	0	100.0
Lot 13, initial extract of anterior pituitary, stockyard run of cattle	7.5	75	35	18	17	51.4
	10.0	75	21	12	9	57.1
	12.5	75	23	20	3	87.0
	17.5	75	34	33	1	97.1
Cattle lactogenic 39	5.0	75	3	0	3	0.0
	10.0	75	12	5	7	41.7
	12.5	75	22	20	2	90.9
	20.0	75	4	4	0	100.0
	40.0	75	4	4	0	100.0
Schering 46-51-p <sub>2</sub> -4	10.0	75	10	5	5	50.0
	15.0	75	10	8	2	80.0
	20.0	75	2	2	0	100.0
Cl <sub>3</sub> 41-70	10.0	75	12	4	8	33.3
	13.5	75	11	5	6	45.5
Schering 48-hy-ex.4	7.5	75	11	6	5	54.5
	15.0	75	5	5	0	100.0
	20.0	75	6	6	0	100.0
Lac. la-40	10.0	75	11	0	11	0.0
	12.5	75	5	1	4	20.0
	20.0	75	8	4	4	50.0
	25.0	75	4	3	1	75.0
	37.5	75	4	3	1	75.0
	50.0	75	3	3	0	100.0
L-1-41-60	15.0	75	12	6	6	50.0
L-1-41-70	20.0	75	13	8	5	61.5
Thyr. 2a-40	10.0	75	13	0	13	0.0

TABLE 14. COMPARATIVE MAMMOGENIC AND LACTOGENIC HORMONE ASSAYS ON A GROUP OF ANTERIOR PITUITARY PREPARATIONS

Preparation	Mg. Extract Per Mammogenic Unit	I. U. of Lactogen Per Mg. of Extract	I.U. of Lactogen Per Mammogenic Unit
Lot 14, fresh anterior pituitary, pregnant cattle	35.0	0.06	2.1
Lot 15, fresh anterior pituitary, pregnant cattle	35.0	0.06	2.1
Lot 13, fresh anterior pituitary, stockyard run of cattle	44.0*	0.06	2.6
Lot 14, acetone-ether dried anterior pituitary, pregnant cattle	15.0	0.3	4.5
Lot 15, acetone-ether dried anterior pituitary, pregnant cattle	15.0	0.3	4.5
Lot 13, acetone-ether dried anterior pituitary, stockyard run of cattle	17.6*	0.3	5.1
Lot 13, initial extract of anterior pituitary, stockyard run of cattle	7.5	4.5	33.8
Cattle lactogenic 39	10.0	4.0	40.0
Schering 46-51-p <sub>2</sub> -4	10.0	12.0	120.0
CI <sub>3</sub> 41-70	13.5	12.0	162.0
Schering 48-hy-ex.4	7.5	30.0	225.0
L-la-40	20.0	12.0	240.0
L-l-41-60	15.0	18.0	270.0
L-l-41-70	17.6*	20.0	352.0

\*Corrected to basis of 50 per cent positive responses from 61.5 per cent.

Shedlovsky (1943), can detect extremely small amounts of hormone contaminant that will give physiological responses with sensitive assay technics. If, for instance, extremely small amounts of lactogen were added to pure casein, would the physical-chemical methods for determining purity of proteins be able to detect such contamination in view of the extremely small amount of lactogen required to give

positive responses with the sensitive intradermal test (Lyons and Page, 1935)?

The case seems more complete for the separateness of the mammo-genic factor from the thyrotropic and gonadotropic hormones. A thyrotropic-gonadotropic extract, Thyr. 2a-40 (Table 13), which contained ten chick units of thyrotropin per mg. and four chick units of gonadotropin per mg., failed to elicit any mammary growth when assayed at the ten-mg. level, a total of 100 c. u. of thyrotropin and 40 c. u. of gonadotropin. These amounts of hormone greatly exceed the amount present in a mammo-genic unit of any of the other three extracts assayed for gonadotropic and thyrotropic hormones. The comparative content of mammo-gen, thyrotropin and gonadotropin of these four extracts are presented in Table 15. The chick units of thyrotropin per mammo-gen unit varied from 1.31 to at least 100, a differential of at least 80 times, while the chick units of gonadotropin varied from 0.095 to at least 40, a differential of at least 400 times. It is thus rather apparent that the mammo-genic factor is not associated with either the thyrotropic or gonadotropic hormones.

Extracts were not available which were known to consist predominantly of adrenotropin or growth hormone, so it was not possible to make any comparison of mammo-gen with these two factors.

TABLE 15. COMPARATIVE MAMMOGENIC, THYROTROPIC AND GONADOTROPIC HORMONE ASSAYS ON A GROUP OF ANTERIOR PITUITARY PREPARATIONS

Preparation	Mg. Extract Per Mammo-genic Unit	Thyrotropic*		Gonadotropic*	
		C. U. Per Mg. Extract	C. U. Per Mammo-genic Unit	C. U. Per Mg. Extract	C. U. Per Mammo-genic Unit
Cl <sub>3</sub> -41-70	13.5	0.097	1.31	0.007	0.095
Lot 13, acetone- ether dried anterior pituitary, stockyard run of cattle	17.6**	0.278	4.89	0.110	1.936
Lot 13, initial extract of anterior pituitary, stock- yard run of cattle	7.5	2.943	22.07	0.776	5.720
Thyr. 2a-40	No response at 10 mg.	10.000	At least 100	4.000	At least 40

\*Chick Units

\*\*Corrected to basis of 50 per cent positive responses from 61.5 per cent

4. Discussion.—As the pituitary is subject to intensive study as to its physiological activities, new functions are being found. It is of prime interest to determine then if the newly-observed activity

of the pituitary is ascribable to some already identified hormone or whether it may be put in the class of new hormones. Until such time as the factor responsible for a certain activity has been chemically isolated and purified, our only tool in determining its possible identity with other hormones is the use of comparative assays on various types of extracts.

The use of comparative assays on various types of pituitary extracts was used in this study to determine the possible identity of the mammogenic lobule-alveolar growth factor with other anterior pituitary hormones.

Although these results cannot be considered as conclusive, they do indicate that the lobule-alveolar growth factor is not identical with lactogen, thyrotropin or gonadotropin.

#### D. Progesterone-like Activity of Some Steroid Compounds and of Diethylstilbestrol in Stimulating Mammary Lobule-Alveolar Growth.

1. **Review of Literature.**—Following the purification, isolation and synthesis of progesterone, it was shown that this hormone stimulated progestational proliferation of the endometrium, induced sexual receptivity in the guinea pig and also stimulated the growth of the mammary lobule-alveolar system. For a time it was thought that progesterone was specific for these functions, but now numerous other compounds are known to have these same properties, but to a lesser degree than progesterone.

Both testosterone and methyl testosterone have been shown to have slight progesterone-like activity in stimulating progestational proliferation of the endometrium (Klein and Parkes, 1937). Emmens and Parkes (1939) reported that a new synthetic hormone, pregnenolone (ethinyl testosterone or anhydro-hydroxy-progesterone), possesses about one-tenth the activity of injected progesterone in promoting progestational proliferation of the rabbit endometrium, and that it was equally potent by mouth or injection. Definite progestational proliferation was produced in the endometrium of post menopause women with pregnenolone administered orally after preliminary priming with estradiol benzoate (Salmon, Walter and Geist, 1939). An adrenal-cortical compound, desoxycorticosterone and its acetate, have been shown to promote progestational proliferation of the endometrium (Van Heuverswyn et al., 1939, and Leatham and Crafts, 1940).

Hertz, Meyer and Spielman (1937) have reported on the specificity of progesterone in inducing sexual receptivity in the ovariectomized guinea pig. However, Van Heuverswyn et al. (1939) found that desoxycorticosterone has one-sixth to one-tenth the activity of progesterone in inducing sexual receptivity. Tortsveit and Mellish (1941) similarly have reported that aqueous extracts of the adrenal cortex



containing the life-maintaining principle produced the copulatory reflex in properly conditioned guinea pigs.

There are a limited number of reports which indicate that substances other than progesterone may cause the development of the lobule-alveolar system. Mixner and Turner (1941b) reported in a preliminary paper that pregnenolone was about one-half as active as progesterone in stimulating alveolar growth. Speert (1940b) found that desoxycorticosterone acetate caused lobule growth in mature spayed monkeys whose glands had involuted following castration. Similarly Chamorro (1940) reported that the injection of desoxycorticosterone acetate plus estradiol benzoate caused lobule-alveolar growth in hypophysectomized male rats. Both substances were inactive alone. Dodds, Lawson and Noble (1938) secured full mammary gland growth with diethylstilbestrol in the guinea pig. Lewis and Turner (1941a, 1941b, 1942b, 1942c) also secured limited proliferation of the lobule-alveolar system of mice, rats, guinea pigs, rabbits and goats with diethylstilbestrol. Selye, McEuen and Collip (1936), Nelson and Merckel (1937), Astwood et al. (1937), Noble (1939), Reece and Mixner (1939), and Folley et al. (1939) reported on the ability of testosterone to stimulate the growth of the mammary lobule-alveolar system.

The following section reports the results of a study to determine the relative activity of progesterone, other steroids and diethylstilbestrol in stimulating mammary lobule-alveolar growth in the ovariectomized mouse. These relations as determined are taken to indicate the relative ability of these materials to stimulate the increased production of the mammogenic lobule-alveolar growth factor by the pituitary of the assay animal.

**2. Experimental.**—Progesterone, pregnenolone, desoxycorticosterone acetate, dehydroandrosterone, diethylstilbestrol, acetoxy-pregnenolone, methyl testosterone and testosterone were assayed for their ability to stimulate lobule-alveolar growth (Table 16 and Fig. 7) using the assay method already developed. Progesterone and pregnenolone assays (with and without estrone) were reported earlier in Part B of the Experimental Section. They are repeated here so that they may be compared directly with the other compounds.

Progesterone was the most potent substance assayed for its ability to stimulate lobule growth, followed by pregnenolone which possessed one-half the potency of progesterone according to the assay data.

Both desoxycorticosterone acetate and dehydroandrosterone possessed considerable potency also, having roughly one-third the activity of progesterone.

Four mg. of diethylstilbestrol were required for an assay response. This dose of material caused a slight weight decrease in the animals over the ten-day assay period. A six-mg. dose, however, severely decreased the body weights of the assay animals, and this was reflected

TABLE 16. LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE TO SOME STEROID COMPOUNDS AND DIETHYLSTILBESTROL

Compound		Estrone, Total Dosage, I. U.	Number of Mice	Lobule-Alveolar Responses		
Name	Total Dosage, Mg.			+	-	Per Cent Positive
Progesterone	0.025-0.25	133	16	0	16	0.0
	0.50	133	16	4	12	25.0
	0.75	133	12	5	7	41.6
	1.00	133	10	6	4	60.0
	1.25	133	12	10	2	83.3
	1.50	133	3	3	0	100.0
Pregneninolone	1.0	133	12	1	11	8.3
	1.5	133	12	3	9	25.0
	2.0	133	12	7	5	58.3
	2.5	133	10	9	1	90.0
	5.0-10.0	133	12	12	0	100.0
Desoxycorticosterone acetate	2.0	75	12	4	8	33.3
	3.0	75	9	5	4	55.6
	4.0	75	12	8	4	66.7
	5.0	75	7	6	1	85.7
Dehydroandrosterone	2.0	75	12	4	8	33.3
	3.0	75	12	5	7	41.7
	5.0	75	11	9	2	81.8
Diethylstilbestrol	2.0	75	12	1	11	8.3
	4.0	75	12	6	6	50.0
	6.0	75	12	4	8	33.3
Progesterone	3.33	-	24	2	22	8.3
	4.67	-	24	5	19	20.8
	6.00	-	26	15	11	57.7
	7.33	-	24	17	7	70.8
Pregneninolone	2.5-5.0	-	10	0	10	0.0
	7.5	-	8	2	6	25.0
	10.0	-	12	5	7	41.7
	12.5-20.0	-	16	16	0	100.0
Acetoxy-pregnenolone	5.0	75	8	0	8	0.0
	10.0	75	12	4	8	33.3
Methyl testosterone	5.0	75	7	0	7	0.0
	10.0	75	12	2	10	16.7
Testosterone	2.0	75	4	0	4	0.0
	4.0	75	4	0	4	0.0
	6.0	75	3	0	3	0.0
	10.0	75	11	0	11	0.0
Testosterone propionate	5.0	75	8	0	8	0.0
	10.0	75	6	0	6	0.0

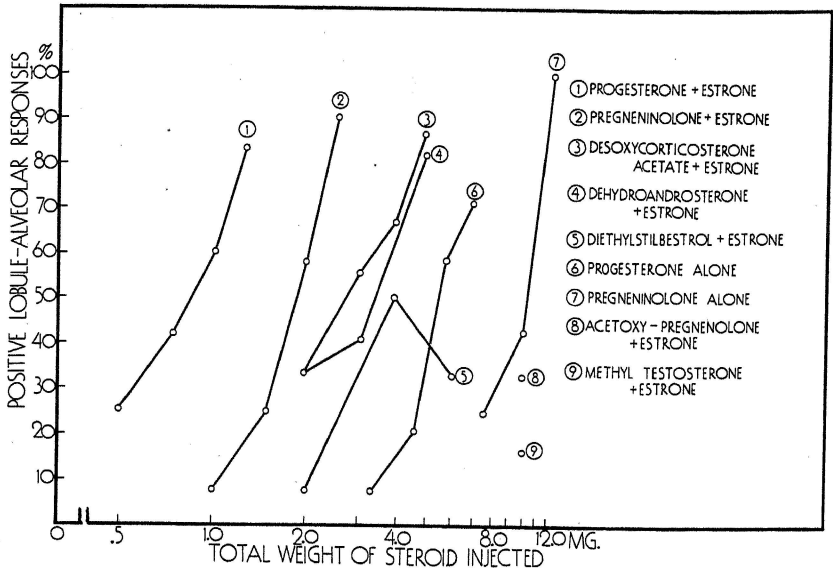


Fig. 7.—Comparison of progesterone and other progesterone-like compounds in their ability to stimulate the mammary lobule-alveolar systems of ovariectomized mice, plotted on arithlog paper.

in the reduced response secured with the higher dosage. It appears that while diethylstilbestrol will stimulate lobule-alveolar growth in reasonably small amounts, it is very toxic at higher levels and therefore at dosages which should cause good alveolar growth the toxic properties predominate to the disadvantage of mammary gland growth.

Selye (1941) reported that acetoxy-pregnenolone, an intermediary product in the Steiger-Reichstein synthesis of desoxycorticosterone acetate possessed corticoid activity only slightly less than desoxycorticosterone acetate. In this study ten mg. of acetoxy-pregnenolone with estrone caused four of twelve mice to respond with lobule-alveolar growth. This would make it approximately one-sixteenth as potent as progesterone or one-fifth as active as desoxycorticosterone acetate in stimulating lobule-alveolar growth.

Ten mg. of methyl testosterone caused two of twelve mice to respond with alveolar growth indicating that it is approximately one-twenty-fifth as active as progesterone in this respect.

Although it has been widely shown that testosterone stimulates lobule-alveolar growth in certain animals, these experiments indicate that testosterone plus estrone is ineffective in stimulating lobule-alveolar growth in the mouse in dosages as high as ten mg. during a ten-day period.

**3. Discussion.**—The relative activity of progesterone, pregnenolone, desoxycorticosterone acetate and methyl testosterone in promoting mammary lobule-alveolar growth corresponds in a rough way to the relative activity of these substances in stimulating progesterational proliferation of the endometrium as reported in the literature. It appears that the early idea on the specificity of progesterone must be revised as it is now apparent that numerous steroids have progesterone-like activity in varying degrees.

It is interesting to note the failure to secure lobule-alveolar growth with testosterone or the propionate in the mouse reported in these experiments as contrasted to the numerous reports in the literature of positive results with other species.

### **E. The Influence of Certain Adverse Conditions on the Growth of the Mammary Gland.**

**1. Review of Literature.**—Many studies have been reported indicating that variations in such environmental factors as light, temperature, and diet influence the secretion of the anterior pituitary hormones. However, in several instances it has been shown that these same factors do not adversely influence the ability of the end organs such as the thyroid and gonads to respond to their direct growth stimuli. Thus in the case of underfeeding, Breneman (1940) has shown that the testes are responsive to less gonadotropic hormone than glands of animals on a normal ration, and similarly Stevens (1940) has shown that the thyroids are responsive to less thyrotropic hormone than normal-fed animals.

This same problem enters into the question of whether it is necessary to postulate a pituitary mammary growth factor because in most instances it is impossible to secure mammary growth with ovarian hormones in the absence of the pituitary. It is acknowledged that hypophysectomy constitutes an unfavorable factor which interferes with food intake and many other body processes, and thus it might be difficult to secure mammary growth in such animals with the ovarian hormones.

Astwood, Geschickter and Rausch (1937) restricted the diet of normal rats and at the same time injected estrogen. No mammary growth could be secured in such animals. Normal rats on unrestricted diets responded with good mammary growth. They concluded that the ineffectiveness of ovarian hormones in hypophysectomized animals was insufficient evidence for the claim that mammary growth is mediated by the pituitary.

Selye and Collip (1936) secured no mammary growth with estrogen in hypophysectomized rats whose gonads and adrenals were maintained in good condition with a pituitary extract.

Nathanson, Shaw and Franseen (1939) similarly reported that estrogen did not cause mammary growth in hypophysectomized rats

even when a pituitary growth extract was also injected, maintaining normal body weight of the hypophysectomized animals similar to those of controls. They conclude that although nutrition is important, the sex hormones must act through the pituitary to cause mammary gland growth.

Samuels, Reinecke and Petersen (1941) attacked this problem from a different angle. Hypophysectomized animals were force-fed, given thyroid, sodium chloride and adrenal cortical extract. The mammary glands of such animals in which normal body weight was maintained failed to respond to estrogen. They suggested that either changes in metabolism caused by the pituitary were necessary for mammary growth or estrogen acts through the anterior pituitary to cause mammary growth.

Trentin and Turner (1941) have further studied the effect of inanition on responsiveness of the mammary gland to estrogen. If the food intake of male mice was decreased by fifty per cent, the requirement of estrogen for mammary growth was increased by sixteen times. It was significant, however, that mammary growth could be secured with high dosages of estrogen when the food intake was greatly reduced. Thus Astwood et al. (1937) probably could have secured mammary growth with higher dosages of estrogen in rats in which the food intake was limited or restricted.

In view of the above experiments it seemed of interest to determine the influence of an adverse factor (high environmental temperature) upon the responsiveness of the mammary lobule-alveolar system to progesterone and an anterior pituitary extract containing the mammo-genic lobule-alveolar growth factor.

It might be predicted from the above discussion that (1) if progesterone acts indirectly on the mammary gland by means of the pituitary, the high temperature would adversely affect the ability of progesterone to stimulate mammary-lobule growth and that (2) if the pituitary factor acts directly on the mammary gland to cause alveolar growth, the high temperature would *not* adversely affect the ability of the pituitary extract to cause lobule-alveolar growth.

**2. Experimental.**—Groups of assay mice were maintained in constant temperature chambers regulated to 25° and 35° C. respectively. A complete grain ration was fed to all mice *ad libitum*. Some mice were injected with progesterone plus estrone while others were injected with an anterior pituitary extract plus estrone, groups of each being kept in the 25° and 35° chambers. At the 35° C. temperature approximately fifty per cent of the animals on both progesterone and the pituitary extract died during the ten-day assay period. Only the surviving animals are included in the data presented in Table 17. At the more normal temperature of 25° C., 1.25 mg. of progesterone and 75 I. U. of estrone stimulated lobule-alveolar growth in 51.7 per cent of the animals. This is considered a unit response. On the

TABLE 17. EFFECT OF HIGH ENVIRONMENTAL TEMPERATURE ON LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED WITH PROGESTERONE AND A PITUITARY EXTRACT

Injection Materials Name	Estrone,		Environmental Temperature, °C	Number of Mice	Lobule-Alveolar Responses		
	Total Dosage, Mg.	Total Dosage, I. U.			+	-	Per Cent Positive
Progesterone	1.25	75	25	29	15	14	51.7
	1.25	75	35	15	1	14	6.7
Anterior pituitary preparation	12.5	75	25	15	13	2	86.7
	12.5	75	35	8	7	1	87.5
	17.5	75	25	14	13	1	92.9
	17.5	75	35	8	8	0	100.0

same level of hormone injection but at 35° C. the response dropped to 6.7 per cent. Thus the high temperature adversely affected the ability of the anterior pituitary gland to secrete the mammogenic lobule-alveolar growth factor in response to the stimulation of progesterone and estrone. This is in contrast to the results secured with the pituitary extract. Here the groups receiving 12.5 mg. and 17.5 mg. of pituitary extract responded equally well in both the 25° and 35° C. temperature groups. It is apparent that the high temperature in no way inhibited the ability of the mammary glands, as end organs, to respond to their direct growth stimulus.

**3. Discussion.**—Previous experiments indicated that undernutrition in mice and rats constituted an adverse condition which prevented the mammary glands from responding in a normal manner to the injection of estrogen. Under-nutrition may affect the pituitary in such a way that it is no longer stimulated to the increased production of mammogen by normally optimal amounts of estrogen.

Indeed, excess amounts of estrogen itself may constitute an adverse factor. Gardner (1941a) found that large amounts of estrogen inhibited mammary growth. Similarly, a large amount of estrone inhibited the normal effect of progesterone in stimulating lobule-alveolar growth (Table 8). It is not improbable that the pituitary again is the organ which is affected to the disadvantage of mammary growth. This is further borne out by the experiments of Lewis and Turner (1939) in which large amounts of estrone decreased the mammogen content of pituitaries of rabbits as compared with pituitaries of rabbits injected with more optimal amounts of estrone.

In the present experiments high environmental temperature was another of these factors which prevented normal mammary gland stimulation by progesterone and estrone. This high temperature did not affect the normal mammogenic action of pituitary extract plus estrone.

It is suggested that the various experiments on undernutrition, high estrogen administration and high environmental temperature are harmonious with, and contribute to, the concept of the pituitary-mam-mogen theory of mammary gland growth.

#### F. Influence of Thyroxine and Thyroidectomy upon Mammary Lobule-Alveolar Growth.

1. **Review of Literature.**—Thyroid tissue, thyroxine and synthetic thyroprotein have been demonstrated repeatedly to have a stimulating effect upon lactation in dairy animals (Herman et al., 1938, Ralston et al., 1940, and Reineke and Turner, 1942). The effect of thyroxine on the growth of the mammary duct and lobule-alveolar systems is less clear.

Dragstedt, Sudan and Phillips (1924) observed that the complete absence of the thyroid and the parathyroid in the dog did not prevent growth of the mammary gland during pregnancy and secretion of milk after delivery.

Weichert and Boyd (1933, 1934) found that normal female rats fed desiccated thyroid became pseudopregnant while pregnant rats fed thyroid showed a precocious development of the mammary gland which was especially marked from the seventh to the ninth days of pregnancy. There was also an early appearance of secretion.

No mammary growth was obtained in ovariectomized adult rats fed desiccated thyroid (Weichert, Boyd and Cohen, 1934).

Cohen (1935) observed changes in the mammary glands of male rats fed desiccated thyroid. Normal males had poorly defined lumina of alveoli while thyroid-fed males had well arranged lumina of the alveoli.

Leonard and Reece (1941) reported that thyroidectomy in normal, young female rats, in spayed female rats and in estrogen-treated spayed rats caused a thickening of the mammary ducts and an increase in the number of lateral and end buds as compared to appropriate controls. Thyroxine given to these animals prevented these changes.

Smithcors and Leonard (1942) claimed that thyroidectomy in normal immature male rats inhibited mammary duct growth and stimulated lobule-alveolar growth. Thyroidectomy in castrated male rats gave similar results but to a lesser degree. Testosterone propionate or estradiol dipropionate caused inhibition of mammary duct growth but an exaggeration of the alveolar system in thyroid-ectomized, castrated male rats.

Mammary glands of intact male mice fed desiccated thyroid in their feed showed evidence of duct stimulation. Thyroid was ineffective in castrate mice (Gardner, 1942).

The development of an assay technic for substances which stimulate mammary lobule-alveolar growth has made possible the study

of factors which influence such growth. In this section experiments on the influence of thyroxine and thyroidectomy on mammary lobule-alveolar growth stimulation by progesterone and estrone and by a pituitary extract are reported.

**2. Experimental.**—Groups of assay animals were injected with constant amounts of progesterone and estrone over the standard ten-day assay period. Using these groups the effect of injecting varying amounts of thyroxine or of thyroidectomy on lobule growth was determined. The animals in group 1 (Table 18) serve as a control group for the next four groups of animals. In the controls, 35 per cent positive lobule-alveolar responses were secured in twenty animals. The same response was secured when 150 micrograms of thyroxine were given in addition to the progesterone and estrone (Group 2). However, when the thyroxine was increased to 1500 micrograms in group 3, the percentage of positive responses increased to 64.5 per cent, indicating that in some manner the thyroxine was increasing the efficiency of action of the progesterone and estrone. This increase was considered to be significant in comparison with the level of 35 per cent in control group 1. Group 4 which received 4500 micrograms gave only the control level of response. This was interpreted as indicating that there is an optimum thyroxine level for the synergism observed.

TABLE 18 - EFFECT OF THYROXINE AND THYROIDECTOMY ON LOBULE-ALVEOLAR RESPONSES OF CASTRATE FEMALE MICE INJECTED WITH PROGESTERONE AND A PITUITARY MATERIAL

Group Number	Injection Material		Estrone, Total Dosage, Micrograms	Thyroxine, Total Dosage, Micrograms	Number of Mice	Lobule-Alveolar Responses		
	Name	Total Dosage				+	-	Per Cent Positive
		Micrograms						
1	Progesterone	750	7.5	-	20	7	13	35.0
2		750	7.5	150	20	7	13	35.0
3		750	7.5	1500	31	20	11	64.5
4		750	7.5	4500	18	6	12	33.3
5		750	7.5	*	12	1	11	8.3
6		1000	13.3	-	10	6	4	60.0
7		1000	13.3	1500	9	9	0	100.0
		Milligrams						
8	Anterior	7.5	7.5	-	18	9	9	50.0
9	pituitary	7.5	7.5	1500	18	1	17	5.6
10	extract	7.5	7.5	**	14	2	12	14.3
11	-----	-	7.5	1500	10	0	10	0.0

\* Thyroidectomized fifty days before start of injections

\*\* Thyroidectomized five days before start of injections

To determine the activity of progesterone and estrone in the absence of the thyroids, a group of animals (Group 5) were thyroidectomized 50 days before progesterone and estrone injections were



begun and were ovariectomized only shortly before the beginning of injections. Here only one animal of twelve (8.3 per cent) responded with lobule-alveolar growth which further indicates that thyroxine is needed to secure optimal mammary growth.

In groups 6 and 7, the mice received 1000 micrograms of progesterone and 13.3 micrograms of estrone while group 7 received in addition 1500 micrograms of thyroxine. Thyroxine increased the per cent positive responses from 60 to 100, further evidence in confirmation of the previous observations.

As 64.5 per cent positive responses were obtained from 750 micrograms of progesterone plus 1500 micrograms of thyroxine (Group 3) while 1000 micrograms of progesterone plus estrone gave 60 per cent positive responses (Group 6), it would appear that the efficiency of the progesterone was increased about 33.3 per cent by the action of the thyroxine.

In groups 8, 9 and 10 a pituitary mammogenic extract was substituted for the progesterone. Group 8 served as a control for group 9 which received in addition 1500 micrograms of thyroxine and for group 10 in which the animals were thyroidectomized five days prior to the beginning of injections. The thyroxine and thyroidectomy inhibited mammary growth in both cases as the per cent positive responses dropped from 50 to 5.6 and 14.3 per cent respectively.

Group 11 received 1500 micrograms of thyroxine and 7.5 micrograms of estrone as a check on the possible mammary growth activity of the thyroxine under assay conditions. No lobule-alveolar stimulation resulted.

**3. Discussion.**—The enhancing effect of thyroxine on mammary growth when stimulated by progesterone and estrone may be explained in several ways. As thyroxine plays a role in somatic growth both in thyroidectomized (Reineke and Turner, 1941) and normal animals (Koger, Hurst and Turner, 1942), it seems reasonable to expect that thyroxine would also augment the growth of specialized tissues when they are subjected to the action of hormones which normally stimulate their growth as for instance, mammary growth stimulation by progesterone and estrone. Koger, Hurst and Turner (1942) observed that thyroxine in suitable dosage increased the growth rate of normal, young, female mice about 25 per cent over that of non-injected controls. Carcass analysis indicated a greater retention of nitrogen and water but a lesser percentage of fat than in controls. Thus the enhancing effect of thyroxine on mammary lobule-alveolar growth when stimulated by progesterone and estrone may be simply that of superimposing the general protein-growth stimulation of thyroxine upon the more specific mammary lobule-alveolar growth stimulation of progesterone and estrone. Mammary growth being primarily protein in nature, a greater effect might be expected to result.

Another possibility is that thyroxine, through its effect in elevating general body metabolism, accelerates the rate of cellular reactions and thus, although a certain optimal level of progesterone and estrone are necessary to direct the mechanism of mammary growth, the reaction as a whole is accelerated by thyroxine.

The inhibiting effect of thyroxine on mammary growth with pituitary extract (Group 9) was probably due to excessive thyroxine stimulation due to the combined effects of the injected thyroxine and the endogenous thyroxine stimulated by the thyrotropic hormone in the pituitary extract injected.

The thyroidectomized animals (Groups 5 and 10) injected with either progesterone or pituitary extract gave markedly inferior responses as compared with their appropriate controls, indicating in a negative way the need for thyroxine for optimal mammary growth.

### G. Mammary Lobule-Alveolar Growth in Goats.

1. **Review of Literature.**—Both natural and synthetic estrogens and progesterone have been widely used in the stimulation of mammary gland growth in the smaller experimental animals. It has been found generally that the estrogens stimulate primarily mammary duct growth and at times a limited amount of lobule-alveolar growth, while a combination of estrogen and progesterone stimulates complete mammary gland development.

Relatively few experiments on mammary growth using dairy animals have been reported, primarily because of the large size and cost of the animals and the relatively greater amounts of hormone required. Experiments on hormonal stimulation of lactation in dairy animals are more numerous. In some of these lactation experiments attempts have been made to judge the nature and degree of mammary growth on the basis of the amount of milk secreted and by palpation of the udder, histological techniques not being used. As considerable milk secretion can occur from a well extended duct system, the judgment of mammary growth on this basis is liable to considerable error.

De Fremery (1936, 1938) administered estradiol benzoate both subcutaneously and percutaneously on the udder to virgin female goats. He claimed that a growth of the udder to a size comparable with that observed during pregnancy was obtained. He concluded that estrogen alone is sufficient for complete mammary growth in the goat, progesterone being unnecessary. These mammary glands were not examined histologically, size and milk secretion only being used as an indication of udder growth.

Lewis and Turner (1941a, 1942c) reported that the administration of diethylstilbestrol to virgin or parous female goats caused limited growth of the mammary lobule-alveolar system. While in some cases treatment was carried out over extended periods of time, in no case

did the glands appear comparable to the glands of normal, parturient lactating animals.

Folley et al. (1941a) reported that they secured very limited duct and alveolar growth in two castrate male goats with stilbestrol treatment.

Walker and Stanley (1941) obtained fair lactation in two Jersey heifers following the injection of diethylstilbestrol dipropionate over a nine month period. In one of the heifers testosterone propionate supplemented the stilbestrol. They concluded that progesterone was not necessary for complete mammary development in cattle. Since histological examinations of the glands were not reported, the validity of the last statement may be questioned on the basis of insufficient evidence.

As there seems to be some question regarding the necessity of both estrogen and progesterone in stimulating complete mammary lobule-alveolar growth in the female goat, the experiments in the following section were designed to help clarify this subject.

**2. Experimental.**—The goats used in these experiments were normal, virgin females ranging in age from one to two years and weighing from 47 to 87 pounds.

Both the diethylstilbestrol and the progesterone were dissolved in olive oil for injection purposes. When both of these compounds were injected into an animal, they were dissolved together in the same oil. All injections were made subcutaneously, once daily.

The mammectomies performed were done under local anesthesia (two per cent apothesine). The liberal use of sulfanilamide in these operations prevented any serious infections.

Mammary glands were prepared both for micro and macro inspection. The ordinary paraffin microtome sections were cut at about twenty micra and stained with hematoxylin and eosin. Other mammary gland tissues were dissected out as thin sections containing individual branches of the mammary tree. These sections were stained with hematoxylin and mounted in clarite. By examining microtome-cut sections under a 100x microscope and the dissected sections under a 20x dissecting scope, a very good picture of the state of mammary development was secured. Although it was felt that the dissected sections were superior to the microtome sections in judging mammary development, the dissected sections did not photograph well.

In the experiments to be reported, mammary glands of goats injected with diethylstilbestrol were compared with other glands from goats injected with progesterone plus diethylstilbestrol. A constant ratio of one mg. of progesterone to five micrograms of diethylstilbestrol was maintained in all injections involving both compounds.

Goat 518 served as a control in these experiments. The mammary glands of this animal showed only a complex branching of the duct system (Figs. 8 and 9) such as was reported by Turner and Gomez (1936).

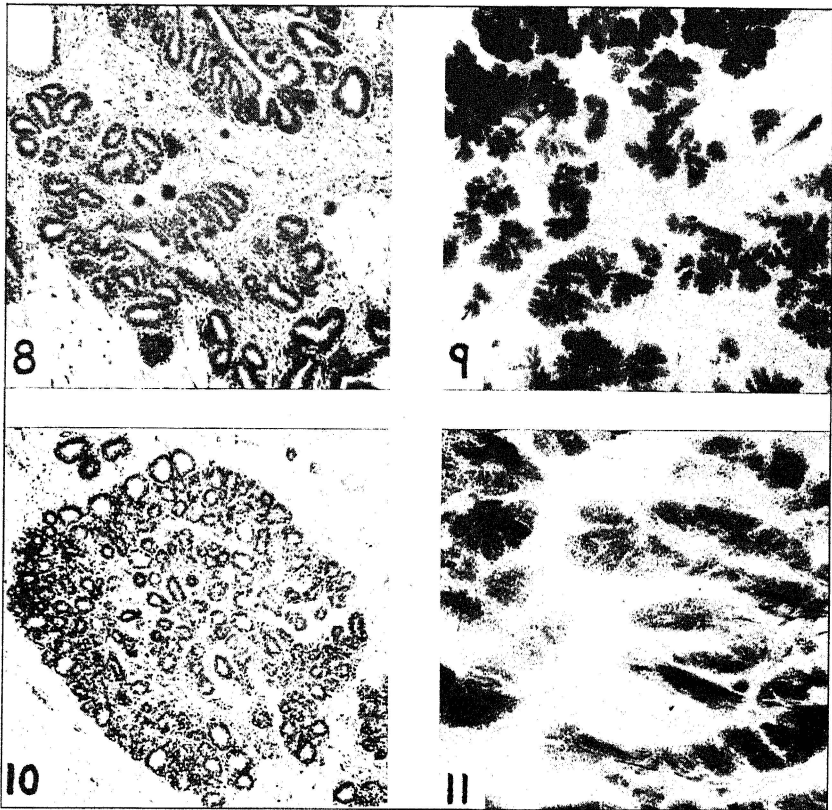


Fig. 8.—A microtome section from a mammary gland of goat 440 which received five mg. of progesterone and twenty-five micrograms of diethylstilbestrol daily for twenty-five days, similar to control goat 518 (58x).

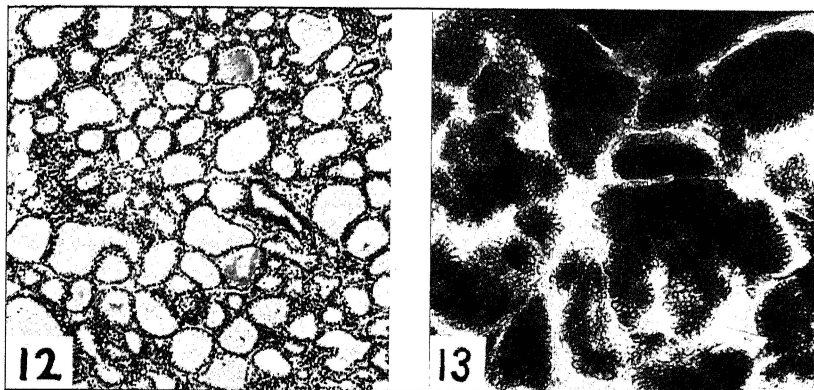
Fig. 9.—Dissection from a mammary gland of normal virgin female goat 518 (13x).

Figs. 10 and 11.—Microtome section (58x) and dissection (13x), respectively, from a mammary gland of goat 443 which received twenty mg. of progesterone and 100 micrograms of diethylstilbestrol daily for sixty days. A considerable development of the lobule-alveolar system has taken place.

Goat 440 received daily five mgs. of progesterone and twenty-five micrograms of diethylstilbestrol. Mammary glands were taken after twenty-five and sixty days of injection. No development was seen in either gland above that of the control (Figs. 8 and 9).

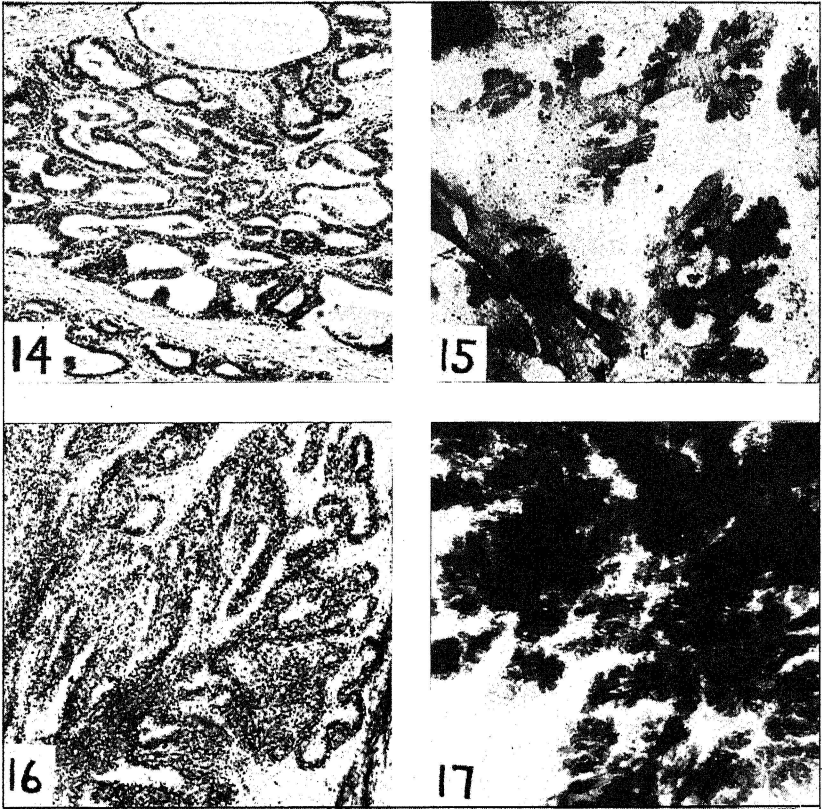
Two other goats (833 and 881) were injected daily with ten mg. of progesterone and fifty micrograms of diethylstilbestrol. Goat 833 died after twenty-five days. Examinations of the glands showed no development above that of the control. Goat 881 was injected for sixty days at which time there was still no observable development of the lobule-alveolar system.

Goats 443 and 198 received daily doses of twenty mg. of progesterone plus 100 micrograms of diethylstilbestrol and thirty mg. of progesterone plus 150 micrograms of diethylstilbestrol, respectively, for sixty days. At this time the mammary glands of these animals (surgically removed) showed a great development of the lobule-alveolar system, characterized by lobules of rather tightly packed, unhypercrophied alveoli (Figs. 10 and 11). Fig. 11 presents very well the type of growth present at sixty days. It corresponds to the glands of goats observed at mid-pregnancy (Turner and Gomez, 1936). It was thought to be of interest to determine the appearance of such a gland if it was stimulated to secretion. Therefore, these goats (443 and 198) received after sixty days of progesterone-diethylstilbestrol treatment a further treatment for twelve days of 0.25 mg. per day of diethylstilbestrol alone. At the end of this time the goats were killed and the mammary glands taken and examined. This additional diethylstilbestrol treatment caused the initiation of secretion and a consequent hypertrophy of the alveoli (Figs. 12 and 13). This action was very marked, and caused an appearance of the gland similar to that seen at parturition (Turner and Gomez, 1936). It might be added that the alveoli were entirely normal in appearance as seen under high-power magnification. These experiments involving progesterone and diethylstilbestrol simulate the normal growth of the mammary gland as seen during pregnancy and the initiation of lactation as observed at parturition.



Figs. 12 and 13.—Microtome section (58x) and dissection (13x), respectively, from a mammary gland of goat 443 which after the treatment described in Figs. 10 and 11 received an additional twelve days injection of diethylstilbestrol at the rate of 0.25 mg. per day. Lactation was initiated, accompanied by alveolar hypertrophy.

Two other goats, 188 and 438, received 0.25 and 0.10 mg. of diethylstilbestrol daily for sixty days, at which time mammary glands



Figs. 14 and 15.—Microtome section (58x) and dissection (13x), respectively, from a mammary gland of goat 188 which received 0.25 mg. of diethylstilbestrol daily, for sixty days. Very slight if any lobule-alveolar growth was stimulated, although a great hypertrophy due to secretion was evident.

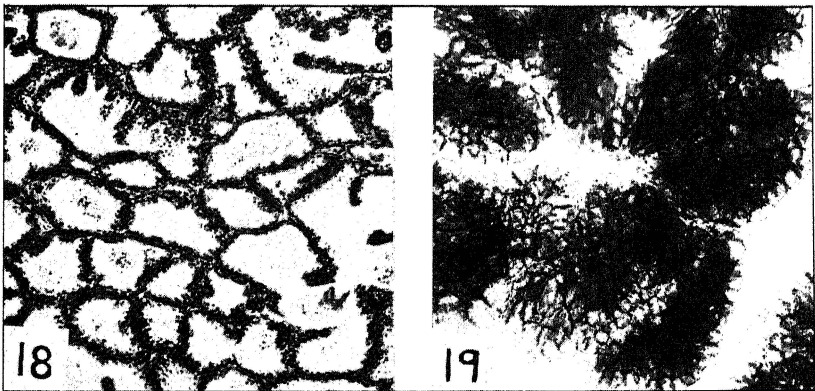
Figs. 16 and 17.—Microtome section (58x) and dissection (13x), respectively, from a mammary gland of goat 569 which received diethylstilbestrol treatment at the rate of 0.25 mg. daily for 138 days. Very slight stimulation of the mammary lobule-alveolar system is evident.

were removed for examination. The gland of goat 188 (Figs. 14 and 15) was characterized by a very great hypertrophy and swelling of the duct system with secretion. These ducts were greatly dilated, small milk cisterns being present in all parts of the gland. There was a very slight development of scattered groups of what appeared to be very large alveoli, not at all similar to that seen in normal lactation. Goat 438 presented a somewhat different picture. The great hypertrophy of the gland as seen in goat 188 was not evident in this goat. Rather a slight development of the gland in the direction of lobule growth had taken place. This early lobule-alveolar growth was not at all comparable to that secured in goats 443 and 198 with progesterone

and diethylstilbestrol in sixty days. Thus of these two goats receiving different amounts of diethylstilbestrol, the one receiving the lesser amount showed the greater degree of lobule-alveolar growth while the one receiving the greater amount showed by far the most secretion.

Another study was made of the mammary glands of a group of goats on a diethylstilbestrol lactation experiment. Goats 258, 351, 176, 355 and 569 were started on diethylstilbestrol injections at the rate of 0.25 mg. per day. On the 90th day of injection milking was started and the injections were continued for thirty additional days, or a total of 120 days. At this time injections were discontinued for a 31-day period and then started again and continued for the balance of the experiment. After the experiment had been in progress 169 days (138 days of which the animals were injected and 79 days of which they had been milked), the highest producing goat (258) had produced a maximum amount of milk per day of 690 ml. while the lowest producing goat (569) had produced a maximum of 56 ml. per day.

On the 169th day of the experiment mammectomies were performed on goats 258 and 569. The low producing goat (569) showed a considerable hypertrophy of the duct system with secretion (Figs. 16 and 17) but there was a conspicuous lack of any degree of development of the lobule-alveolar system. This is in contrast to the condition seen in the high producing goat (258). Here there was an extensive development of the lobule-alveolar system which was greatly hypertrophied by secretion (Figs. 18 and 19). The alveoli of this gland were distinctly abnormal in respect both to size and structure. They were larger than alveoli of normal lactating goats and there was histological evidence that they were still undergoing further division in the process of forming new and smaller alveoli. Furthermore the formed alveoli did not seem to be normal in that papillae of a cellular



Figs. 18 and 19.—Microtome section (58x) and dissection (13x), respectively, from a mammary gland of goat 258 which had the same treatment as goat 569 in Figs. 16 and 17. A rather extensive development of the lobule-alveolar system is seen, with evidence of great secretory activity.



nature could be seen protruding into the lumina of the alveoli, indicating that the alveoli were over one cell thick at many places.

Mammary glands of the three remaining goats on the lactation experiment, 351, 176 and 355, which were intermediate in milk production between goats 268 and 569, were also removed seven days after the first group. These glands were very similar in appearance to those of the best producing goat, 258, (Figs. 18 and 19), although rather less developed on the whole.

**3. Discussion.**—De Fremery (1938) has been quoted by various reviewers (Folley et al., 1941a, and Peterson, 1942) as authority for the statement that estrogen will cause complete development of the mammary glands of goats, progesterone being unnecessary. De Fremery, however, merely demonstrated that goats given a preliminary treatment of estradiol benzoate could be stimulated to good lactation by lactogen. No histological examination of the mammary gland tissue was reported as being made. It is well known that a mammary gland with a well developed duct system can be stimulated to copious lactation with lactogen.

In these experiments it was found that an adequate amount of progesterone together with a small amount of diethylstilbestrol was able to stimulate mammary lobule-alveolar growth in virgin female goats comparable to that seen at midpregnancy (Figs. 10 and 11). By further treating these glands with diethylstilbestrol alone for a short period, lactation was initiated accompanied by a great hypertrophy of the alveoli with secretion such as is seen at the time of normal parturition in goats (Figs. 12 and 13).

The response of virgin female goats to diethylstilbestrol both in regard to mammary growth and the degree of lactation stimulated was extremely variable. In some cases very slight if any stimulation of the lobule-alveolar system could be detected (goats 188 and 569). In other cases a considerable development of the lobule-alveolar system was secured (goats 258, 351, 176 and 355). However, the alveolar development secured in these cases was not histologically typical of that of normal lactating glands. The alveoli were much larger and less dense than normal lactating alveoli. The cells lining the alveoli were often more than one cell deep and atypical papillae-like structures were seen protruding into the lumina of the alveoli.

These experiments are taken to indicate that although diethylstilbestrol may stimulate variable degrees of alveolar development, this development is not typical of that seen during normal pregnancy and lactation. Further, these experiments indicate that in all probability the growth of the mammary lobule-alveolar system occurring during pregnancy is accomplished by the joint action of progesterone and estrogen; and that contrary to previous reports, the goat should not be included in the list of animals in which progesterone is unnecessary to secure the complete and full development of the mammary gland such as occurs during normal pregnancy.



This discussion should not be taken to infer that diethylstilbestrol may not be a suitable agent for the stimulation of milk production in non-breeding dairy animals. It has been shown by Folley et al. (1940, 1941b) Walker and Stanley (1941), Lewis and Turner (1942d) and Reece (1943) that diethylstilbestrol will stimulate varying amounts of milk in dairy animals. Reece (1943) has been most successful to date in securing normal milk production in the cow. A 33 month old Jersey heifer which failed to conceive was injected over a thirteen week period with diethylstilbestrol dipropionate. Milking was then started and later a peak production of 33.7 pounds of milk testing 5.09 per cent fat was secured.

The mechanism by which stilbestrol initiates lactation has been rather well established. In addition to stimulating the growth of the mammary gland, stilbestrol stimulates the pituitary to secrete a greatly increased amount of the lactogenic hormone, thus causing the initiation of lactation (Reece and Turner, 1937, Lewis and Turner, 1941b, and Meites and Turner, 1942).

### DISCUSSION

As these investigations have included a rather wide field of study, it is necessary to relate them to the overall picture of mammary gland growth. As a background for this discussion, a general concept of the important endocrine factors controlling mammary gland growth in the female is schematically presented in Fig. 20.

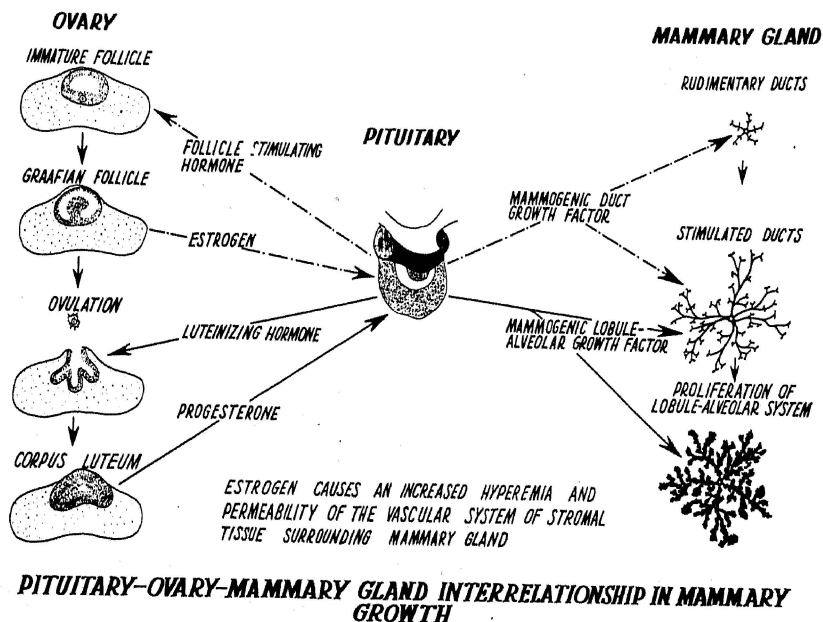


Fig. 20.—Pituitary-ovary-mammary gland interrelationship in mammary growth.

Of the various endocrine glands, only the pituitary and gonads have been shown to be of prime importance in mammary growth. Although a compound, desoxycorticosterone, found in the adrenal cortex, has been shown to stimulate mammary duct (Van Heuverswyn, Folley and Gardner, 1939) and lobule-alveolar growth (Section D), it is not known that the adrenals play any direct part in mammary gland growth. To definitely establish this point, it would be necessary to inject adrenalectomized animals maintained with salt therapy with ovarian hormones or pituitary mammogenic extracts. Positive results would indicate that the adrenals are not necessary for mammary growth.

That the thyroids and parathyroids are not necessary for mammary growth was demonstrated by Dragstedt et al. (1924) who found that completely thyro-parathyroidectomized dogs had a normal hyperplasia of the mammary glands during pregnancy. Of course it is known that experimentally induced hyperthyroidism in the normal female will induce pseudopregnancy and will cause a precocious development of the mammary glands of pregnant rats (Weichert and Boyd, 1933, 1934). There have been other reports indicating that the thyroids have an indirect role in mammary growth. In the experiments reported in Section F of this paper, the thyroids indirectly affected lobule-alveolar growth.

The growth of the mammary gland up to the time of approaching sexual maturity closely follows body growth, indicating that in all probability the mammary gland is largely independent of hormonal control through this period.

However, with the approach of sexual maturity in the female, the anterior pituitary gland begins the increased secretion of a gonadotropic hormone, known as the follicle stimulating hormone, which causes the growth of the ovaries, the maturation of the Graafian follicles and the secretion of estrogen by these follicles. Estrogen has several functions: (1) it stimulates the pituitary to secrete an increased amount of a mammogenic duct growth factor which directly stimulates mammary duct growth; (2) it acts directly upon the mammary gland stromal tissue, increasing its vascularity and also increasing the permeability of the capillaries so that more pituitary mammogen and nutrients reach the region of the growing gland (Section B); and (3) estrogen suppresses the production of the follicle stimulating hormone of the anterior pituitary and stimulates the increased secretion by the pituitary of the luteinizing hormone which induces ovulation and the formation of functional corpora lutea. It has been suggested that the corpora lutea are maintained during pregnancy or pseudopregnancy by a third gonadotropic hormone, luteotrophin, which has been associated with lactogen by some investigators. *A priori* considerations would indicate that luteotrophin and lactogen are not the same hormone as (1) the level of lactogen secretion by the pituitary is low during pregnancy (Reece and Turner, 1937, and Holst

and Turner, 1939), not being significantly higher than in non-pregnant animals, and (2) in certain animals, the cow for instance, estrous cycles are initiated after pregnancy at the height of lactation when the secretion of lactogenic hormone is at its highest level.

The cyclic nature of duct growth, then, is due to the increased estrogen secretion of the ovary during the estrous phase of the cycle, acting both on the pituitary and on the mammary gland stromal tissue.

Evidence has already been presented indicating that estrogen injections increased the pituitary content of a mammogenic factor. Conclusive evidence is not at hand at the present time which will show whether or not this mammogenic duct growth factor is a new hormone, separate from the other recognized hormones. However, Gardner and White (1942) are of the opinion that this factor is lactogen.

Progesterone is produced by the corpus luteum which persists after conception. It is suggested that progesterone stimulates the anterior pituitary to an increased secretion of a mammogenic lobule-alveolar growth factor which directly stimulates mammary lobule-alveolar growth. The estrogen present continues to influence favorably the vascularity and permeability of the capillaries in the mammary gland stroma, and thus not only synergistically influences the activity of the lobule-alveolar growth factor, but directly stimulates the growth of the teat and the mammary gland stromal tissue.

There is no direct evidence at the present time that progesterone does cause the increased secretion of a pituitary mammogenic lobule-alveolar growth factor, other than the facts that (1) progesterone will stimulate lobule-alveolar growth in normal animals, (2) progesterone is ineffective in stimulating lobule-alveolar growth in hypophysectomized animals, and (3) that pituitary materials do contain a lobule-alveolar growth factor. Data reported in this paper indicate that the lobule-alveolar growth factor is not identical with lactogen, gonadotropin or thyrotropin. Other workers, however, have associated lobule-alveolar growth activity with lactogen, adrenotropin and growth hormone. At the present time it still seems reasonable to postulate that there is a specific mammogenic lobule-alveolar growth factor which is separate from the other known anterior pituitary hormones.

Throughout the course of these investigations there has been no reason to question the fundamental thesis that the sex hormones act through the pituitary to promote mammary gland growth in the normal animal both during puberty and pregnancy. However, several recent reports on this subject should be considered in the light of the discussion. Gardner (1940) secured slight mammary growth in hypophysectomized male mice by the injection of desoxycorticosterone acetate, progesterone or estradiol dipropionate. Somewhat more growth was secured with either desoxycorticosterone acetate or progesterone plus estradiol dipropionate. Gardner and White (1942)

secured additional confirmation of these observations. These results have been confirmed in part by Trentin and Turner (1942).

Recently, Leonard (1943) reported that estradiol dipropionate stimulated mammary gland duct end buds in hypophysectomized immature male or female rats if (1) they weighed less than 70 gms. at operation, (2) injections were begun immediately after operation, and (3) glands were examined after ten to twelve days of treatment. He suggested that mammary glands retain for a certain period of post-natal life, potentialities for growth which can be stimulated by estrogen, but after this critical period this potentiality is lost and the cells are no longer affected by estrogen.

It would seem from these reports that the mammary gland in common with several pituitary "target glands" retains for a limited period of time a measure of autonomous activity apart from the pituitary stimulation and control. This activity may be stimulated to a very limited extent by certain of the sex and adrenal hormones. An analogous situation exists between the adrenal and the pituitary (Miller and Riddle, 1942).

Although our knowledge of the exact mechanisms controlling mammary gland growth is incomplete at the present time, it is felt that the theory of direct pituitary control of mammary gland growth as discussed in this paper is in general harmony with the existing data in this complex field of investigation.

### SUMMARY

1. Mammary lobule-alveolar growth responses were secured in castrate virgin female mice with anterior pituitary materials injected over periods of time ranging from four to ten days. These responses were not very predictable or repeatable on re-assay.

2. The simultaneous injection of pituitary preparations and a small amount of estrone greatly reduced the amount of pituitary required to secure alveolar responses and the dosages of pituitary preparations injected were proportional to the per cent positive lobule-alveolar responses secured in groups of assay mice.

3. In the development of an assay method for the mammogenic lobule-alveolar growth factor, a ten-day assay period was found to be optimal.

4. The length of time elapsing between ovariectomy of the assay mice and the beginning of injection affects the mammary response secured, the shorter the time, the greater the mammary response.

5. An assay for the mammogenic lobule-alveolar growth factor was formulated. A mouse unit of this factor was defined as the amount of material required per mouse injected over a ten-day period to obtain minimal lobule-alveolar growth in  $50 \pm 10$  per cent of ten or more castrate nulliparous female mice when a total of 75 I. U. of estrone is simultaneously injected. Injection of the assay animals should start

immediately after ovariectomy unless they are first primed with estrogen for several days preliminary to the start of injections.

6. Estrone in amounts of 40 I. U. to 133 I. U. were found to synergize best with one mg. of progesterone in stimulating mammary lobule-alveolar growth. Greater or smaller amounts of estrone did not give optimum synergism with progesterone.

7. Progesterone or pregneninolone alone caused lobule-alveolar growth. However, five or six times as much was required as if estrogen was also injected.

8. Although 2400 I. U. of estrone was able to completely inhibit the activity of a mouse unit of progesterone (one mg.), it was unable to inhibit the activity of a mouse unit of a pituitary preparation.

9. As a result of a series of experiments with progesterone, pregneninolone, pituitary extracts and estrogen, it was suggested that estrogen enhances the activity of progesterone and pituitary materials in stimulating mammary lobule-alveolar growth by acting directly on the stromal tissue surrounding the mammary gland producing an increased hyperemia and vascularity associated with an increased permeability of the vascular system. This condition would allow a circulating pituitary mammogen to be maximally effective in causing mammary gland growth.

10. Both estradiol benzoate and diethylstilbestrol were able to substitute for estrone in conjunction with progesterone in enhancing mammary lobule-alveolar growth.

11. Assays of various types of pituitary extracts showed that the mammogenic lobule-alveolar growth factor is protein in nature. These assays also indicate that this factor is not identical with lactogen, thyrotropin or gonadotropin.

12. Progesterone, pregneninolone, desoxycorticosterone, dehydroandrosterone, diethylstilbestrol, acetoxy-pregnenolone, and methyl testosterone ranked in the above order in their ability to stimulate mammary lobule-alveolar growth.

13. High environmental temperature inhibited the ability of progesterone and estrone to stimulate mammary lobule-alveolar growth. This same high temperature was unable to inhibit the ability of a pituitary preparation to stimulate lobule-alveolar growth.

14. Thyroxine in suitable amounts increased by about 33 per cent the efficiency of progesterone in stimulating lobule-alveolar growth.

15. Thyroidectomy greatly decreased the efficiency of both progesterone and pituitary preparations in stimulating mammary lobule-alveolar growth.

16. Virgin female goats injected daily with twenty or thirty mg. of progesterone plus 100 or 150 micrograms of diethylstilbestrol, respectively, for sixty days were stimulated to develop mammary glands similar to that seen in midpregnancy. Twelve days additional treatment with 0.25 mg. daily of diethylstilbestrol caused an initiation of

secretion in these mammary glands similar to that seen at the time of parturition.

17. The response of virgin female goats to diethylstilbestrol in regard to mammary lobule-alveolar growth was extremely variable. In some cases very slight stimulation of the lobule-alveolar system was effected while in others a considerable development was secured.

18. Lobule-alveolar growth secured with diethylstilbestrol injections in goats was not histologically typical of that seen in normal lactating glands. The alveoli were much larger and less dense than normal lactating alveoli. Abnormal papillae-like structures were seen protruding into the lumina of the alveoli.

19. The over-all picture of mammary gland development as affected by the various endocrine glands was discussed.

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