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The Experimental Induction of Growth of the Cow's Udder and the Initiation of Milk Secretion

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CONTENTS

SUMMARY

This experiment concerned the induction of growth of the mammary glands of the cow's udder by hormone injection, followed by experimental induction of milk secretion. A total of 20 heifers were used. These included 11 of low fertility, three sets of normal twins, four freemartins, and two normal animals.

Sixteen of these animals were injected daily with a mixture of 100 mg. of progesterone and 100 ug. of estradiol benzoate for a period of 180 days to induce lobule-alveolar mammary gland growth. Four animals were fed 10 mg. daily of diethylstilbestrol in place of the estrogen.

Milk secretion was then stimulated by the injection of 3 mg. of estradiol benzoate daily for 14 days or more. All of the animals came into milk production except three freemartin heifers. The rise in milk production continued for periods of 5 to 14 weeks. The mean daily maximum milk yield was 22.7 pounds.

On the basis of all comparisons, it was concluded that the milk yield of these animals, which were induced to lactate experimentally, was from 80 to 90 percent of that which they might have produced had they calved normally.

It is interesting to note that many of the heifers of low fertility (had failed to conceive after repeated insemination) were bred after the induction of lactation and a number of animals gave birth to normal calves.

ACKNOWLEDGMENTS

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The Experimental Induction of Growth of the Cow's Udder and the Initiation of Milk Secretion

C. W. TURNER

INTRODUCTION

The productivity of dairy cattle, to a considerable extent, is determined by the number of milk secreting cells in the cow's udder. If the total number of cells is limited then no matter how intensely milk secretion is stimulated by various hormones, milk production will be limited by the size of the udder. In a study of the factors involved in the inheritance of milk production, the genetic-endocrine factors influencing normal growth of the mammary glands in the four quarters of the cow's udder become of special significance.

Thirty years ago practically nothing was known about the anatomy of the cow's udder or of the changes which occurred in the cow's udder during pregnancy to prepare it to secrete milk at calving time. The Missouri Agricultural Experiment Station has been a leader in research concerned with studies of the anatomy of the cow's udder (Turner, 1952) and with the endocrine aspects of mammary gland growth. Knowledge of the hormones involved in mammary gland growth have been aided greatly by studies involving the use of small experimental animals.

As more and more was learned concerning the hormones which stimulated mammary gland growth in experimental animals, it was our ambition to extend our observations to the dairy cow.

To understand the problems involved, it is necessary to review briefly the observations concerning normal development of the mammary gland and the hormones involved in mammary gland growth.

In many experimental animals and in heifers, the initial growth has been shown to be branching tubules or ducts. These ducts extend outward from the teats or, in the case of cattle, extend from the milk cisterns into the udder which, at this time, is composed of fatty tissue. The stimulus for the extension of these ducts comes with each estrus cycle. Comparable growth of the duct system can be induced experimentally by the injection or feeding of the ovarian hormone called estrogen (synthetic type, diethylstilbestrol).

When animals become pregnant, there is further extension of the duct system but, in addition, there is a new type of growth called the lobule-alveolar

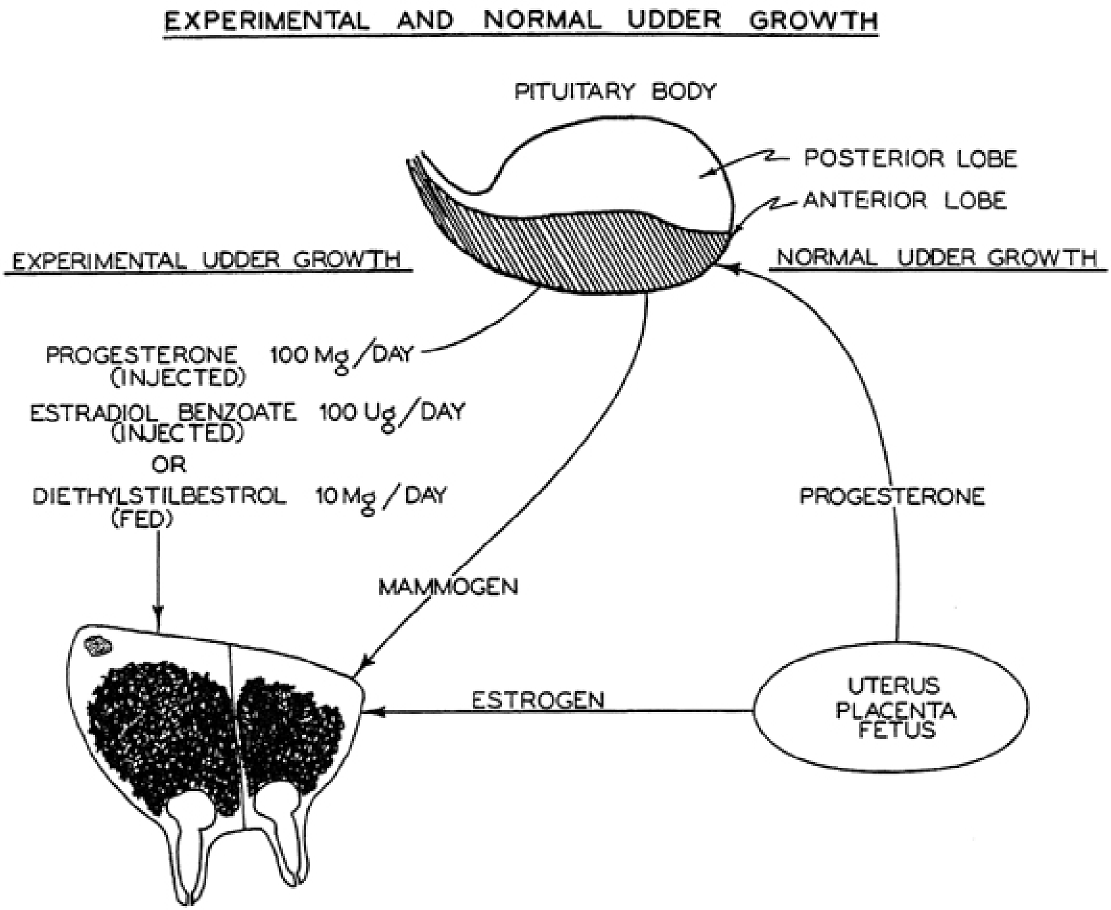

Fig. 1—During pregnancy, estrogen and progesterone are secreted by the ovary and placental membranes (afterbirth). The estrogen acts directly upon the mammary glands, increasing the amount of blood flowing to the udder and the permeability of the blood capillaries, permitting increased amounts of blood plasma to nourish the growing cells. The progesterone secreted by the corpus luteum and placental membranes acts upon the pituitary and stimulates an increased secretion of mammogen. Mammogen flows to the udder, stimulating the growth of the lobule-alveolar system.

The study demonstrated that the estrogen and progesterone normally secreted by the uterus, placenta and fetus of the pregnant heifer can be injected into heifers, in the amounts indicated, to stimulate udder growth without breeding them.

system. At the sides and the ends of the duct system numerous spherical cellular structures develop, called alveoli, with the cells facing a cavity called the lumen. While the cells lining the duct system are capable of secreting milk, the bulk of the milk produced is secreted by the cells of the alveoli. The growth of the lobule-alveolar system continues during pregnancy. Cells which will secrete

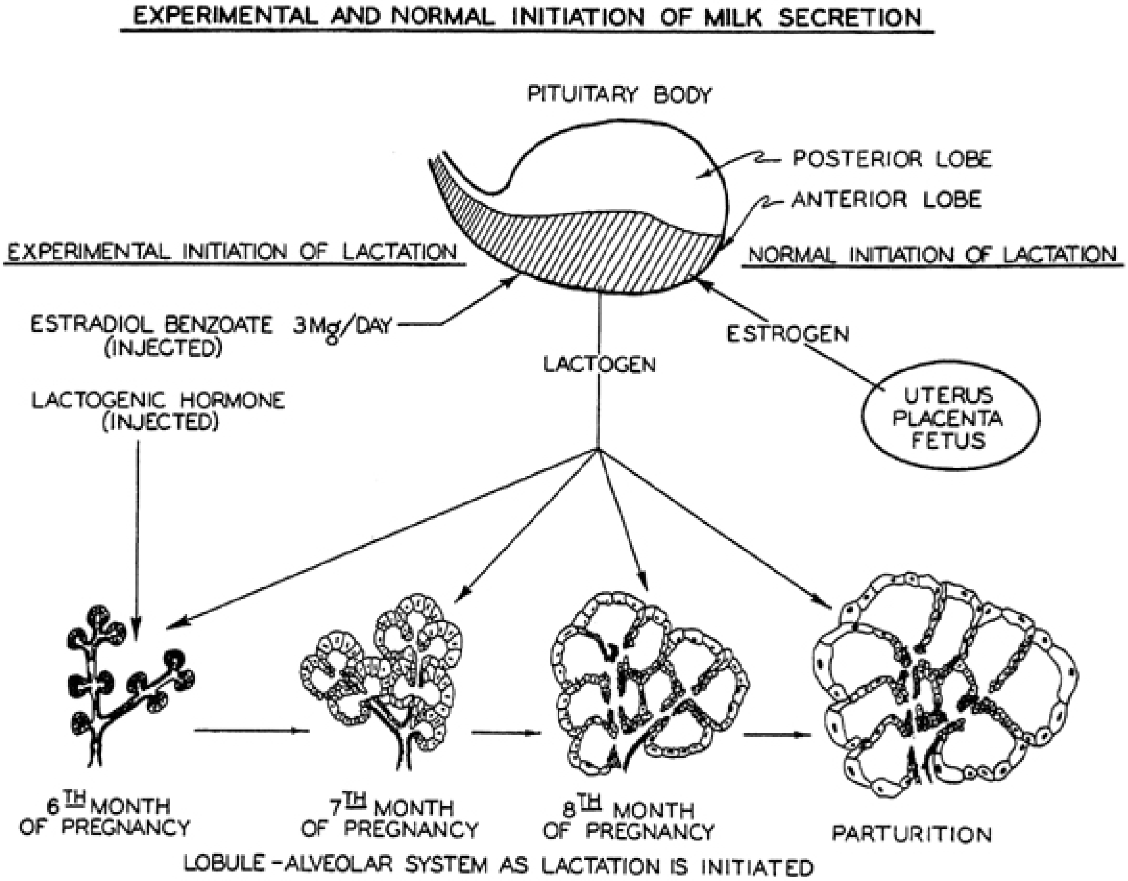

Fig. 2—In normal pregnant animals, the increased secretion of estrogen (shown in Fig. 3) stimulates the secretion of lactogen by the pituitary. Lactogen, in turn, stimulates the cells of the alveoli to secrete milk. Under experimental conditions, when the growth of the udder has been stimulated by estrogen and progesterone, lactation can then be induced by the daily injection of estrogen for a period of about two weeks. The filling of the alveoli with milk causes the marked enlargement of the udder observed at this time.

milk during the following lactation period are formed. The growth of the lobule-alveolar system is under the influence of two hormones, estrogen and progesterone, which are first secreted by the ovaries of the pregnant animal. Later in pregnancy the blood levels of these two hormones are augmented by increasing secretion from the placental membranes (Fig. 1).

During the final one-third of pregnancy, the milk secretion cells are gradually stimulated to begin secreting milk. Until this time the cells of the alveoli are small and the lumen do not appear. As secretion is initiated, the cells elongate with contained milk (Fig. 2). The milk, or colostrum as it should be called, passes from the cells and begins to fill the lumen of the alveoli. As more and more colostrum is secreted, the udder of the heifer begins to show external enlargement and "bagging-up." This enlargement results from the secretion of colostrum. During the last 2 or 3 weeks of pregnancy the pressure of the contained colostrum may cause swelling and an edematous condition of the udder and surrounding subcutaneous areas.

The stimulus of milk secretion in late pregnancy has been shown to be due to the increased secretion of estrogen by the placenta which, in turn, stimulates the increased secretion of the lactogenic hormone of the anterior pituitary.

The lactogenic hormone has a very important influence upon the alveolar cells of the udder. This hormone stimulates the enzyme systems of the cells making it possible for the cells to use the milk precursors from the blood and convert them into the various unique constituents of milk. Thus, blood glucose is converted into lactose, the blood amino acids are converted into casein, and the blood fats and acetic acid are converted into milk fat. Without the lactogenic hormone, milk secretion does not take place.

The Problem of Experimentally Stimulating Mammary Gland (Udder) Growth in Cattle: In spite of the extensive research concerning the role of the two ovarian hormones (estrogen and progesterone) in stimulating the growth of the lobule-alveolar system in experimental animals, the problem of determining an effective dosage for cattle was very great. The following facts were considered in deciding upon the amounts of the two hormones which were used in our initial experiments.

Estrogen and Progesterone: Our earlier studies (Mixner and Turner, 1943) had shown that the primary effect of estrogen in promoting lobule-alveolar growth concerned the increase in blood supply to the mammary gland. It was suggested the increased permeability of the capillaries permitted a greater flow of blood plasma to the intercellular spaces of the glands, thus increasing both the nutrient supply and the concentration of circulating hormones effective in mammary gland growth. Thus, estrogen is believed to act locally upon the glands. In small animal experiments, with increasing levels of estrogen and a constant optimal amount of progesterone, it has been shown that insufficient amounts of estrogen may limit lobule-alveolar growth (Damm and Turner, 1957). On the other hand, excessive amounts of estrogen may be detrimental to the growth process due to the stimulation of milk secretion. It is necessary that optimal amounts of estrogen be administered. Many years ago (Turner *et al.,* 1930) the estrogen excreted in the urine of cows in early pregnancy was shown to be low in amount. This was confirmed in recent work in which the estrogen in both the urine and feces was determined (El-Attar and Turner, 1957).

The level of progesterone used was based upon the requirements for progesterone to maintaine pregnancy in dairy heifers from which the corpora lutea had been removed. The observations of Raeside and Turner (1950), Uren and Raeside (1951), and McDonald *et al.* (1952) indicated that progesterone at the rate of 100 mg./day/1000 pounds body weight would maintain early pregnancy in the absence of the corpora. It is quite possible that greater amounts of progesterone are secreted daily and, further, there may be considerable variation in the daily secretion rate of progesterone by individual cows. Thus, dairy cattle which develop large glands during pregnancy may secrete far more progesterone per day than is required for the maintenance of pregnancy.

However, for our preliminary experiments it was decided to inject 100 mg. of progesterone per day. To synergize with the progesterone, the estrogen level decided upon was based upon the observations in experimental animals. Previous studies had shown, in the case of the mouse (Elliott and Turner, 1953; Yamada *et al.,* 1954; and Damm and Turner, 1957), rat (Kirkham and Turner, 1954), and dog (Trentin *et al.,* 1952), the ratio of the two hormones for optimal synergism was of the order of 1 part of estrogen to 1000 or more parts of progesterone. Therefore, to synergize with 100 mg./day of progesterone it was decided to inject 100 μg./day of estrogen/1000 pounds body weight.

In place of injecting the estrogen, a few heifers have been fed diethylstilbestrol at a level of 10 mg. per day with the progesterone by injection. This level of diethylstilbestrol was fed in our initial experiment because it exhibited a pronounced effect upon the growth of the teats and duct system of beef animals (Turner, 1956a) when it was fed to them to increase rate of body growth.

It should be emphasized that the levels of estrogen and progesterone administered are tentative and further adjustment will be made in the light of the results.

Time Required to Stimulate Complete Mammary Gland Growth: Extensive studies on the period during which mammary gland growth occurs in normal pregnancy indicate that the growth of the lobule-alveolar system continues during the first two-thirds of pregnancy. Following this period, the cells begin to secrete milk. In recent work in our laboratory it has been shown that growth of the mammary gland in rats continues during the latter part of pregnancy and early lactation as well.

In these preliminary experiments it was decided to stimulate the growth of the heifers' udders by the injection of the two hormones for a period of 180 days; though a shorter period of injection would be effective in stimulating considerable growth.

Experimental Initiation of Lactation: The secretion of milk is gradually initiated during the final 100 days of pregnancy. This is caused by the increased secretion of the lactogenic hormone by the anterior pituitary. It has been shown in experimental animals that after mammary gland growth is complete, the injection of the lactogenic hormone will quickly stimulate the cells of the glands to secrete milk. This procedure has been demonstrated in heifers, bringing them into lactation after udder growth has been stimulated. However, the amounts of lactogenic hormone required are rather large. Due to the scarcity of the lactogenic hormone, this procedure would not be practical.

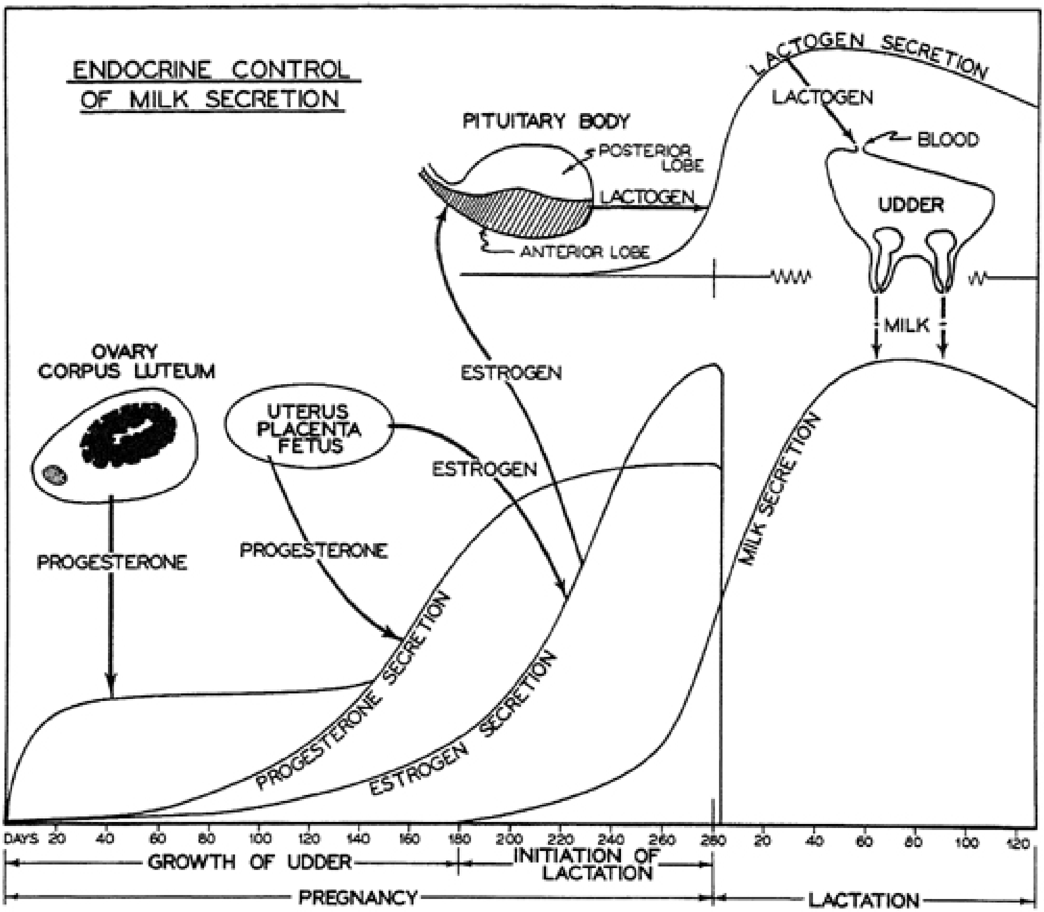

Fig. 3—During pregnancy, two hormones, estrogen and progesterone, are secreted by the ovary and the corpus luteum, and later by the placenta. These hormones stimulate growth of the milk secreting cells of the udder. In late pregnancy estrogen is secreted in increased amount and causes the pituitary to secrete lactogen which, in turn, causes the cells of the udder to begin to secrete milk. Following parturition, the secretion of lactogenic hormone is further increased by the regular discharge of the hormone at each milking. This causes the rise in milk secretion until a peak is reached at about 60 days.

It seemed more desirable to initiate experimental lactation in the heifers by the mechanism that causes the initiation of normal lactation in late pregnancy (Fig. 3). The early observation by Reece and Turner (1936-37) that estrogen stimulated increased secretion of the lactogenic hormone, followed by the work of Meites and Turner (1948), suggested that the normal increase in lactogenic hormone secretion in late pregnancy was due to increase in the secretion of estrogen at that time.

The rise in estrogen secretion in late pregnancy in heifers has been confirmed by recent work (El-Attar and Turner, 1957). Possibly the estrogen secreted gradually stimulates an increase in the number and secretory activity of the acidophilic cells of the anterior lobe of the pituitary. The increased lactogen secreted, in turn, stimulates the initiation of milk secretion (Fig. 3).

There is increasing evidence that estrogen also stimulates the secretion of the growth hormone which is secreted by acidophilic cells. Thus, at the time of parturition, the acidophilic cells are prepared to secrete increased amounts of both lactogenic and growth hormones. Evidence of the great importance of the growth hormone in stimulating intense milk secretion has been presented (Turner, 1956b; Turner *et al.*, 1957).

A recent study indicated estrogen also stimulates an increase in secretion of the thyrotropic hormone and, in turn, an increase in secretion of thyroxine. If further study of this problem confirms these preliminary observations, the key importance of estrogen in stimulating the lactation process will be demonstrated (Pipes *et al.*, 1958).

In normal pregnancy estrogen secretion begins to increase markedly only after 160 to 180 days. It has been observed that the level of estrogen is high by the 200th day of pregnancy and continues high until parturition.

To simulate the effect of estrogen during late pregnancy in initiating the lactation process, the levels of estrogen and progesterone used to cause the growth of the udder were discontinued and 3 mg. of estrogen daily was administered for a period of two weeks or longer if lactation had not been stimulated by that time.

Index of Mammary Gland Growth: The type and extent of mammary gland growth has been observed following the sacrifice of experimental animals and examination of whole mounts of the glands or, more recently, by the determination of the amount of desoxyribosenucleic acid (DNA) present in the glands (Damm and Turner, 1957). This latter method is based upon the observation that each mammary gland epithelial cell contains a constant amount of DNA (Griffith and Turner, 1957). As the mammary gland lobule-alveolar system is stimulated to growth during pregnancy by the ovarian hormones of pregnancy, the number of cells increases and, correspondingly, the amount of DNA, which can be determined quantitatively by a chemical method. It is thus possible to estimate very accurately the extent of mammary gland growth.

The value of the individual cow precludes a direct estimation of the extent of mammary gland growth at the end of the period of experimental growth stimulation. The best alternative appeared to be an indirect and less precise index—the maximum daily milk yield (weekly average) following the initiation of lactation by means of estrogenic hormone.

It is realized that this index of mammary gland growth may be inaccurate for two reasons. First, the best method (amount and duration of estrogen stimulation) of initiating lactation is still subject to experimental determination. Second, it must be assumed that the number of epithelial cells present is directly related to the yield of milk.

This assumption might be true if all the hormones influencing the intensity of milk secretion were being secreted in optimal amounts. However, cows differ in their rates of secretion of lactogenic, thyrotropic, growth, and other hormones influencing the intensity of lactation. As a result maximum milk yield may vary to some extent due to the influence of these hormones as well as due to the extent of gland growth. In spite of these considerations, maximum milk yield appeared to be the best index available for our study.

Description of Cattle Used: Most of the animals used were heifers of low fertility being discarded from the University herds due to failure to conceive after a number of services. Several sets of twins were available of normal fertility. A few freemartin heifers were included. Details of the data on each animal are in Table 1.

Management: All lactating animals were milked twice per day. The concentrate mixture was the same as that used in the University dairy herd. The amount of concentrate was usually fed up to the animal's capacity. The quality of alfalfa hay varied, but an attempt was made to obtain high quality roughage. The roughage was fed outdoors in a bunker.

The animals were kept under dry lot conditions. They were subject to outdoor environmental conditions all seasons of the year.

Estimations of the daily thyroxine secretion rates of these animals were made at intervals during the entire period the animals were in the herd (Premachandra *et al.,* 1958).

TABLE 1--DATA ON EXPERIMENTAL CATTLE

Herd No.	Breed	Date of Birth	Experimental Growth of Udder Started	Age at Initiation of Lactation (Months)	Body Weight At Start (Lbs.)	Body Weight At End (Lbs.)	Remarks
310	Jersey	9/22/51	5/25/54	31	878	961	Low fertility
580	Jersey	6/13/51	6/11/54	36	840	902	Low fertility
583	Jersey	12/9/51	7/6/54	43	790	914	Low fertility. One blind quarter
585	Jersey	4/24/52	7/6/54	25	825	922	Low fertility
402	Holstein	7/5/51	6/11/54	35	1260	1399	Low fertility. Twin to No. 401
160	Jersey	5/30/50	2/7/55	61	987		Low fertility
544	Jersey	6/-/51	2/7/55	48	947		Low fertility
159	Jersey	12/2/53	7/1/55	24	539	766	Fertile twin of No. 158
G42	Jersey	1/21/55	9/15/56	28	732	967	Fertile twin of No. G41
24	Jersey	2/1/55	9/15/56	27	650	800	Fertile twin of No. 76
180	Guernsey	2/15/54	2/15/55		732	889	Freemartin
18	Guernsey	4/23/54	3/1/56	29	927	1048	Freemartin
102	Guernsey	7/23/55	11/1/56		668	889	Freemartin
110	Guernsey	8/17/55	11/1/56		637	732	Freemartin
601	Holstein X Jersey cross	10/11/56	5/1/58	25	650		Normal
38	Guernsey	8/26/54	5/23/58	51	1185		Normal

RESULTS

Growth Phase: For the first 180 days the heifers were injected once daily, subcutaneously, with 1.2 ml. sesame soil containing a mixture of 100 mg. progesterone and 100 μg. of estradiol benzoate. At the end of this period no attempt was made to estimate the extent of mammary gland growth stimulated for the reasons mentioned previously. Externally, there was little change in the size of the udders during this period. This was expected since the ducts and lobule-alveolar systems invade the preformed fatty substance of the udder (Turner, 1952). Slight lengthening of the teats usually occurred. The skin of the udders showed some evidence of increased hyperemia.

During the period of the injection of these hormones, as during pregnancy, estrous cycles were inhibited. None of the undesirable features exhibited by cattle administered estrogen alone such as mounting and pelvic or vaginal changes were observed. Body weight gains were satisfactory. In spite of the long continued daily subcutaneous injection of hormones, no undesirable reaction of the skin at the site of the injections was noted.

Lactation Phase: At the end of 180 days, the injection of the mixture of estrogen and progesterone was discontinued. The next step was to attempt to initiate milk secretion in the previously grown udders. For this purpose 3 mg. daily of estradiol benzoate was injected subcutaneously. Injections were continued for 14 days or longer.* Twice daily milkings at about 7 a.m. and 4 p.m. were commenced the day after the first injection. The mammary secretion at first consisted of a sticky, honey-like material, which rapidly developed a colostrol appearance and gradually changed to normal appearing milk. A marked increase in milk yields commenced between the third and sixth days in most of the heifers. In these animals the injection of estrogen was stopped after 14 days. Some heifers still weren't giving much milk after 14 days. Such animals were injected an additional seven days or longer (Fig. 4).

A total of 16 heifers received this treatment (Table 1). Of this group seven heifers were of low fertility, i.e., they were discarded from the herds of the University for failure to conceive after repeated breeding. Two were normal heifers. In this group, one heifer (No. 402) was a twin to a heifer which was normal as to breeding performance. Three heifers were normal twins. Of these, two sets were diagnosed as monozygous and one dizygous. Their normal mates were bred at the time experimental treatment was initiated to serve as comparisons of productive ability. Four freemartin heifers were included in this study.

*While estrogen and progesterone were administered together, the cows showed no signs of heat (estrus). However, when the progesterone was discontinued and 3 mg. of estradiol benzoate was injected, the animals all uniformly came into intense heat. At first the heifers were allowed to run together, but excessive sexual activity (riding) resulted in injury and, in one case, a broken pelvis. It is necessary, therefore, to isolate the animals during the period of estrogen treatment to prevent injury to the animals.

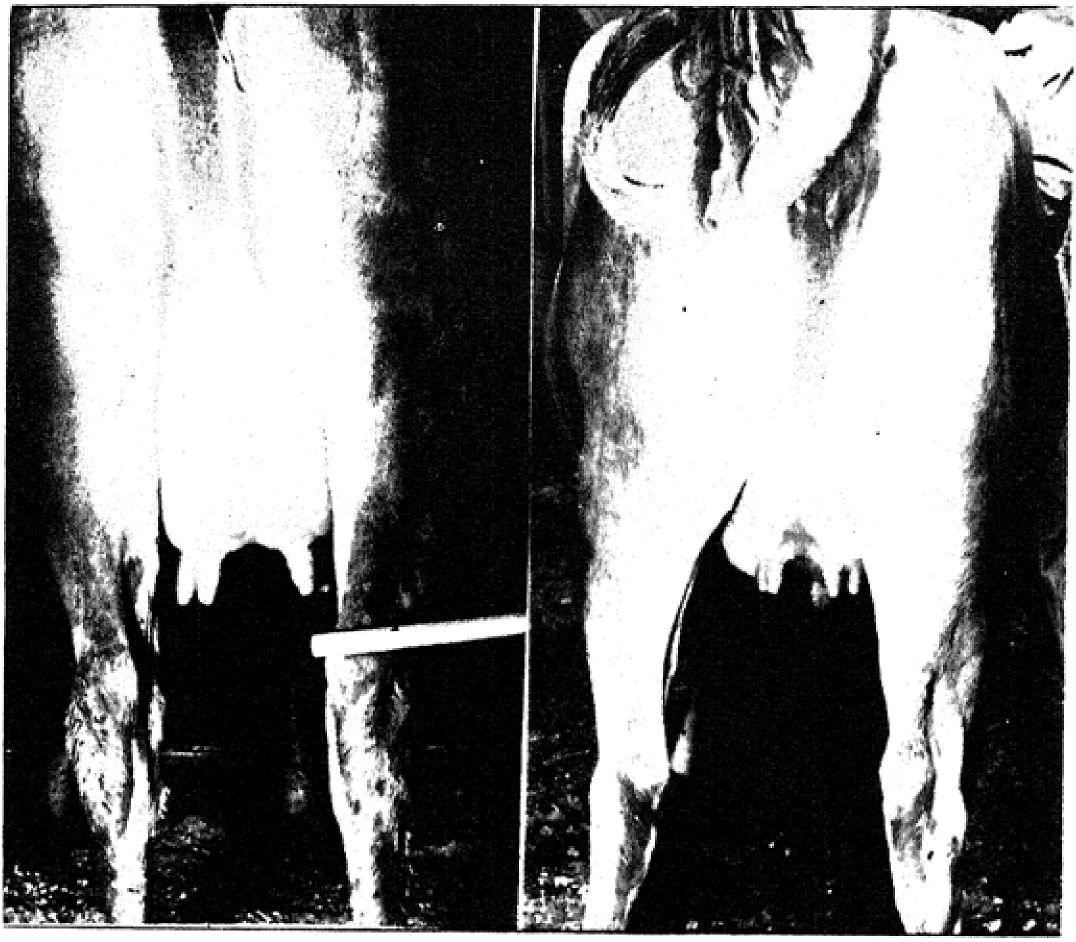

Fig. 4—The udder of the heifer at left has been stimulated for experimental growth of the lobule-alveolar system during a period of 180 days. Little change in external appearance of the udder was noted during this period. The lack of external change is due to the fact that the growth of the lobule-alveolar system penetrates the fatty pad of the udder.

The udder of the heifer at right shows great enlargement resulting from experimental stimulation of the lactation process.

Twelve heifers showed rapid increases in milk production following the administration of estrogen, whereas three of the four freemartins failed to secrete milk. While the estrogen was administered for a period of 2 or 3 weeks, the rise in milk production continued for periods ranging from 5 to 14 weeks (Table 2 and Figs. 5-9).

Within a few days following the initiation of estrogen treatment and concurrent with the initiation of milk secretion, marked edema of the subcutaneous tissue of the udder was observed. The edematous condition extended anteriorly from the udder to the navel. The condition persisted during the entire period of estrogen injection but gradually subsided following the cessation of injections.

TABLE 2--MAXIMUM MILK PRODUCTION EXPERIMENTALLY STIMULATED
(First Induced Lactation)
(Growth of udder stimulated with estradiol benzoate, 100 ug. and progesterone, 100 mg. daily for 180 days. Lactation then stimulated by estradiol benzoate, 3 mg./day, for 14 days or longer.)

Herd No.	Breed	Time to Reach Maximum Milk Production (Week)	Milk Yield at Maximum (Lbs./Day)	Fat Content at Time of Maximum Production (%)	FCM Maximum (Lbs./Day)
310	Jersey	9	18.2	6.4	25.3
580	Jersey	8	22.9	5.1	27.2
583	Jersey	9	12.2	5.1	13.9*
585	Jersey	5	18.2	5.7	22.3
402	Holstein	14	33.3	3.7	31.3
160	Jersey	8	27.3	4.6	29.8
544	Jersey	10	29.6	3.5	27.4
159	Jersey	12	22.4	3.8	21.7
G42	Jersey	6	19.6	7.7	22.6
24	Jersey	12	24.2	3.5	22.4
180	Guernsey		(Freemartin - lactation not induced)		
18	Guernsey	4	13.1	3.9	13.0
102	Guernsey		(Freemartin - lactation not induced)		
110	Guernsey		(Freemartin - lactation not induced)		
601	Holstein X Jersey cross	11	23.0	4.0	23.0
38	Guernsey	7	24.7	5.4	29.8
	Average (not including No. 18)		23.0		24.7

* One blind quarter

This reaction of estrogen is believed to be due to increased hyperemia and permeability of the capillaries of the udder and skin, permitting an increased influx of blood plasma into the glands and skin. Estrogen produces a similar phenomenon in the uterus, resulting in a marked increase in uterine weight.

The increased flow of blood to the udder, which bathes the alveolar cells with a rich nutrient supply and lactogenic hormone, plays an important role in the rapid initiation of milk secretion.

The rising segments of the lactation curves were somewhat more prolonged than in cows calving normally. It will be recalled that milking was commenced in the experimental animals as soon as the lactation-inducing hormone was injected. On the other hand, in normal animals the cows receive the lactation stimulus (estrogen) in late pregnancy and this hormone is withdrawn at parturition. During this period the animals "bag up" and are prepared to lactate following parturition. If the experimental animals had been allowed to "bag up" during the first 14 days of estrogen treatment, their lactation curves would probably have been quite comparable to those calving normally.

The maximum daily milk yield (based on a weekly average) and its F.C.M. equivalent for each animal is presented (Table 2). The yield of milk varied from 12.2 pounds for cow No. 583, with three functional quarters, to 33.3 pounds for

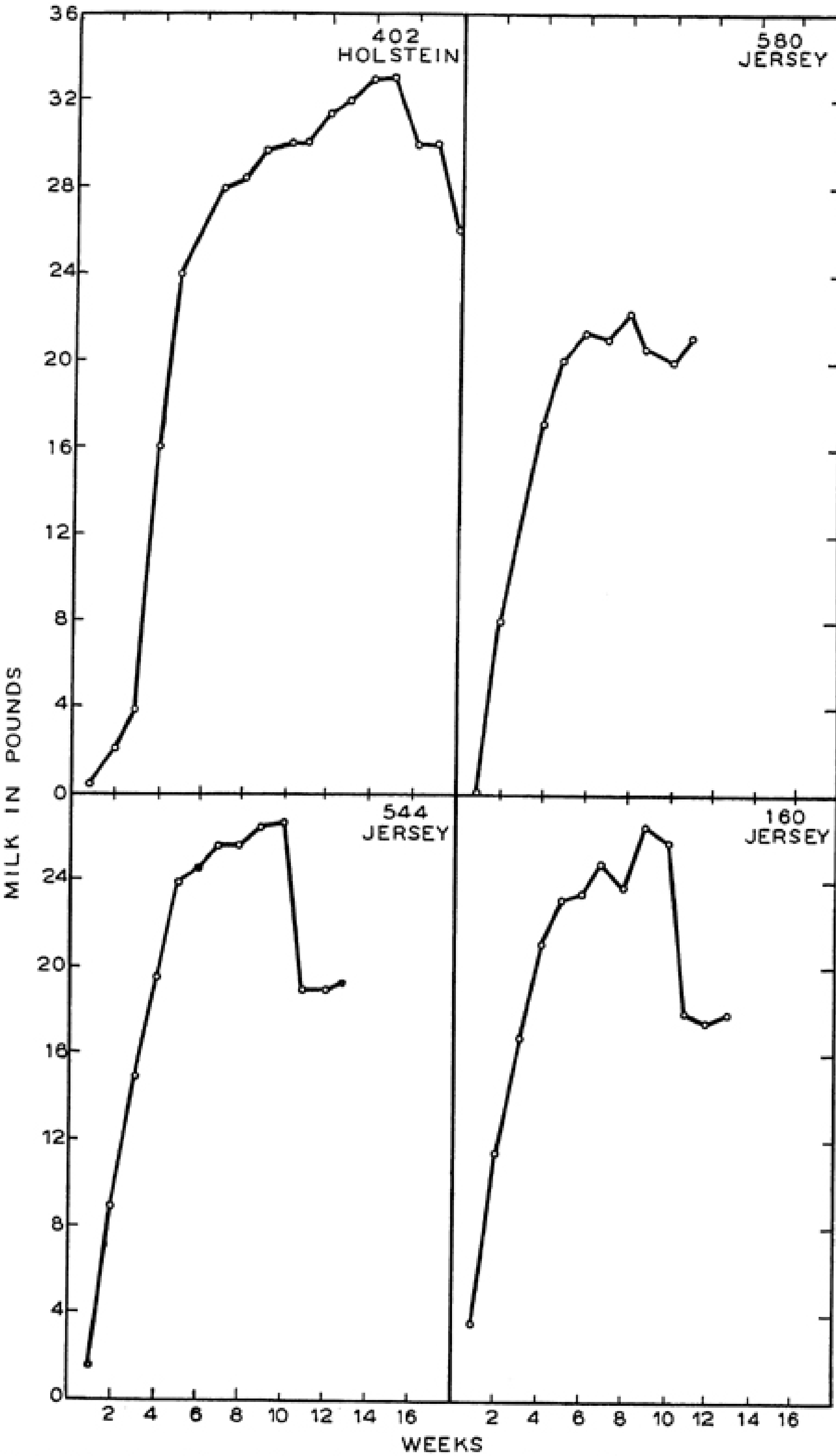

Fig. 5—Following the udder development phase, lactation was initiated by the daily injection of 3 mg. of estradiol benzoate for 14 days or more. Milk secretion began within a week, in most cases, and continued to increase for many weeks after estrogen injections were stopped (see Table 2).

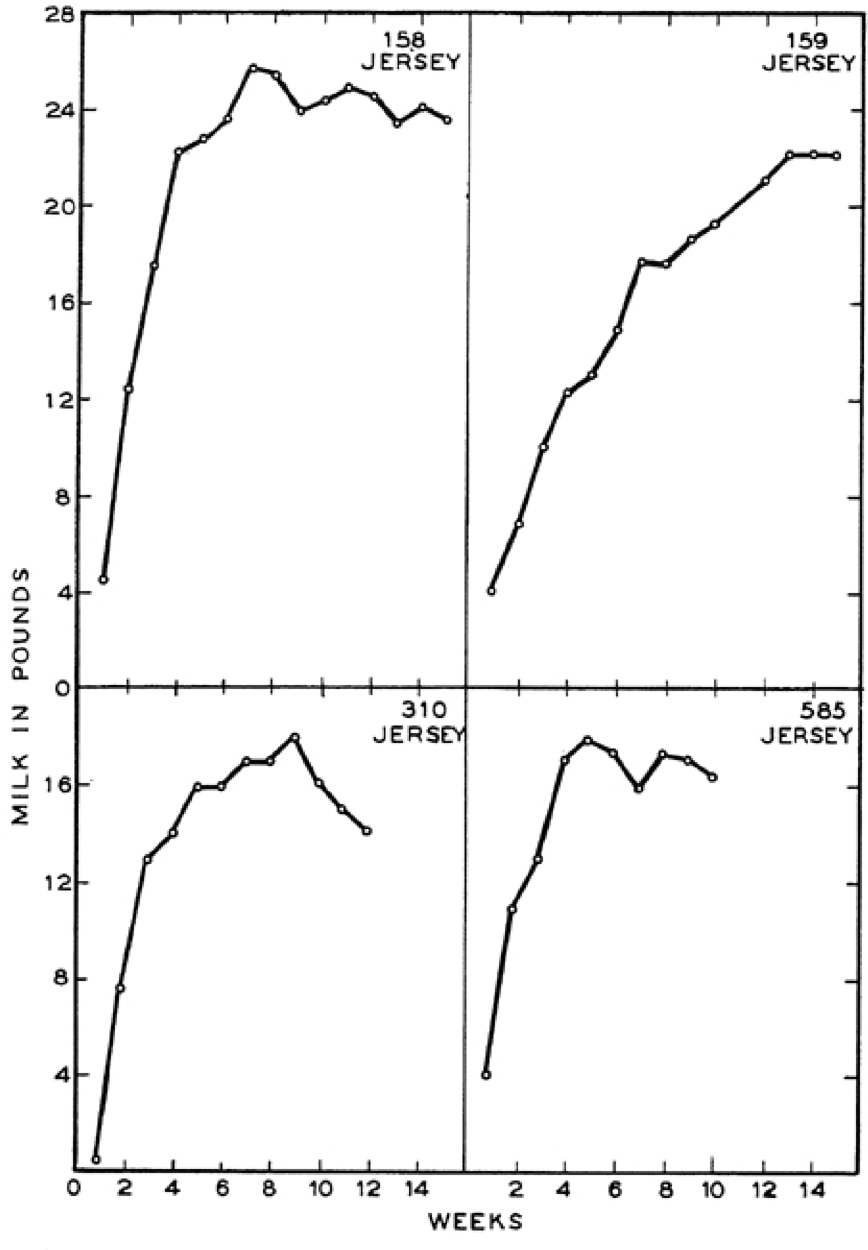

Fig. 6—Jersey 158, a twin of 159, calved and lactated normally. The other three curves are the rising segments of the lactation curves of three heifers that were induced to lactate experimentally with 3 mg. of estradiol benzoate for 14 days, after experimentally stimulating udder growth. These curves indicate the difference in normal and experimental initiation of lactation. No. 159 produced 85.2 percent as much milk at her peak as did her twin No. 158. No. 158 and 159 have been diagnosed as monzygous twins.

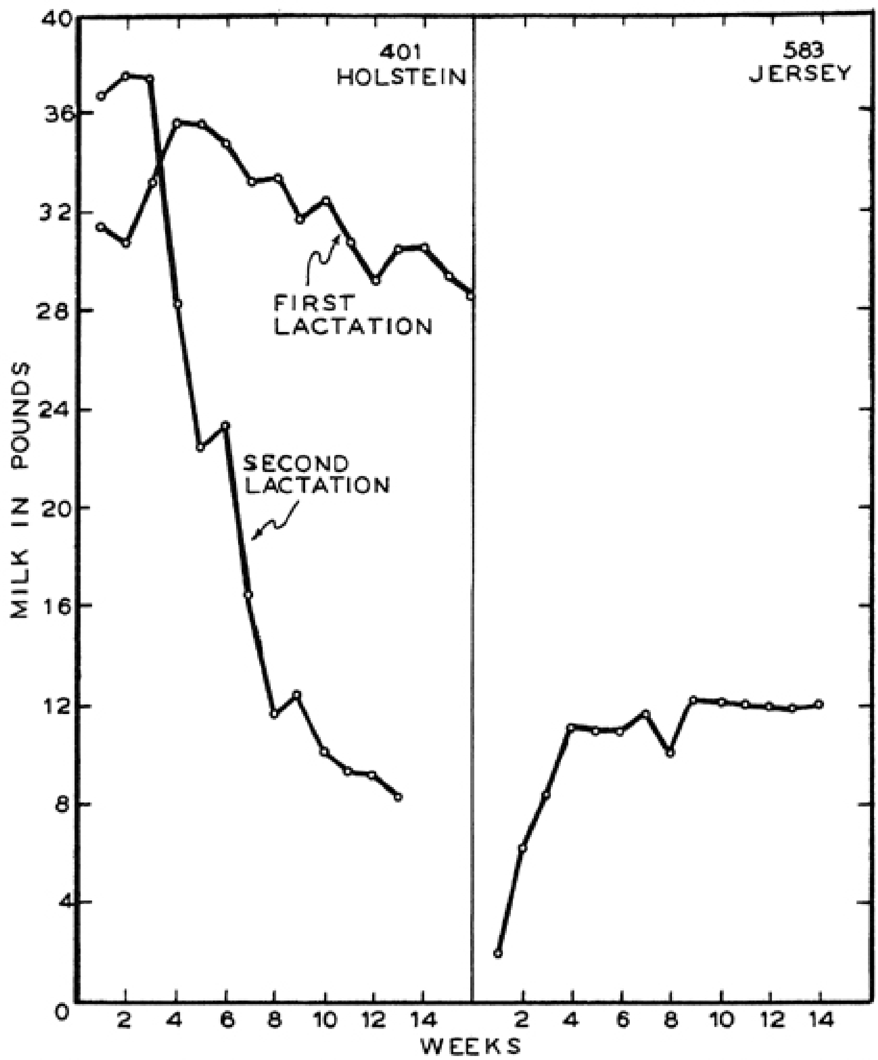

Fig. 7—At left are the first and second lactation curves of Holstein No. 401 after normal pregnancies in the University herd. She was a twin of No. 402 which was induced to lactate experimentally (see Fig. 5). The maximum milk production of No. 402 was 93.0 percent as great as that of No. 401 in her first lactation. No. 583, with three functional quarters, had the lowest maximum milk yield under experimental conditions.

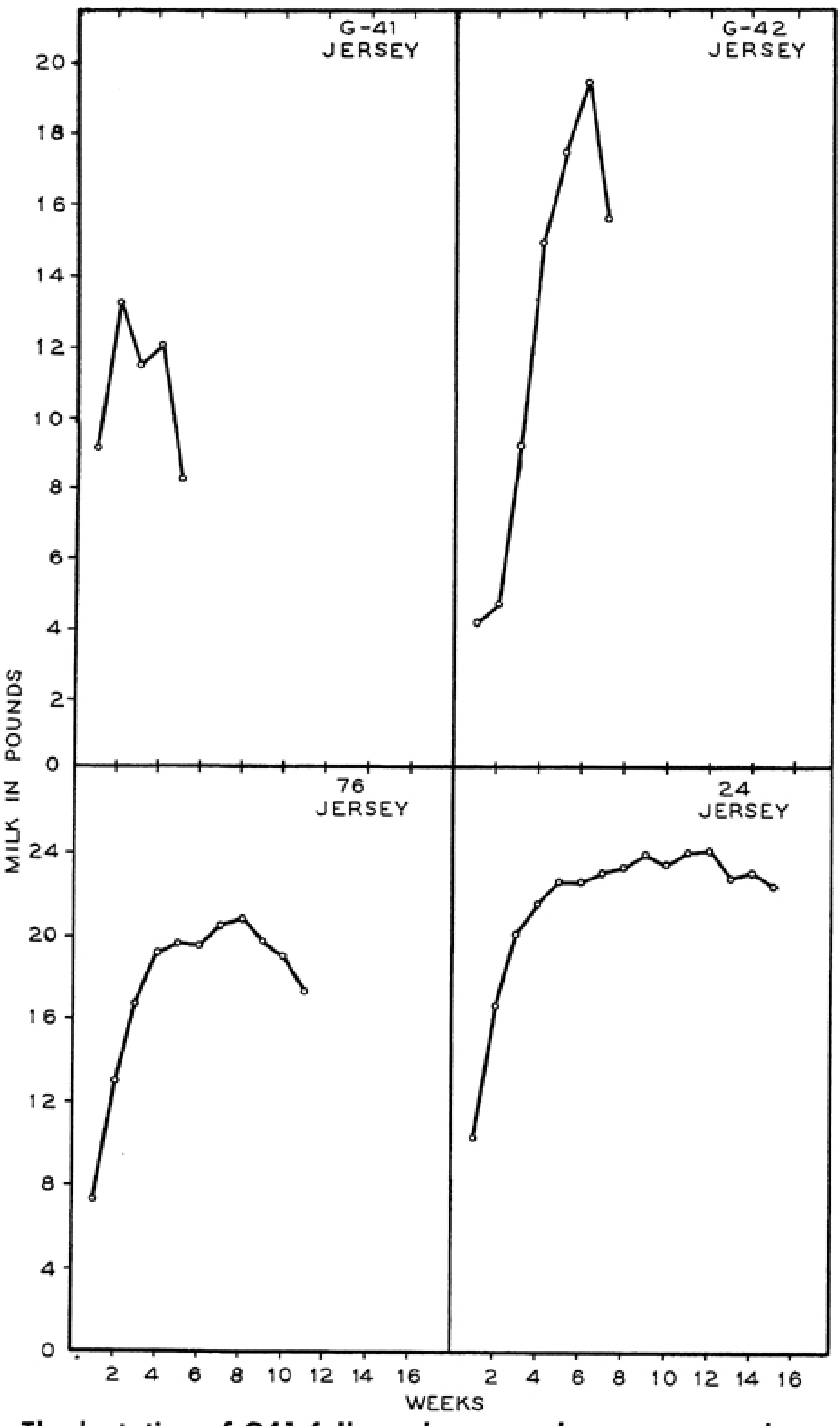

Fig. 8—The lactation of G41 followed a normal pregnancy, whereas G42 was induced to lactate experimentally. G42 produced 147.4 percent more milk than the control twin at the maximum. No. 76 was a twin of No. 24 The lactation of No. 76 followed a normal pregnancy,whereas No. 24 was induced to lactate experimentally. No. 24 produced 115.8 percent more milk than the control twin at the maximum. G41 and G42 have been dianosed as monozygous twins, whereas No. 76 and 24 have been diagnosed as dizygous twins.

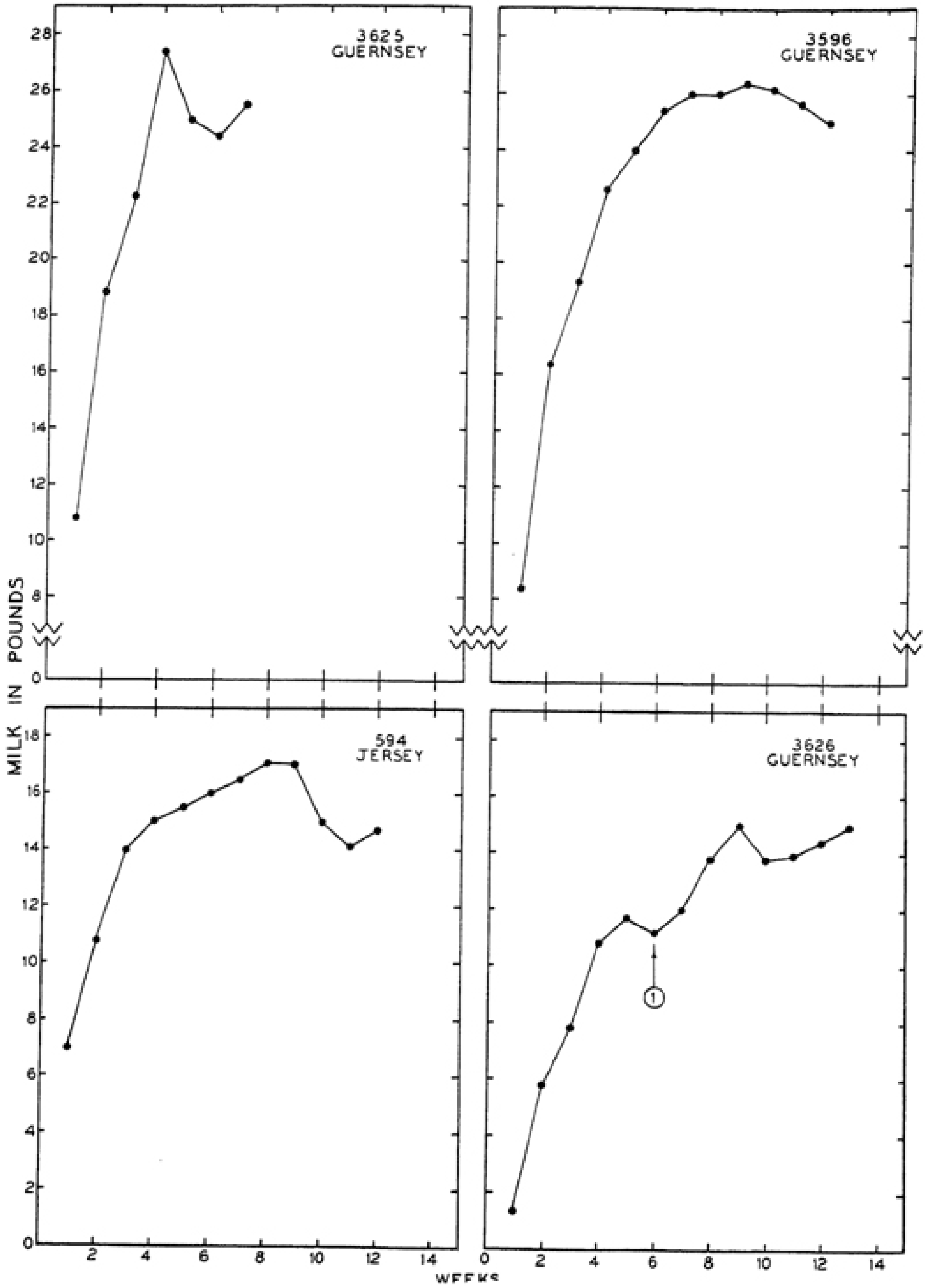

Fig. 9—These are rising segments of the lactation curves of sterile heifers whose udders were stimulated to growth by the injection of 100 mg./day of progesterone and by 10 mg./day of diethylstilbestrol administered orally. Lactation was stimulated by the daily injection of 3 mg. estradiol benzoate for 2 weeks. In the case of No. 3626, the rise in milk yield stopped at the sixth week. She was given a further injection for an additional week and showed a further response (1).

cow No. 402. The mean maximum milk production for the 12 heifers was 23.0 pounds and its F.C.M. equivalent, 24.7 pounds of 4 percent milk.

Of the four freemartin heifers given similar treatment only a single animal secreted significant amounts of milk (No. 18), reaching an average level of 13.1 pounds of milk during the fourth week. An explanation of our failure to induce mammary gland growth or to initiate milk secretion is not yet available.

A discussion of the relation of the amounts induced experimentally to those which might have been induced following normal parturition follows.

Feeding Diethylstilbestrol in Place of Estrogen by Injection: The ultimate objective of our research is to stimulate udder growth and initiate milk secretion by incorporating the necessary hormones in concentrate rations for cattle. This objective cannot be realized immediately due to the fact that progesterone is relatively inactive, biologically, when given orally. During the past several years a number of chemical compounds have been synthesized which are claimed to have biological activity similar to progesterone yet are more effective orally. These compounds are now being studied in our laboratory to determine the hormones most effective orally in stimulating growth of the mammary glands of experimental animals. The most promising of these compounds will then be tested to determine their effectiveness in stimulating growth of the udders of cattle.

In the meantime, orally effective synthetic estrogens related to diethylstilbestrol are readily available at a reasonable cost. Preliminary experiments thus seemed desirable to determine the amount of diethylstilbestrol administered orally which would synergize with injected progesterone at the level previously used. The level of diethylstilbestrol fed was 10 mg. per day. This level, when fed to beef cattle, has been shown to stimulate body growth as well as growth of the teats and mammary duct system (Turner, 1956a).

The present experiment was identical in every respect to the one described previously except 10 mg. of diethylstilbestrol per day was added to the concentrate mixture in place of the daily injection of 100 μg. of estradiol benzoate. Lactation was then initiated by 3 mg. daily of estradiol benzoate for two weeks.

Description of Heifers. Four sterile heifers were used, three Guernseys and one Jersey. Dates of birth, ages at time of initiation of lactation and body weight are given in Table 3. Note that considerable gain in body weight occurred during the injection period, indicating favorable effects of the hormones of

TABLE 3--DATA ON EXPERIMENTAL CATTLE

Herd No.	Breed	Date of Birth	Date at Start of Injection	Age at Initiation of Lactation (Months)	Body Weight At Start Lbs.	Body Weight At End Lbs.	Gain Lbs.
3596	Guernsey	8/8/52	4/1/55	38	987	1219	232
3625	Guernsey	12/6/52	2/1/56	44	1263	1423	160
3626	Guernsey	12/6/52	4/19/55	35	1263	1376	113
594	Jersey	8/16/53	12/15/55	34	744	1069	325

pregnancy upon growth in the absence of the fetus, fetal membranes and fluids of pregnancy.

Each animal was injected with 100 mg. of progesterone per day and fed 10 mg. of diethylstilbestrol per day for 180 days. Differences in body weight were not taken into consideration in regard to the amount of either hormone administered. Since the two smaller heifers made greater gains in body weight, it is possible that better lactational effects would have been induced if the two hormones had been administered in relation to body weight.

The external appearance of the udders during the growth phase was essentially unchanged, similar to that of the udders described previously. This is believed to indicate that the diethylstilbestrol at the 10 mg. level synergized with the progesterone to promote lobule-alveolar growth without inducing lactation.

At the end of the phase of udder growth stimulation (180 days), the two hormones were stopped abruptly and 3 mg. of estradiol benzoate per day was administered for a period of 14 days. At the same time, regular milking was begun. The size of the udder quickly increased as copious milk production was initiated. Maximum milk production was reached in 7 to 10 weeks (Table 4 and Fig. 9). No. 3626 reached a peak at the sixth week. Estradiol benzoate was administered for an additional week and a further increase in milk production was stimulated. This observation suggests that all cows should be given a further week or more of estrogen stimulation at their first peak of milk production to determine if a further increase might be obtained.

TABLE 4--PRODUCTION AFTER INITIATION OF LACTATION
(First Induced Lactation)
(Heifers fed 10 mg. diethylstilbestrol and injected with 100 mg. progesterone daily for 180 days. Lactation then stimulated by estradiol benzoate, 3 mg./day for 14 days.)

Herd No.	Breed	Time to Reach Maximum Milk Production (Week)	Milk Yield at Maximum (Lbs./Day)	Fat Content at Time of Maximum Production (%)	FCM Maximum (Lbs./Day)
3596	Guernsey	9	26.3	4.35	27.4
3625	Guernsey	10	30.0	3.90	29.6
3626	Guernsey	9	14.9	5.30	17.8
594	Jersey	7	16.5	5.60	20.5
	Average		21.9		23.8

Table 4 gives the maximum daily milk yield (weekly average) and its F.C.M. equivalent for each animal. The yield of milk varied from 14.9 pounds to 30.0 pounds. The mean maximum milk production for the 4 heifers was 21.9 pounds and its F.C.M. equivalent, 23.8 pounds. This average production compares quite favorably with the mean maximum milk production of 23.0 pounds and its F.C.M. equivalent of 24.7 pounds.

These limited data indicate that 10 mg./day of diethystilbestrol administered orally synergizes as well with a 100 mg./day injection of progesterone as a 100 μg./day injection of estradiol benzoate in stimulating growth of the udders of heifers.

It is not inferred that optimal levels of either estrogen or progesterone are yet known. It is hoped that much greater growth and maximum lactation can be stimulated as optimal and synergistic levels of the two ovarian hormones are deermined.

Productivity of Cows Following Normal Pregnancy: Since a number of the cows of low fertility conceived during the experimentally induced lactation and subsequently had one or more normal lactation periods, it is possible to compare records of experimental and normal lactations (Table 5). In addition, several sets of twins were included in our study, with one of each pair being induced to lactate experimentally and its mate being bred and permitted to lactate normally. Some of these were later reversed, that is, after a twin that had undergone an experimental lactation was bred for a normal lactation.

In the case of the twins where experimental and normal lactations were at the same age, the records are directly comparable. In individual cows, where the normal lactation followed an experimentally induced lactation the animals were at least one or two years older and age correction factors are necessary before comparisons can be made. Since most of the cows were Jerseys, conversion factors for the 2-year-old equivalent were used (Ragsdale *et al.*, 1924).

Two years	1.000
Three years	.862
Four years	.770
Five years	.726
Six years	.706

Methods of Comparison of Normal and Experimental Milk Yield: The range in maximum daily milk yield (week average) of the cows whose udder growth and lactation were initiated experimentally is given in Tables 2, and 4. To properly evaluate these data, an estimate is needed of the maximum daily milk yield these animals would have attained after a normal period of pregnancy.

The ideal comparison would involve the use of monozygotic (identical) twins whose productive capacity under similar nutritional and environmental treatment would be expected to be very similar. If one twin is induced to lactate normally and the second twin, experimentally, the comparison of maximum production is highly significant. Comparison of dizygotic twins is also of value, although of less significance.

Milk yields of the same animal after experimental stimulation and after normal pregnancy also serve as a basic of comparison. As pointed out above, corrections must be made for the effects of age changes involving body weight and developmental effects.

TABLE 5--MAXIMUM MILK PRODUCTION FOLLOWING NORMAL PREGNANCY

Herd No.	Breed	Time to Reach Maximum Production (Weeks)	Age (Years)	Milk Yield at Maximum: Actual (Lbs./Day)	Milk Yield at Maximum: Corrected to 2-Year-Old Equivalent (Lbs./Day)	Fat Content at Time of Maximum Production %	F.C.M. at Maximum (Lbs./Day)	Remarks (Refers to Sequence of Lactation)
580	Jersey	9	6	34.3	24.2	4.0	34.3	1st exp., 2nd normal
583	Jersey	13	5	24.7	17.9	4.8	26.7	1st exp., 2nd normal
585	Jersey	3	4	33.8	26.0	5.3	40.4	1st exp., 2nd normal
585	Jersey	9	5	42.8	31.1	5.7	53.7	3rd normal
402	Holstein	8	4	63.4	48.8	5.2	74.9	1st exp., 2nd normal
158	Jersey (twin 159)	7	2	26.3	26.3	3.7	24.6	1st normal, 2nd exp.
158	Jersey (twin 159)	5	4	33.6	25.9	1.8	24.0	3rd normal
159	Jersey (twin 158)	7	3	34.9	30.1	3.6	32.8	1st exp., 2nd normal
160	Jersey	4	4	28.6	22.0	4.8	32.3	1st exp., 2nd normal
G41	Jersey (twin G42)	2	2	13.3	13.3	2.9	11.1	1st normal
3596	Guernsey	3	5	36.1	26.2	4.9	41.0	1st exp., 2nd normal
594	Jersey	12	4	20.9	16.1	5.5	25.6	1st exp., 2nd normal
601*	Jersey	4	3	29.9	25.8	6.1	39.3	1st abortion, 2nd normal
76	Jersey (twin 24)	14	2	20.9	20.9	4.7	23.1	1st normal

* This no. 601 was later used on a Holstein-Jersey cross bred heifer.

Finally, comparisons can be made with the average maximum daily milk yield of paternal and maternal half-sisters. Naturally such comparisons are subject to the range of variation in the group. The experimental animal might have been as poor as the lowest normal producer or as high or higher than the highest normal half-sister.

The production of the experimental animals will be compared with these various indices so far as they are available (Table 6).

Cow No. 310 produced, experimentally, a maximum of 18.2 pounds of milk. She had 17 half-sisters in the University herd which averaged 27.6 pounds of milk at their maximum with a range of 20 to 39 pounds. Thus she produced only 65.9 percent as much as the average of her half-sisters.

Nos. 580 and 583 were half-sisters. The 18 half-sisters in the University herd produced an average of 26.2 pounds of milk at their maximum with a range of 7 to 40 pounds. No 580 produced 22.9 pounds of milk, or 87.4 percent as much as the average for the half-sisters. No. 583 produced only 12.2 pounds from 3 functional glands or 46.6 percent of the average production of the half-sisters. However, after a normal pregnancy she produced 17.9 pounds of milk (2-year-old equivalent). Thus her experimental production was 68.2 percent as much as her normal production.

No. 585 had two half-sisters which produced an average of 14.3 pounds of milk. She produced 18.2 pounds of milk, which is 127.3 percent of the half-sister production. She also had two normal pregnancies. By these comparisons her experimental production was 58.2 to 69.2 percent of her normal production.

No. 402's experimental production can be compared three ways. Her experimental production was 84.7 percent of the average production of her 24 half-sisters, 93.0 percent of that of her twin sister, No. 401, and 68.2 percent of what she produced after a normal pregnancy.

No. 159 produced 85.2 percent as much as her monozygous twin sister (No. 158) and 74.4 percent as much as she produced after a normal pregnancy.

No. 160 produced 27.3 pounds of milk at the peak of her experimental lactation and 22.0 pounds in a normal lactation corrected to 2-years, making her experimental lactation 124.1 percent of her normal lactation.

Cow G42 produced 19.6 pounds of milk in an experimental lactation. Her monozygous twin, G41, in a comparable normal lactation produced only 13.3 pounds of milk at the maximum. Thus, G42 produced 147.4 percent of the normal twin's production.

Cow No. 24 produced 24.2 pounds of milk in an experimental lactation while her dizygous twin, No. 76, produced 20.9 in a normal lactation. The experimental production was 115.8 percent of the normal production.

Cow No. 3596 produced 26.3 pounds of milk in an experimental lactation. In a subsequent normal lactation her corrected milk yield was 26.2 pounds. The experimental production was 100.4 percent of the normal lactation.

Cow No. 594 produced 16.5 pounds in an experimental lactation and 16.1

TABLE 6--COMPARISONS OF EXPERIMENTAL AND POTENTIAL PRODUCTIVITY

Herd No.	Comparison of Related Animals		Experimental Lactation		Lactation After Normal Pregnancy		
	Type of Comparison	Maximum Milk Production Lbs./Day (Range)	Maximum Milk Production (Lbs./Day)	Comparison %	Maximum Milk Production (Lbs./Day)	Corrected to 2-Year-Old Equivalent	Comparison %
310	17 Half-sisters	27.6 (20-39)	18.2	65.9			
580	18 Half-sisters	26.2 (7-40)	22.9	87.4			
583	18 Half-sisters	26.2 (7-40)	12.2	46.6	24.7	17.9	68.2
585	2 Half-sisters	14.3	18.2	127.3	38.8 (1st. preg.)	26.3	69.2
585					42.8 (2nd. preg.)	31.1	58.5
402	24 Half-sisters	39.3 (22-54)	33.3	84.7	63.4	48.8	68.2
402	Twin (401)	35.8	33.3	93.0			
159	Twin (158)	26.3	22.4	85.2	34.9	30.1	74.4
160			27.3		28.6	22.0	124.1
G42	Twin (G41)	13.3	19.6	147.4			
24	Twin (76)	20.9	24.2	115.8			
3596			26.3		36.1	26.2	100.4
594			16.5		20.9	16.1	102.5

pounds in a normal lactation, when corrected for age. The experimental production was 102.5 percent of the normal lactation.

Thus, three cows, (Nos. 160, 3596 and 594) reached a higher level of production in their experimental lactation than they did during a following normal lactation (age corrected). Four other cows (Nos. 583, 585, 402 and 159) produced about 70 percent of the normal milk yield in their experimental lactation.

In a comparison of four sets of twin sisters, one of which was stimulated to lactation experimentally while the other lactated after a normal pregnancy, two undergoing experimental lactation exceeded the maximum production of their sisters (G42 and 24) and two (402 and 159) produced from 85.2 to 93 percent as much.

The comparison of experimental milk yield with the average normal production of half-sisters showed a wide range, varying from 46.6 percent to 127.3 percent for the experimental animals.

Considering all the factors influencing milk production when the records are not concurrent, it seems reasonable to conclude that the productivity of the experimental animals, in which udder growth and lactation was induced by the ovarian hormones, was at least 80 to 90 percent of the natural production.

Thyroxine Secretion Rate of Experimental Animals: The heifers in the experimental herd were used concurrently in our study of the development of a method for determining thyroxine secretion rate of cattle (Pipes *et al.,* 1957). Estimations of the thyroxine secretion rate were made at intervals throughout the year. These data are presented and discussed in a separate publication (Permachandra *et al.,* 1958). They show a marked season variation in thyroxine secretion rate in individual animals. It has been suggested that low thyroxine secretion rate in cattle may limit their milk secreting capacity. Further, the fact that nine of the animals reached maximum production during the summer months when thyroxine secretion was low probably limited their production. While many records will be required before the relationship of thyroxine secretion rate and milk yield can be shown, the present comparison is of interest (Table 7).

In the future an attempt will be made to have the experimental cows reach their maximum production in the winter season to avoid the drastic influence of summer on thyroxine secretion rate.

TABLE 7--COMPARISON OF MAXIMUM MILK YIELD AND SEASONAL THYROXINE SECRETION RATE

Herd No.	Breed	Month of Maximum Milk Yield	FCM at Maximum (Lbs./Day)	Daily Thyroxine Secretion Rate (Mg./1000 Lbs. Body Weight)	
				Winter*	Summer**
310	Jersey	March	25.3	4.0	1.0
580	Jersey	April	27.2	6.0	1.0
583	Jersey	August	13.9***	5.0	4.0
585	Jersey	July	22.3	4.0	--
402	Holstein	February	31.3	6.0	1.0
160	Jersey	July	29.8	7.0	1.0
544	Jersey	July	27.4	7.0	4.0
159	Jersey	February	21.7	7.0	2.0
G42	Jersey	June	22.6	5.0	1.8
24	Jersey	July	22.4	6.0	2.8
3596	Guernsey	November	27.4	6.0	2.0
3625	Guernsey	October	29.6	4.0	1.0
3626	Guernsey	December	17.8	2.0	1.0
594	Jersey	September	20.5	5.0	1.5
601	Holstein X Jersey cross	July	23.0	5.0	--
38	Guernsey	July	29.8	4.0	1.5

* Average of 3 to 4 estimations made during months of Dec. to April.
** Average of 2 to 3 estimations made during months of May to Sept.
*** One blind quarter.

Reproductive Performance of Heifers of Low Fertility: Nine of the heifers included in this study came from the University herds as a result of poor reproductive performance. Heifers failing to conceive upon repeated services were included (Table 8). No attempt was made to breed these animals following their admission to the experimental herd. The experimental induction of lactation was started immediately. However, following the induction of lactation, when these animals came into heat they were inseminated artificially with semen from bulls of the University herd.

The fact that many of these heifers conceived is of considerable interest. Following conception some of these heifers carried their calves to term; others failed to maintain viable fetuses. The following observations may furnish clues to some of the causes of infertility. Note that only one heifer failed to conceive; however, one heifer showed fetal resorption after about 90 days. In two other heifers fetal death occurred during pregnancy, resulting in mumified fetuses eventually being expelled. Possibly, these three heifers failed to maintain viable fetuses due to abnormal secretion of the hormones of pregnancy, estrogen and progesterone, associated with their initial low state of fertility. The presence of two mummified fetuses in a group of nine heifers of low fertility is in contrast to the herd experience reproted by Erb and Morrison (1957) in which 2607 cows with 9994 reproductive periods produced 32 mummified fetuses which represented 1.1 percent of the cows and 0.43 percent of the parturitions.

TABLE 8--REPRODUCTIVE PERFORMANCE OF HEIFERS OF LOW FERTILITY

Herd No.	Breed	No. of Services	Gestation Days	Reproductive Record	Remarks
310	Jersey	2	60 - 90	Fetus resorbed	
580	Jersey	3	280	Mummified fetus	Fetus expelled with stilbestrol
580	Jersey	1	303	Calf alive	Parturition difficult - calf died
583	Jersey	2	285	Calf alive	Died during delivery
583	Jersey	2	251	Calf alive	Died during delivery
858	Jersey	2	270	Calf alive	Normal calf
402	Holstein	1	274	Calf alive	Normal calf
402	Holstein	2	268	Calf alive	Normal calf
160	Jersey	1	279	Calf alive	Normal calf
544	Jersey	Never conceived			
3596	Guernsey	6	170	Mummified fetus	Fetus expelled with stilbestrol
3596	Guernsey	6	277	Calf alive	Normal calf
594	Jersey	8	293	Calf alive	Normal calf

Regrowth of the Udder Following Normal or an Experimentally Induced Lactation: All of the data presented up to this point is concerned with the experimental induction of growth and lactation in heifers which had not lactated previously. In many of the heifers of low fertility, it was possible to induce conception and a normal lactation followed. To date, in only a few cows has an attempt been made to regrow the udder experimentally and induce a second experimental lactation.

A number of interesting problems arise in connection with this phase of lactation. Normally, cows are bred about the third or fourth month of lactation. Lactation and pregnancy thus occur concurrently. Can the experimental treatment be started at a comparable period of lactation and induce a rise in lactation comparable to that observed following parturition? Is a dry period necessary or can the increase in milk yield be induced while lactation is going on?

These problems are being investigated in experiments currently in progress.

Table 9 lists observation on four cows in which lactation was induced after one or more previous lactations. Jersey cows No. 310 and No. 158 failed to secrete appreciable amounts of milk. Holstein cow No. 402 was induced to produce about the same amount of milk at her maximum as she had produced in her first experimental lactation (see Table 2) but much less than she had produced after a normal pregnancy. Jersey No. 601 produced 39.3 pounds of F.C.M. after a normal pregnancy (Table 5) and only 19.5 pounds of F.C.M. after experimental stimulation.

Reasons for the reduced response in these cows following previous experimental and normal lactations require further study.

TABLE 9--MAXIMUM MILK PRODUCTION EXPERIMENTALLY STIMULATED
(Second or Later Lactation)

Herd No. & Breed	Time to Reach Maximum Production (Weeks)	Milk Yield at Maximum (Lbs./Day)	Fat Content at Time of Maximum Production (%)	FCM at Maximum (Lbs./Day)	Remarks*
310 Jersey	4	3.1	5.7	3.9	Second induced lactation
158 Jersey	8	13.6	2.4	10.3	Normal pregnancy
402 Holstein	11	31.4	4.5	33.8	Normal lactation
601 Jersey	8	18.7	4.3	19.5	Normal lactation

* Indicated type of preceeding lactation

Effect of Increasing Levels of Estrogen During Progesterone Treatment: During the course of the experiments described and after the level of estrogen to be used was decided upon (100 ug./day), study of the total secretion of estrogen in the urine and feces of pregnant cows was conducted (El-Attar and Turner, 1957). It was observed that the estrogen excreted at the 100th day of pregnancy greatly exceeded the amount of estrogen being injected. While considerable amounts of the estrogen observed in the urine and feces might be in a metabolized or conjugated form and far exceed the effective blood estrogen level, it seemed desirable to determine the effect of increasing levels of estrogen with progesterone upon the udders.

In a small group of heifers, it was decided to increase the estrogen from 1 to 3 mg. daily during the last 30 days of the growth period. In other words, at the 150th day the progesterone was continued at the 100 mg. level, but the estradiol benzoate was increased from 100 ug. to 1 mg. daily for 10 days, to 2 mg. for 10 days, and then to 3 mg. for 10 days. The udders were observed for indications of swelling and initiation of milk secretion. It was considered that if udder swelling occurred, it would indicate that estrogen was overriding the physiological effect of the progesterone and causing the initiation of lactation, whereas if the estrogen caused no noticeable effect upon the udders, such levels of estrogen might be tried in subsequent experiments.

Of four heifers tested, two showed no change in their udders when 1 to 3 mg. of estradiol benzoate were given during successive 10 day periods. One heifer showed evidence of bagging up after 8 days on 1 mg., and a second heifer showed slight bagging up during the injection of 3 mg.

While these observations are limited, it seems clear that if levels of estrogen in the range of 1 to 3 mg. daily were injected with the progesterone, lactational stimuli would be imparted which might be detrimental to normal mammary gland growth in some animals.

In future trials slightly higher levels of estrogen may be tried in synergism with progesterone to determine whether greater growth of the udder may be stimulated.

DISCUSSION

The experiments described are part of a project to determine the role of the various endocrine glands and their hormones upon the growth of mammary glands and the secretion of milk. A theory used to guide our experiments is that cattle secreting optimal levels of the hormones which stimulate growth and multiplication of the milk secreting cells of the udder will have udders with the capacity for large production of milk. If one or more hormones influencing udder growth are secreted in limited amounts, the size of the udder will be limited.

The problem faced by dairy endocrinologists is two-fold. First, it is necessary to determine the hormones which directly and indirectly stimulate the growth of the udder. Second, it is necessary to develop methods for the determination of the secretion rate of each hormone involved.

The present experiments have as their objective the determination of the hormones which stimulate the growth of the mammary glands of the cow's udder. Unfortunately, we do not have satisfactory methods of determining the secretion rate of the hormones involved. Consequently, it is necessary to seek by trial and error, the levels of hormones which will produce growth of the udder equal to that produced by normal pregnancy. Eventually, as optimal levels are found, we hope that growth in excess of that obtained normally will be stimulated by experimental procedures.

The reasons for selecting the amount of estrogen and progesterone used in the present experiment were given in the introduction. It might have been desirable to determine the effect of estrogen alone upon lobule-alveolar growth, since a few investigators have suggested that estrogen is sufficient to grow the cow's udder (see Petersen, 1948; Folley, 1956). This conclusion was based upon the observation that natural or synthetic estrogens would stimulate limited lactation in heifers. Our study indicates that the lactation following estrogen stimulation is dependent upon the presence of preformed mammary gland cells in the udder and does not indicate that estrogen stimulates more than duct growth.

In experimental animals, conclusive evidence has been presented indicating that lobule-alveolar growth in normal animals requires the synergistic action of the two ovarian hormones, estrogen and progesterone. There are no good reasons to believe that the same two hormones are not required by the cow for normal growth of the udder.

The problem of optimal estrogen and progesterone dosage in experimental stimulation of lobule-alveolar growth, is very great. Because of the great size of the gland and the value of individual animals it did not seem feasible to attempt to examine individual cows directly for the extent of gland growth produced. The long period of pregnancy and the expectation of the requirement of long periods of injection of the hormones for comparable growth also limit the number of animals which can be studied.

A limited amount of research has been conducted with dairy cattle using estrogen and progesterone to stimulate udder growth. In the research conducted at Michigan State University the hormone was administered as implanted pellets (Meites *et al.,* 1950; Meites *et al.,* 1951; Reineke *et al.,* 1952). Such studies are difficult to evaluate. In the first place, the daily rate of absorption from pellets is high at first, then gradually declines as the size of the pellet decreases. Second, absorption from the pellets may be reduced markedly by the formation of connective tissue around the capsules (called encapsulation). Third, body fluids may infiltrate the pellets and slow down the absorption (called ghost formation).

Thus, the daily level of hormones effective in udder grówth is difficult to evaluate. Another problem with pellets in which estrogen and progesterone are combined is the relative proportions of the two hormones being absorbed daily. If the amount of estrogen absorbed is excessive, the biological effectiveness of progesterone is reduced. On the basis of our studies it was believed that the amount of progesterone absorbed from pellets would be grossly deficient and that the effects observed where pellets were used were due primarily to the estrogen content of the pellet.

For these reasons, in spite of the convenience of pellets, the injection of the two hormones seemed advisable. Preliminary studies using this basic plan have been reported by Hancock *et al.* (1954) and Williams *et al.* (1955). A preliminary report of the present experiments has been presented (Turner *et al.,* 1956, 1957).

While estrogen and progesterone have been considered the two hormones involved in mammary gland growth and are effective in stimulating growth in normal animals, it should be pointed out that their role is not entirely direct action upon the mammary gland. The action of estrogen upon teat growth and upon the vascular supply of the udder is believed to be direct, but the action of progesterone has been considered indirect, acting upon the anterior pituitary and the secretion of mammogen (Mixner and Turner, 1943). Recently, in our laboratory, it has been shown that the hormone, relaxin, can substitute completely for progesterone in stimulating lobule-alveolar growth (Wada and Turner, 1958, 1959). These data are believed to indicate that estrogen and progesterone may act to stimulate endogenous relaxin secretion and that relaxin may stimulate the secretion of mammogen. It is possible, of course, that both progesterone and relaxin can stimulate the secretion of mammogen by the pituitary.

Many investigators have questioned the separate identity of the mammogenic hormone for the reason that it has not been possible to separate the lactogenic and mammogenic hormones. Recently, however, it has been possible to extract from the anterior pituitary a fraction rich in mammary lobule-alveolar growth stimulating properties which is essentially free of lactogenic hormone (Damm and Turner, 1958, 1959). While it will not be possible to stimulate the growth of a cow's udder with mammogenic hormone due to the large requirements, it is important to appreciate the role of mammogen in the process and that the ovarian hormones used in our experiments are effective only in the presence of an intact anterior pituitary gland.

With the completion of the growth phase of mammary gland development, the second problem is one of initiating the secretion of milk. The hormone which initiates the synthesis of milk in the glandular epithelium is the lactogenic hormone of the anterior pituitary. This hormone could be injected directly and start milk secretion but since lactation can only be sustained by the continued secretion of the hormone, a more effective method is to employ the hormone, estrogen, which normally stimulates the pituitary to secrete lactogen.

In normal pregnancy, the secretion of estrogenic hormone increased markedly toward the end of the period. It is believed that the rise in estrogen plays a role in parturition and the initial stimulus of lactogen secretion. In our initial experiments, 3 mg./day of estradiol benzoate has been injected for a period of 2 weeks or longer. Whether this is an optimal amount cannot be stated at this time although its effectiveness has been demonstrated. Diethylstilbestrol or other synthetic estrogens could be fed in place of the natural estrogen used and their most effective level would be determined.

As indicated in the introduction, the ultimate objective of our research is to feed the hormones necessary to stimulate the growth of the udder and milk secretion. This objective is nearer attainment as a number of synthetic progesterone-like compounds which are orally effective are being available. We hope to test one or more of these compounds with cattle in the near future.

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