Induced lactation in non-pregnant ewes

by Robert A Bellows

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Animal Industry at Montana State College
Montana State University

Abstract:
An experiment in two parts has been conducted to study the artificial induction of lactation in sheep. Part I was an exploratory trial during 1957 involving 15 dry, non-pregnant, three to six year old females and one yearling castrate male. Treatments consisted of various levels of estrone and diethylstilhestrol with and without progesterone. Prolactin was given alone or in combination with one or both steroids in four treatments. Oxytocin treatment was given to one ewe. Secretion was produced on ten treatments but was not collected.

Part II was an experiment in artificially inducing lactation conducted during 1958, involving 11 head of dry, six to seven year old ewes. Treatment I was a graded dose of estrone; Treatment II a 1:40 estradiol to progesterone ratio. Treatments III, IV, and V were respectively: 1:40, l:80, and l:200 ratios of diethylstilhestrol to progesterone. Treatments VI, VII, VIII, and IX were O.25 mg, 0.50 mg, 1.00 mg and 5.00 mg of diethylstilhestrol respectively. Treatment X was 3 c.c. peanut oil injected every other day, with Treatment XI being udder manipulation with no injections.

All injections were given intramuscularly, every other day, in a peanut oil carrier for 27 days.

Measurable secretion (more than 0.30 gm.) was obtained from the ewes on Treatments I, IV, V, VI, VII, VIII, and IX, with peak daily productions being 29.90 gm., 306.81 gm., 29.70 gm., 5.50 gm., 142.43 gm., 610.52 gm., and 406.69 gm. respectively. A small amount of honeylike secretion was obtained from Treatment XI. This as well as the secretion obtained from Ewe Number V was present at the start of the experiment and was not due to treatment. Secretion was collected from four normal ewes that had just lambed for a colostrum sample and from ten normal ewes that had lambed earlier for an advanced lactation sample. Chemical analyses for fat, protein, lactose, ash, and total solids on all samples showed the secretion from the treated ewes to be lower than normal colostrum in all components. As lactation proceeded the secretion rapidly assumed the composition of normal milk; both chemically and physically.

Routine histological examinations were conducted on Ewes Number I, IV, IX, X, and XI. Ocular micrometer measurements on alveoli and lumina revealed percent increase over the control average alveoli diameter to be 24.23, 112.37, 156.19, and 246.39 percent for Treatments I, IV (right and left half), and IX respectively* Percent increase over the control average lumina diameters were 117.16, 414.18, 502.24, and 728.38 percent for Treatments I, IV (right and left half), and IX respectively.

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ABSTRACT

An experiment in two parts has been conducted to study the artificial induction of lactation in sheep. Part I was an exploratory trial during 1957 involving 15 dry, non-pregnant, three to six year old females and one yearling castrate male. Treatments consisted of various levels of estrone and diethylstilbestrol with and without progesterone. Prolactin was given alone or in combination with one or both steroids in four treatments. Oxytocin treatment was given to one ewe. Secretion was produced on ten treatments but was not collected.

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INDUCED LACTATION IN NON-PREGNANT EWES

INTRODUCTION

Lactation, as defined by Webster, is "the secretion and yielding of milk by the mammary gland." The process by which milk is snythesized by the mammary gland has intrigued the mind of man for many centuries and the possibility of inducing lactation in the non-pregnant or virgin female is a field in which much experimental work has been done.

Udder development, and subsequently lactation, may be induced through two general treatment regimes: by estrogen, either natural (estrone or estradiol) or synthetic (diethylstilbestrol), given alone, or by estrogen and progesterone given separately with simultaneous treatment or in a synergistic ratio.

Whether or not the female steroid hormones are directly responsible for the initiation of lactation is the subject of some controversy. According to Evans (1947), lactation is initiated by an anterior pituitary hormone, prolactin. This hormone is also responsible for so called maternal behavior patterns in rats (Hoskins 1956).

An exploratory trial was initiated on April 4, 1957, into the possibility of artificially inducing lactation in sheep. Experimental procedure and results are presented in Part I of this thesis.

Results obtained from the preliminary study made in 1957 and study fields obtained through further literature review were used to set up a second trial started on April 3, 1598. The procedure and results obtained are presented in Part II of this thesis.

LITERATURE REVIEW

There is general agreement that estrogen and progesterone are needed in sufficient amounts to cause duct and alveolar development in the mammary gland of the mammalian female (Petersen 1939). Harrow and Mazur (1954) state that after parturition the inhibiting influence of the placenta is removed. Lactogen (or prolactin) secretion from the anterior pituitary is then initiated and milk production is fostered.

Since discovery of the potent synthetic estrogen diethylstilbestrol (commonly called stilbestrol) in 1938 by workers in England, it has been tested on rats, guinea pigs, goats, cattle, fowls, and humans, according to Marshall et al. (1948). Stilbestrol has generally been found to cause proliferation of mammary tissues, false heat, and ovarian quiescence in many mammals. Relaxation of the broad ligaments and distortion of the rump and tailhead are common overdosage symptoms in cattle.

Many workers have used diethylstilbestrol to induce lactation. Other studies have been conducted to determine its effect on established lactation.

It is well known that the first secretion from the mammary glands is of a different consistency and composition than normal milk for the given species. The term "colostrum" has been given to this initial secretion. It is higher in total solids, protein, fat, and ash than milk secreted later in the lactation period (Espe and Smith 1952).

The appearance of a true colostrum in mammals induced to lactate via hormonal treatment is not so clear cut. Extensive analyses con-

ducted by Perrin (1955) with identical twin dairy heifers indicated the first secretion is of a colostrum nature in protein and ash content only. Fat and lactose content was initially low and gradually increased to that of normal milk. Results similar to these were also found by Folley et al. (1941a) to be the case in goats that were induced to lactate.

The amounts of stilbestrol given and the mode of treatment has varied greatly among experiments conducted. The injection method appears to be the most efficient and gives the most rapid response. The injections have usually been given at least three times a week with some workers reporting more rapid response when given daily. There seems to be little difference in the effect of subcutaneous injections.

Lambourne (1956) reports that the injection of stilbestrol in levels varying from 5 to 80 milligrams (mg.) in 13 barren Romney ewes resulted in marked udder development within 48 hours after injection. Strong orphan lambs were placed with them and were allowed to suckle. The lambs seemed to eat well and showed no adverse effects, but the ewes showed no interest in the lambs and had to be restrained to allow suckling.

Lewis and Turner (1942) found that 0.25 mg. of stilbestrol was sufficient for lactation in goats while 5 mg. or 20 times this amount was necessary when given orally. In the same experiment they found that 4 mg. per day or 16 times the injected amount was necessary to cause lactation when it was applied externally to the udder in a petroleum salve or alcohol medium.

Mixner et al. (1944) found that a daily dose of 0.25 mg. of stilbestrol constitutes a lactation stimulating dose in non-parous goats while dosages varying from 1 to 4 mg. per day were progressively inhibitory to lactation in lactating goats. As judged by the lactation-inhibiting effect, oral administration is only about 1 percent as effective as subcutaneous administration.

Mixner further states that the lactation stimulating effects of small dosages are presumably due to its ability to stimulate the secretion of the lactogenic hormone by the anterior pituitary. The lactation inhibiting effects are believed to be correlated with increased adrenal cortical activity resulting in an increased rate of deamination of the precursors of milk protein. They also suggest that the hormones of the adrenal cortex play a role in regulating the course of the rising segment of the lactation curve.

Spielman et al. (1941) treated four lactating cows with 10 to 100 mg. diethylstilbestrol in cottonseed oil at irregular intervals by intramuscular injections. An increase of 1 to 1.5 percent in butterfat and lactose was obtained. There was no apparent effect on total milk production, total protein, or total casein. Blood fat levels rose 100 or more milligrams percent and there was a significant increase in blood sugar. The increase in butterfat began to decline about 48 hours after termination of the injections and had returned to normal in five days. Blood sugar remained high for a longer period of time. That amount of stilbestrol needed to give the preceding results was about 50 to 150 mg. per cow. The latent period was about three days.

Eaton and Simmons (1953) studied the effect of stilbestrol induced lactation in dairy goats. Treatment was started in May on kids 13 to 16 months old. Subcutaneous injections of 0.5 mg. stilbestrol in 1 milliliter (ml.) of oil were given three times a week for about 50 days. Several began secreting milk after the fourth or fifth injection. The peak producers gave 2.5 to 3.0 pounds per day. Production then fell gradually to less than one pound and milking was discontinued. Lactation curves resembled those for normal lactation except that the production was lower and did not continue as long. Production ranged from 0 to 360 pounds of milk in 185 days. Afterwards the natural production ranged from 3 to 1500 pounds of milk and lasted from 10 to 419 days. Untreated control production ranged from 127 to 1600 pounds and lasted for 118 to 306 days. The animals were later bred and only three failed to conceive. The authors concluded that the injections and the resulting prebreeding lactation had no harmful effect on future estrus and breeding.

The effect of diethylstilbestrol on the mammary glands of the mouse, rat, rabbit, and goat was studied by Lewis and Turner (1941). Subcutaneous injections of 0.167 to 0.500 mg. per day caused extensive duct proliferation in male mice. Mammary development did not proceed further in spayed, virgin female mice similarly treated. Oral administration of the stilbestrol required six times more to obtain results similar to these. Castrate male rats required a higher dosage than did mice. Subcutaneous injections of 0.4 gm. per day produced extensive duct development in male rabbits. After 40 to 60 days of treatment,

early lobule development was apparent. Percutaneous treatment was also effective. Two male rabbits were implanted. They had well developed lobule-alveolar systems and responded well to lactogen treatment in 90 days. Normal females tended to lactate on stilbestrol alone.

Subcutaneous injection of stilbestrol into virgin goats caused abundant and prolonged lactation from the lobule-alveolar glands. Subcutaneous injections followed by implantation caused no mammary gland development in castrate male goats; however, teats did enlarge.

The effect of diethylstilbestrol treatment on animals in established lactation has been studied. Folley et al. (1941) conducted experiments on the chemical enrichment of cow's milk by administering stilbestrol and its dipropionate ester. A rise in non-fatty milk solids was obtained. Butterfat increased only slightly. Milk production declined in some and not in others. These workers postulated that the threshold for inhibition of milk secretion was apparently higher than that for enrichment.

Hawkins and Autrey (1957) fed 15 lactating cows diethylstilbestrol at levels of 0, 0.068, and 3.400 micrograms (mcg.) per pound of feed. These treatments did not significantly effect (a) the level of 4 percent fat corrected milk, (b) percent of non-fatty solids, (c) gross efficiency of milk production, (d) changes in body weight of cows, or (e) efficiency of utilization of available total digestible nutrients. The levels of stilbestrol fed in this study provided estrogenic activity that lie within the range of that found in forages. It was further stated it is therefore unlikely that the increase in quality of milk in

non-fatty solids when cows change from dry feeds to succulent young forage, is due to estrogenic activities.

The effect of stilbestrol on lactating cows and non-lactating heifers has also been studied by Matus (1952). He treated 33 non-lactating heifers with an 800 mg. stilbestrol implant at one time. Symptoms of nymphomania, crepitation in the pelvis, elevation of sacral bone, and relaxation of the sacroiliac ligament developed; three pregnant cows aborted within a few days. After 118 to 271 days, he removed the pellets and milk production increased to 2488.8 liters. He calculated the amount absorbed and found it to be about 200 to 450 grams (gm.) but there was no relation to this amount and the amount of milk produced. After implant removal the nymphomania and high tailhead disappeared. In his second group, in which the pellets were not removed, the total milk production was 110.1 liters per animal.

Subterranean clover (Trifolium subterraneum) is a plant containing rather high levels of estrogenic substances. Clover infertility disease, as reported by Chamberlain and Habel (1957) is characterized by abnormal lactation in wethers and non-pregnant ewes. In wethers particularly there is considerable udder and teat development, with the teats sometimes resembling those of a ewe in full milk. Small quantities of a fluid similar to ewe's milk can be drawn from them. It is, however, unwise to regard this symptom alone as definite evidence of the disease, as it may occur on pastures with no subterranean clover.

Doan and Lyons (1954) studied the effect of 45 daily injections

of estrone, progesterone, and pituitary mammotropin on the mammary glands of C_3H male mice that had been castrated at six weeks of age. No changes occurred after a total of 22.5 cubic centimeters (c.c.) of the sesame oil carrier, 45 mg. progesterone, or 45 mg. of pituitary mammotropin had been given. Following treatment with 92.5 mcg. of estrone alone or combined with 45 mg. of pituitary mammotropin, good duct and fair alveolar growth occurred with the formation of a few small lobules. The combination was slightly more effective than estrone alone. Extensive duct development, abundant alveolar growth and a few large lobules were formed after 92.5 mcg. of estrone plus 45 mg. of progesterone were given. These hormones in combination with 45 mg. of pituitary mammotropin could develop in the male, mammary glands similar to those of females in midpregnancy.

There appears to be a synergistic ratio existing between progesterone and estrogen for another treatment regime to induce lactation. Yamamoto and Turner (1956) gave daily injections of 9 mcg. of estradiol benzoate for 20 days and found that it induced extensive development of the mammary duct system of male rabbits, but only negligible lobule formation. With male rabbits pretreated with estrogen to develop the duct system, the daily injection of 15 mcg. of estradiol benzoate combined with 1 mg. of progesterone (synergistic ratio 1:67) induced extensive development of the lobule-alveolar system as indicated by the DNA content and by visual inspection of whole mounts of the glands. These observations were interpreted to indicate that the optimum synergistic ratio of the ovarian hormone in the lobule-alveolar development

in the rabbit differs widely from the ratio of 1:1000 or more seen in the mouse, rat, and dog.

Work done by Nellor and Reineke (1956) in inducing lactation in goats was quite successful. Their work consisted of various levels of hormone injections including stilbestrol alone (1.750 mg.) or stilbestrol (1.750, 0.875, or 0.070 mg.) in combination with 70 mg. progesterone injected as a crystalline suspension once weekly for nine weeks into immature and adult anestrous female goats. Each treatment was terminated with two weekly injections of 1.75 mg. stilbestrol. The last three groups resulted in ratios of progesterone to stilbestrol of 40:1, 80:1, and 1000:1 respectively. The milk production plateau was reached within 30 days for the 40:1 and 80:1 hormonal ratios; the 1000:1 ratio group did not plateau until 60 days post-treatment. In the stilbestrol group, lactation increased very slowly and did not reach a peak until six months post-treatment. The most uniform response was obtained with the 80:1 and 40:1 ratios where all animals responded with a production peak average of 1,061 and 862 ml. daily, respectively, the range in production being 683 to 2,190 ml. daily. The highest average production was realized in three does in the 1000:1 group (1,683 ml.), but only three does of five responded at this ratio. There were no detectible differences in the degree of response shown by kids, yearlings or aged does in any of the treated groups. Retreatment of the two kids that failed to respond at the 1000:1 ratio, with nine weekly injections at the 80:1 ratio resulted in mammary growth and lactation comparable to that in animals treated initially at this

level. There was a great latitude in the ratios that can induce mammary development and lactation in goats, but it would seem that the 80:1 ratio was more effective in establishing peak production in the shortest time, whereas all treated groups produced the same average amount of milk daily seven months post-treatment.

Turner et al. (1956) reported studies made with six Jerseys and one Holstein that had failed to conceive; also one set of Jersey twins (identical) and one freemartin Guernsey. To induce lobule-alveolar mammary gland growth in these animals, injections were made daily with a mixture of 100 mg. of progesterone and 100 mcg. of estradiol benzoate for 180 days. During this period there were few external signs of change of the udder except slight lengthening of the teats and hyperemia of the skin. Estrual cycles were inhibited; mounting and pelvic changes observed when estrogen alone was injected were not observed. To induce milk secretion, 3 mg. of estradiol benzoate were injected daily for a period of 15 days or more. A marked increase in milk yield commenced from the third to sixth days in the Jerseys but was delayed in the Holstein. The Guernsey freemartin failed to come into copious lactation. Maximum milk yields were reached in 5 to 14 weeks after the beginning of stimulation. On the basis of various comparisons, it was concluded that the maximum production of milk rather closely approached the milk yield which might have been obtained had they calved normally. The failure of the Guernsey freemartin to respond to the treatment could not be explained. Although complete chemical analyses of the milk secreted by these heifers were not made, evidence

for the normality of milk composition furnished by pairs of identical twins under similar experimental treatment indicated that the milk stimulated by this method would have been normal.

Work with these heifers was continued by Turner et al. (1957) in which they fed 10 mg. per day of diethylstilbestrol, followed by injections of 0.4 mg. L-thyroxine per 100 pounds body weight. This was then followed by 50 mg. per day of growth hormone. The treatment was started after the heifers had begun to decline in milk yield, so this study was made on established lactation. It was assumed that if the hormone given either arrested the normal decline or increased the intensity of milk secretion it would indicate the animal's secretion of the hormone was limiting its milk secretion capacity.

Feeding of the stilbestrol for four weeks tended to arrest the decline in milk yield during the period and in some animals caused a slight increase in milk yield. In two cows in late lactation, prolonged feeding of this hormone caused the maintenance of the level for periods of 9 to 10 weeks. The authors suggested that low levels of estrogen tend to prevent the decline in the secretion of the lactogenic hormone of the pituitary.

The injection of the L-thyroxine for periods of 4 to 9 weeks in some heifers produced a marked rise in milk yield whereas in others the effect was small. These data are believed to indicate the degree to which the endogenous rate of secretion of thyroxine was limiting the milk producing capacity of the individual heifer. When the injections were stopped suddenly, the yield of milk declined rapidly for about two

weeks then increased to the level which might have been expected at that stage of lactation. During the periods of exogenous thyroxine administration, the secretion of endogenous thyroxine is prevented, but returns rapidly to the normal rate when thyroxine injections are stopped.

Daily injection of 50 mg. of growth hormone for a period of one week stimulated a marked increase in milk yield in 5 to 8 cows injected; this increase was sustained for periods of 2 to 8 weeks. Three cows showed no response. Variation in response to growth hormone was interpreted as indicating the degree to which the intensity of milk secretion was limited by the rate of endogenous secretion of the growth hormone.

On the basis of the responsiveness of each animal to the three hormones given, an analysis of the genetic-endocrine causes of their variability in milk production was suggested. Possible mating of animals on the basis of the adequacy of the rate of secretion of the hormones that make possible high milk production rather than total production.

The relationship existing between the ovarian hormones and prolactin has also been studied. Meitis and Sgouris (1952) injected 960 International Units (I.U.) of estrone and 1 I.U. of progesterone daily for 25 days to induce optimal mammary development in 63 albino rabbits of both sexes, intact and castrate. From the 25th to 35th days, all but three rabbits were injected with 40 to 50 I.U. of prolactin daily, with or without one or both of the steroid hormones. At

the end of the 35th day, the rabbits were killed and the mammary glands exposed and rated for intensity of lactation. The extent of mammary growth induced was determined by injecting radioactive phosphorus (P^{32}) four hours prior to slaughter, and then making radioautographs of the mammary glands. The rabbits given prolactin and both steroids during the last ten days of the experiment had practically no milk in their mammary glands. Those injected with prolactin alone had mammary glands filled with milk. Those receiving prolactin and progesterone showed no inhibition in lactational response and only slight inhibition was noticed in those receiving prolactin and estrone. Radioautographs indicated the presence of intensive mammary growth in the rabbits treated with both steroids throughout the 35-day period but not in those given both steroids for the first 25 days only. It was concluded that in the doses employed, estrone and progesterone together can effectively inhibit the milk stimulating action of prolactin in the rabbit; whereas progesterone alone is ineffective and estrone is only slightly effective in this respect. Meites and Sgouris (1954) studied the effects of altering the balance between prolactin and ovarian hormones on initiation of lactation in rabbits. They ovariectomized 36 young rabbits and after 10 to 14 days injected them with 0.096 mg. of estrone and 1 mg. progesterone for 25 days to induce optimal mammary development. From the 25th to 35th days, three groups of six rabbits each were continued on the same doses of steroid hormones together with 2, 4, and 8 mg. of prolactin daily, respectively. Three other groups of six each were injected with 2 mg. prolactin daily with 0, 0.25, or

0.50 mg. of the two steroid hormones used previously. On the 36th day the rabbits were killed and their mammary glands were exposed and rated visually for lactational response. In the first three groups, lactation was practially inhibited when only 2 mg. of prolactin were injected daily in the presence of the two steroid hormones, while 4 or 8 mg. daily elicited good lactational responses. In the second three groups the greatest amount of milk was secreted when 2 mg. of prolactin were given daily without the steroid hormones, whereas the same dose of prolactin in the presence of either 0.25 or 0.50 mg. of the steroid hormones produced a smaller milk flow. It was concluded that the antagonism between mammary growth and lactation is relative, depending on the balance between the levels of prolactin and the two ovarian hormones in the body. When the mammary growth stimulus exerted by the two ovarian hormones is greater than the lactational stimulus of prolactin, milk flow will be inhibited and vice versa.

Work has been done by Conde and Barsantini (1954) on the mechanism of the inhibition of lactation during pregnancy. They produced pseudopregnancy in rats by treating them for 15 days with estradiol benzoate, and then divided them into four groups. The controls received the estradiol for three more days and the other animals were castrated. In group II the estradiol treatment was continued for 15 days more. Progesterone was given to group III, and estradiol benzoate plus progesterone to group IV. Another group of rats was treated with chorionic gonadotropin during 18 days and a last group treated in the same way was castrated and treated with progesterone during three days. The conclu-

sion of the authors is that the suppression of one or both of the hormones of the ovary allows the action of prolactin on the mammary gland and the beginning of the secretory period.

Sgouris and Meites (1953) removed the inguinal mammary glands from 17 rats on the 12th to 15th day of gestation and from 19 rats on the 4th day postpartum. The glands were homogenized and 100 mg. samples were incubated with 0.2 mg. of Squibb prolactin (20 to 25 I.U. per mg.) at 38 $\pm$0.1° C. for one hour. The tissues were then removed and assayed in White Carneau pigeons for prolactin activity. The mammary homogenates from the lactating rats inactivated 65.6 percent of the prolactin compared with 19.5 percent for the tissue from the pregnant rats. When corrected for milk content the former tissue showed eight times as much capacity to remove prolactin as the latter. These data suggest that the mammary glands of pregnant rats cannot utilize prolactin to any marked extent, and this contributes to the absence of copious lactation during gestation in the rat.

The specificity of the lactogenic hormone in the initiation of lactation has been demonstrated by Bergman and Turner (1940). Their results indicate that the primary function is to initiate and maintain established lactation. They found that anterior pituitary extracts, rich in the thyrotropic and other hormones, but containing only traces of the lactogenic hormone, do not possess the ability to initiate lactation in doses as high as could be tolerated. This fraction does, however, have a supplementing effect on established lactation.

Some interesting findings are the effects of stilbestrol and pro-

lactin on chickens. Godfrey and Jaap (1950) tested 15 mg. of diethyl-stilbestrol in 1 ml. of sesame oil injected subcutaneously in 37 "broody" hens. The "broodiness" was interrupted in 28 cases. They also tried doubling the dose (30 mg.) and found that the "broodiness" was interrupted in 113 out of 115 birds. The birds were put on adequate rations and started to lay again. The authors state that the mode of action may be that of surpressing lactogen from the anterior pituitary.

Nalbandov and Card (1942) induced complete "broodiness" in Cornish, White Rock, and Brown Leghorn males with purified lactogenic hormone. They brought out the fact that it took five times as much of the hormone to bring about "broodiness" in Leghorns as it did in the Cornish male. The White Rocks were intermediate in their response. The authors state that it might be possible to differentiate between genetically broody and non-broody males by the amount of hormone required.

According to Carlisle (1954) the effect of mammalian lactogenic hormone on lower chordates, such as the dogfish, is that of a gonadotropin. He states that this activity resides in the hormone itself and not in any impurity.

A rather interesting article has been written by Elliot and Turner (1954) dealing with what they term the "mammary spreading factor". Their investigations in rats, mice, guinea pigs, and rabbits have shown that the increases and the periods at which peak amounts of the spreading factor were observed to be present in the mammary glands during pregnancy closely parallel the growth phase of mammary gland

development, reaching a maximum between mid and two-thirds of gestation. During the last one-third of pregnancy, when the cells of the mammary glands begin secretion, amounts of the spreading factor decline. They state that it appears that the elaboration or activation of the spreading factor practically ceases as secretory activity begins.

Wallace (1953) made some interesting observations on mammary development in calves and lambs. He found that mammary development in sheep from two months of fetal age to four months after birth was similar to that in dairy cattle. Castration at birth had little effect on mammary growth. Treatment of males with estrogen gave rise to enlarged teats, dilated cisterns and ducts, and some secretion. In sheep, removal of the ovaries at birth had no apparent effect on mammary development whereas in heifers it nearly caused cessation. Estrogen stimulated gland formation in all the female lambs and restricted the normal spreading of mammary tissue into the udder. In heifers, in addition to inducing gland formation, it promoted spreading of tissue into the udder pads. This might possibly indicate a different hormone source in sheep.

Wrenn and Sykes (1953) induced six month old dairy heifers to lactate by injecting 2 mg. of stilbestrol in olive oil three times a week for 20 weeks. This was designated as Group I. Group II were heifers, seven months old, that were injected three times a week with 2 mg. of stilbestrol plus 8 mg. progesterone in the olive oil carrier. Two weeks after the last injection of estrogen and progesterone the administration of pituitary hormones was began in the following sequence:

(a) prolactin, growth hormone, followed by crude extract; (b) growth hormone, then growth hormone plus prolactin followed by crude extract; (c) thyrotropin then growth hormone followed by crude extract. These were aqueous solutions injected intramuscularly over a period of about six weeks. Milk production was found to be much greater in heifers in which the udder was developed with stilbestrol than in heifers in which a combination of stilbestrol and progesterone was used. Prolactin had only a slight, if any, stimulatory effect upon the induced lactations. Thyrotropic hormones stimulated lactation markedly. The greatest stimulation was obtained by administration of a growth hormone preparation. These effects were stated by the authors to be partly a result of thyrotropic hormone contamination of this preparation, but the experiments indicate that growth hormone has galactopoietic effects.

There is a large accumulation of literature regarding this so-called "growth hormone" with many tending to indicate that growth may be only one of the functions of this hormone. Bremby and Hancock (1955) gave daily subcutaneous injections of growth hormone to identical twin cattle during: (a) a 12 week therapy period over the peak phase of lactation; (b) a four week therapy period at the latter end of lactation. In both trials a marked increase in milk and butterfat production resulted. There was no change in either milk or blood composition due to growth hormone therapy as such in the treated animals. Along with the increased milk and fat yields, there was an apparent increase in the efficiency of production.

Mitchell et al. (1954) gave varying doses of partially purified

somatotropin to intact and hypophysectomized guinea pigs. He obtained no somatic growth or change in various organ weights. This hormone was proven highly active in rats.

Jordan and Shaffhausen (1954) studied the effect of somatotropin on milk yield in ewes by injecting 25 mg. of somatotropin in 5 c.c. of normal saline solution intramuscularly. In trial one, ewes gave 40 percent more milk following the treatment period than the controls. There was a significant increase in fat percent and a non-significant decrease in non-fat solids. In trial two, milk yield was up 22 percent and a 2.3 percent increase in fat test during the 21 days of treatment. After the treatment was stopped, milk yield and fat test retarded to near pretreatment levels within 3 to 10 days. Feed consumption was up. To obtain all the milk 10 units of oxytocin were injected.

Besides estrone, progesterone, prolactin, and somatotropin there are many other compounds that will effect lactation. Johnson and Meites (1956) studied the effects of cortisone on lactation in rats. The rats were divied into three groups with one group receiving injections of saline only, the second received 1 mg. of cortisone daily, and the third received 0.5 mg. of cortisone daily. Throughout the experimental period the controls showed no significant change -- either in weight gain of the young or in weight loss of the dams. The lot receiving 1 mg. of cortisone daily had a significant weight loss in the dams but maintained the young at nearly the same rate of gain as the controls. On the other hand, the dams receiving 0.5 mg. of cortisone daily had a slight weight loss, but the weights of the young were

greatly increased over those of the controls and the 1 mg. lots. The authors state that these data support the view that the glucocorticoids play an active role in lactation.

Again working with the corticoids, Cowie and Tindal (1955) obtained virtually complete maintenance of lactation in the rat following adrenalectomy on the fourth day of lactation, by injecting 100 mcg. daily of 9 alpha-chlorohydro-cortisone acetate. Partial maintenance was observed with 9 alpha-flurohydro-cortisone acetate, (100 mcg. daily), aldosterone (50 mcg. daily) or hydrocortisone (1 mg. daily).

Smith (1954) studied relaxin extracts in combination with estradiol or a combination of estradiol and progesterone which had not previously given any lobule-alveolar growth of the mammary gland of immature, ovariectomized rats. The glands of mature rats were considerably less responsive to relaxin than those of immature rats. Mammary glands of hypophysectomized rats showed negative ductal-lobular response to relaxin-steroid combinations, while those of adrenalectomized rats showed only a reduction in total area. An intensely positive response was noted in thyroidectomized rats injected with relaxin, progesterone, and estradiol. He stated that this effect can be accounted for almost entirely by an increased response to estrogen alone. The alveolar structures developed by the steroid-relaxin combinations showed evidence of secretion after a single injection of prolactin.

The drug reserpine has been observed to influence the function of several endocrine glands. Meites (1957) was able to confirm that reserpine can initiate lactation in rabbits by injecting 1 mg. reserpine

per kilogram of body weight into pseudopregnant rabbits. One week later milk could be expressed from the nipples of the reserpine treated rabbits, but not the controls. In no instance was the lactation response induced by reserpine equal to that seen after parturition. The mechanism by which it initiates milk secretion in rabbits is unknown, but it may stimulate secretion and/or release of prolactin from the anterior pituitary.

Soykova-Pachuerova et al. (1954) administered dried placenta orally and increased milk secretion in 86.2 percent of 210 lactating mothers. Beef placenta preparations were also active. The active principle seemed to be a non-protein.

It is quite well accepted that the suckling stimulus can both induce and maintain lactation. Some work done by Cotes and Cross (1954) on the influence of suckling on food intake and growth of some adult female rats was interesting. Changes in body weight and food intake were studied for 14 days postpartum in primiparous rats. The growth increments in suckled rats with galactophores cut to prevent milk withdrawal in normally lactating rats were greater than those in unsuckled controls. The growth increments in rats with galactophores cut were accompanied by parallel increases in food intake. Injection of 3 I.U. of prolactin daily did not produce these changes in body weight and food intake in non-suckled rats. The main factor in the extra growth of lactating rats is an increase in food intake in excess of the metabolic needs for milk secretion, induced by the stimulus of suckling.

After studying all these compounds, one wonders just where they

function. Work done by Williams and Turner (1954) with the localization of I^{131} tagged prolactin in rabbit mammary glands is applicable to this question. The lactogenic I^{131} tagged hormone was injected intraductally into the mammary glands at the end of pseudopregnancy. After 45 to 95 minutes the glands were removed and the cell components separated. The radioactive hormone was localized, to a great extent, in the particulate fractions of the mammary cells. The nuclei and supernatent portions of the cell contained little hormbne. These data suggest that the site of activity of the hormone is primarily associated with the mitochondria and microsomes and, therefore, the particulates of the mammary gland cells play important roles in their synthetic activity.

PROCEDURE

Part I

An exploratory trial was initiated to study the artifical induction of lactation on April 4, 1957.

This experiment involved 15 head of dry, three to six year old ewes and one yearling castrate male sheep. All animals were of Rambouillet, Columbia, or Targhee breeding. The ewes had produced lambs and lactated normally in previous years.

All animals were kept in one pen in the north wing of the Montana State College sheep barn. Each animal had an aluminum ear tag for individual identification.

Hay of average to poor quality alfalfa or mixed grass hay was fed ad libitum in a common feed bunk in the center of the pen. Salt and water were readily available to the animals at all times.

A vasectomized ram equipped with a marking harness was placed in the pen to determine if any of the ewes were in heat.

Palpations were made on the mammary gland of each ewe to detect any gross abnormality, such as mastitis infection or large connective tissue areas due to previous mastitis infections. All animals assigned a treatment appeared normal throughout the experimental period.

The experimental treatments for 1957 are shown in Table I.

The estrone source was a commercial preparation of the crystalline compound in aqueous suspension, 5 mg. per c.c. Two commercial preparations of diethylstilbestrol were used. One being an oil

Table I. Treatments 1957.

Ewe and Treatment Number	Treatment
I	12.50 mg. Estrone
II	25.00 mg. Estrone
III (Wether)	12.50 mg. Estrone
IV	50.00 mg. Diethylstilbestrol (oil)
V	50.00 mg. Diethylstilbestrol (aqueous)
VI	25.00 mg. Diethylstilbestrol (oil)
VII	25.00 mg. Diethylstilbestrol (aqueous)
VIII	25.00 mg. Diethylstilbestrol (oil) 15.00 mg. Diethylstilbestrol implant
IX	5.00 mg. Diethylstilbestrol (oil)
X	5.00 mg. Estrone. Five days. 5.00 mg. Progesterone. Five days. 400 I.U. Prolactin
XI	5.00 mg. Estrone. Ten days. 5.00 mg. Progesterone. Five days. 10.00 mg. Progesterone. Four days.
XII	5.00 mg. Estrone. Ten days. 5.00 mg. Progesterone. Six days. 10.00 mg. Progesterone. Four days.
XIII	Graded dose estrone 1 mg. first day, 2 mg. second day to 5 mg. fifth day. 5 mg. Progesterone. Five days. 400 I.U. Prolactin.
XIV	Graded dose estrone as in XIII 5 mg. Progesterone plus 3 mg. estrone. Five days. 400 I.U. Prolactin.
XV	600 I.U. Prolactin.
XVI	0.75 c.c. Oxytocin. One day. 2.00 mg. Estrone. One day.

suspension, 25 mg. per c.c., and the other being a suspension in isotonic sodium chloride solution, 15 mg. per c.c. The progesterone was a commercial preparation in a sesame oil carrier, 25 mg. per c.c. Prolactin was obtained in crystalline form and was mixed with the supplied aqueous diluent at time of use. The resulting solution contained 200 I.U. per c.c.

All treatments were given as intramuscular injections, except the diethylstilbestrol implant. This was a 15 mg. pellet implanted subcutaneously near the base of the left ear.

The ewes were milked and udder size observed each time an injection was given. The secretion was removed from the udder but was not collected. All data regarding the secretion color, amount, and consistency were obtained by visual observations.

The gross udder development was "measured" by visual appraisal.

RESULTS AND DISCUSSION

Part I

Before experimental treatment was begun, each ewe was "milked". This was done to find if the ewes were really dry in the sense that no secretion of any kind could be obtained from the teats. The ewe on Treatment V (50.00 mg. stilbestrol in aqueous suspension) gave approximately 10 c.c. of a thin, whitish fluid which was present throughout the experimental period but gradually diminished until on April 19, 1957, after 15 days of treatment, there were only a few drops obtained.

Similar results were obtained with the ewe on Treatment XII (5.00 mg. estrone for ten days; 5.00 mg. progesterone for six days followed by 10.00 mg. progesterone for four days). This ewe had a very small udder but when the left half was milked a brownish fluid was obtained for five days in succession. On the fifth day, the secretion was more clear than that obtained at any previous time. No secretion was obtained on the sixth day but on the seventh day following initiation of the trial, a few drops of the brownish fluid were again obtained.

No initial secretion was obtained from any of the other ewes or from the wether on Treatment III. (12.50 mg. of estrone).

Detailed accounts of the treatments and the observations at time of injection are given in the Appendix, pages 73 to 84.

In referring to these tables, the reader will note that the ewes producing a milk-like secretion (Treatments I, II, X, XI, and

XIV) showed a lapse of from 48 to 96 hours from beginning of treatment to the appearance of the milk-like secretion.

The secretion obtained from the variously treated ewes ranged from a fluid that was clear and watery to secretion that was rather thick and brown. The milk-like secretion was white, but was not of a true milk consistency; it was somewhat thick and slightly sticky. The secretion designated as "colostrum-like" was yellow to yellowish-white in color, rather thick, and quite sticky.

The udder size was observed each time after the secretion was removed. It was difficult to determine if the udder had actually enlarged or was distended with unremoved secretion. A more true indication of udder development was the firm glandular tissue lying embedded in the more flabby udder tissue. Palpation of this tissue appeared to give a more valid picture of udder change.

The ewe on Treatment I (12.50 mg. estrone) was marked by the vasectomized ram on April 15, 1957. Whether the ewe was actually in heat or whether the ram mounted her by reasons of sexual prowess only is not known.

The results reported in this experiment tend to indicate that the injected prolactin's effect was masked by the animals' own prolactin secretion that was stimulated by the estrogen treatment. In Ewe Number X (5 mg. estrone for five days followed by 5 mg. progesterone for five days followed by a total of 400 I.U. of prolactin) the mammary secretion was quite clear when the first 200 I.U. of prolactin injection was given. The next day when the second injection

was given, the secretion was milk-like. On the day following the second injection, the secretion was milk-like but of a much smaller amount.

Treatment XIII (graded dose of estrone for five days; 5 mg. progesterone for five days followed by 400 I.U. of prolactin) shows no effect from the 400 I.U. of prolactin but as will be noted by referring to the Appendix, page 82, no secretion was present. As the udder showed very little development, this result could be in agreement with work done by Mizano et al. (1955). Their work dealt with the effect of prolactin, injected intraductally on the mammary gland growth, mainly on alveolus formation. They found that in glands having well developed duct systems with some alveoli, prolactin may promote alveolus growth and induce secretion. In glands with moderately developed duct systems without alveoli, prolactin first induces alveolus formation and subsequently may cause secretion. In glands with slightly developed or rudimentary duct systems without alveoli, growth of ducts is promoted. From this one would have reason to conclude that the udder development present in the trial reported herein had not progressed to the state where the prolactin could initiate the milk secretion.

The ewe on Treatment XIV (graded dose of estrone for five days; 5 mg. progesterone plus 3 mg. estrone for five days followed by 400 I.U. prolactin, see Appendix page 83) stopped secreting after the injection of 200 I.U. prolactin. Whether or not this amount was enough to upset the delicate lactation hormonal balance or if the

animal would have stopped secreting at this point without the treatment is not known.

It would appear that an estrogen was capable of inducing lactation in these trial sheep, even without the udder development associated with normal lactation.

PROCEDURE

Part II

A continuation of the project of inducing lactation in non-pregnant ewes was initiated on April 3, 1958.

This experiment involved eleven head of dry, six to seven year old ewes. These ewes had produced lambs and lactated normally in previous years.

The ewes were examined carefully at the beginning of the experiment for the presence of any gross udder abnormalities. The ewes were then randomly assigned a treatment number from one to eleven, with the numbers and the corresponding treatment shown in Table II.

The hormones used in all injections were purchased in crystalline form. The desired amount of the crystalline compound was carefully weighed into test tubes and dissolved in 2 c.c. of diethyl ether. Commercial peanut oil was then added to this solution and mixed thoroughly. The ether was evaporated out of the oil by placing the test tubes in a water bath heated by a steam plate. The temperature of the preparations did not exceed 60° C. at any time. The solutions were then cooled and the tubes were sealed tightly until needed.

All treatments were given as intramuscular injections. The ratio treatments were devised so as to give the animal the desired amount of estrogen plus the 70 mg. of progesterone every sixth day. The diethylstilbestrol treated ewes received the indicated amount of hormone every second day.

The ewe on Treatment X was the control on the peanut oil carrier,

with a 3 c.c. intramuscular injection being given every other day.

Treatment XI was also a control treatment. The ewe was set up and the udder "milked" in the same manner for approximately the same length of time as were the hormone treated ewes.

Table II. Treatments for 1958.

Ewe and Treatment Number	Treatment
I	Graded dose estrone; 1 mg. on first day to 5 mg. the fifth day
II	1:40 estradiol to progesterone ratio. (1.75 mg. estradiol + 70 mg. progesterone)
III	1:40 diethylstilbestrol to progesterone ratio (1.75 mg. diethylstilbestrol + 70 mg. progesterone)
IV	1:80 diethylstilbestrol to progesterone ratio (0.875 mg. diethylstilbestrol + 70 mg. progesterone)
V	1:200 diethylstilbestrol to progesterone ratio (0.35 mg. diethylstilbestrol + 70 mg. progesterone)
VI	0.25 mg. diethylstilbestrol
VII	0.50 mg. diethylstilbestrol
VIII	1.00 mg. diethylstilbestrol
IX	5.00 mg. diethylstilbestrol
X	Control. 3 c.c. peanut oil injected every other day
XI	Control. Manipulation of the udder every day with no injections.

All ewes were milked every other day, once a day, or twice a day depending on the amount of secretion produced. All secretion of sufficient amount was collected, observed as to color and consistency, and weighed. The samples were then stored in a refrigerator at a tempera-

ture that was sufficient to freeze many of the samples.

In addition to milk obtained from treated ewes, colostrum was collected from four normal ewes for the "colostrum sample". Milk was also collected from ten normal ewes that had lambed from four to six weeks previously for the "advanced lactation sample". These samples were used as controls on chemical analyses.

Fat and total solids content were determined by the Mojonnier Method (Mojonnier and Troy 1925). Protein content was determined colormetrically (after Wu 1922, modified by Folin and Ciocalteu 1927, and Greenberg 1929). Ash content was determined by first evaporating the milk to a total solids basis on a steam plate. The samples were then placed in an electric oven and ashed at 600° to 650° C. until all traces of carbon residue were removed. Composite ash samples from normal colostrum, normal advanced lactation, and Treatments I, IV, and IX were analyzed separately. Calcium, phosphorus, potassium, and iron content for these samples was determined by appropriate chemical analyses.

Udder growth or change was determined in much the same manner as in 1957. Glandular tissue diameter and change in size was recorded to the nearest one-fourth inch. Colored slides (35 m.m.) were taken of each ewe at the start and termination of the experimental period. Weekly pictures were taken of ewes showing marked udder development.

Ewes on Treatments I, IV, IX, X, and XI were slaughtered at what was as close to production peak as possible. The udders were removed, the secretory tissue measured, and fixed in Bouin's Solution. Routine

histological examination was then made to determine the extent of the alveolar development. Alveoli and lumen measurements were made with an ocular micrometer.

At time of slaughter the reproductive tracts and adrenal glands were collected. External appearance was observed in an effort to detect the presence of any gross abnormality.

Udder infusions were made into udders removed from two non-treated, normal, lactating ewes.

The infusions were made with liquid Latex 571 plus Aquarex WAQ stabilizer. The pigments were the rubber pigments made especially for this type of latex, specifically, Rubber Red 2BL, Rubber Blue CPL, and Toluidine Yellow WD.[1/]

The infusion liquid was compounded as follows:

Neoprone from Type 571 Latex 50% solids	200 parts
Aquarex WAQ, 33% in 67% distilled H_2O	9 parts
Color, pigments mixed to give desired color Prepared in a 1% solution of NaOH or KOH	4 parts (suggested)

This mixture was infused into the veins, arteries, and ductal-alveolar systems of the udder. The pressure needed to obtain the desired penetration was sufficient to reach the smaller portions of the lumen network. The colors of the infused latex were: veins, blue; arteries, red; and the duct-alveolar system, yellow.

1/ These compounds were obtained from the E. I. Du Pont De Nemours and Co., Elastomers Department, 2930 East 44th Street, Los Angeles 58, California.

Corrosion was completed by suspending the udder in a solution of concentrated hydrochloric acid.

RESULTS AND DISCUSSION

Part II

Secretion was obtained from eight of the treated ewes and one of the controls. Seven of these produced measurable amounts (more than 0.30 gm.).

Udder and secretion observations

The complete record of the injections and observations of all treatments are shown in the Appendix, pages 85 to 104. Daily production data are shown in Table III.

At the start of the experiment, 0.77 gm. of secretion was removed from the udder of Ewe Number I (graded dose of estrone). This was a white fluid with a slightly yellowish tint. Its consistency was that of thick cream. Two days later when the 2 mg. injection of estrone was given, the secretion was of similar consistency but somewhat more white in color. By the third day the secretion was of a definite milk color. The consistency was fluid in nature but was still more viscous than normal milk. The viscosity appeared to be caused by cell debris.

No secretion was obtained from the ewe on Treatment II (1:40 estradiol to progesterone ratio) at any time. On the fourth day after the start of the treatment a small amount of firm tissue seemed to be appearing in the udder. Whether or not this was glandular tissue is not known. Neither the udder nor the firm tissue appeared to change for the remaining 20 days of treatment.

A small amount of secretion was obtained from the ewe on Treatment III (1:40 diethylstilbestrol to progesterone ratio) at the be-

Table III. Daily production.

Ewe	I	IV	V	VI	VII	VIII	IX
Date	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
4/3/58	.77	3.14	29.70	Not coll.	None	7.04	Not coll.
4/5	2.84	.36	17.97	3.55	None	3.17	3.10
4/6	29.90	Not coll.	Not coll.	Not coll.	Not coll.	Not coll.	Not coll.
4/7	22.00	.32	8.70	3.88	2.20	29.02	45.18
4/8	11.56	1.10	5.00	3.94	5.52	26.92	50.58
4/9	Slaughtered 4/9	1.27	2.98	2.33	12.05	30.30	50.80
4/10		.82	2.72	2.28	11.88	20.90	62.72
4/11		1.93	.94	.78	16.08	57.52	70.45
4/12		1.04		1.16	23.36	71.48	105.43
4/13		1.32		1.24	26.94	93.71	101.63
4/14		3.66		2.60	37.39	151.02	157.20
4/15		8.44		3.43	35.83	170.42	178.51
4/16		15.42		1.62	38.16	209.16	277.50
4/17		25.82		1.94	62.64	246.72	236.28
4/18		44.96		Not coll.	54.38	278.45	228.59
4/19		52.03		4.72	55.11	308.45	406.69
4/20		94.45		Not coll.	72.78	399.22	200.06
4/21		81.16		5.50	67.34	348.92	124.06
4/22		114.90		Not coll.	78.54	419.53	Slaughtered
4/23		124.23		4.73	87.07	468.16	4/21
4/24		126.80		Not coll.	85.66	456.90	
4/25		210.90		3.90	104.74	514.44	
4/26		306.81		Not coll.	142.43	610.52	
4/27		256.91		3.14	98.48	538.22	
4/28		249.09				505.42	
4/29		Slaughtered 4/28				Not coll.	
4/30						724.19	

ginning of the experiment. This fluid was thin, sticky, and honey-colored. There was not enough secretion obtained throughout the experimental period to give an accurate weight. Secretion was obtained for the first six days of treatment. The fluid became slightly less yellow and more cloudy with the amount obtained becoming smaller each time the ewe was milked. No secretion was obtained from April 11 until April 25, 1958. At this time, and on April 27, 1958, a few drops of secretion were obtained similar in appearance to that obtained at the beginning of the experiment.

On April 7, 1958, and April 10, 1958, a string of white material was obtained from the right teat. It was soft but rather tough and was similar to flesh in consistency. No tests were conducted to determine the composition or origin of this material.

Four days after Ewes Number II and III were removed from their respective treatment regimes, they were given an injection of 5 mg. diethylstilbestrol. Two days later, a few drops of rather clear secretion were obtained from the teats of both animals. At this time they were given another 5 mg. injection of diethylstilbestrol. On May 5, or two days following the second injection, milk-like secretion could be expressed from the teats of both animals. The animals were again observed on May 9. The udders at this time were enlarging and the milk-like secretion was still present. The secretion was not collected.

At the beginning of the experiment the ewe on Treatment IV (1:80 diethylstilbestrol to progesterone ratio) had a small but firm udder.

A small amount (3.14 gm.) of secretion was obtained from the left half. This secretion was a thin cloudy fluid but not a milk-like secretion. For the following 12 days the secretion amount remained quite small. The appearance of the secretion changed on April 19, 1958, 16 days after treatment start, to a more viscous fluid, yellowish-white in color. From this sample throughout the experimental period a definite cream layer would form when the tube containing the secretion was placed in a refrigerator over night.

The glandular tissue in the right half of the udder appeared to enlarge more rapidly than that in the left for approximately 15 days. From that time, the left half enlarged markedly and began secreting profusely. On April 21, 1958, or 18 days after treatment start, the glandular tissue in the right half was slightly smaller and on April 22, 1958, the tissue had regressed to the size similar to that at the beginning of the experiment. The right half produced not more than 20 c.c. secretion total amount; all other secretion was obtained from the left half.

At the beginning of the experiment the ewe on Treatment V (1:200 diethylstilbestrol to progesterone ratio) had an udder that was flabby but exhibited some initial development. When she was milked, 29.70 gm. of secretion wereobtained. This secretion was a thin, slightly cloudy, honey-colored fluid. As the treatment was continued the secretion became slightly more cloudy. The amount decreased to only a few drops and was not collected after April 11, 1958. This secretion was not felt to be due to the hormonal treatment but was similar in appear-

ance to the udder secretions described by Hammond (1927).

Some change was noted in the glandular tissue from time to time. There was a slight enlargement for 14 days. Thereafter the udder regressed slightly and remained so throughout the experiment.

Initial udder development of Ewe Number VI (0.25 mg. diethylstilbestrol every other day) was small and flabby with the glandular tissue being firm in both halves. A small amount of cloudy secretion was present but was not collected. The ewe was milked once a day from April 7, 1958 until April 17, 1958. The remainder of the period secretion was removed every other day.

The secretion obtained was similar to normal colostrum in color and consistency throughout the trial. When placed in the refrigerator over night, a white "cream" layer would form at the top of the tube and a thin, yellow fluid would appear below.

Glandular tissue enlarged slightly for 11 days. Thereafter enlargement ceased and the udder decreased in size throughout the remainder of the experiment.

The ewe on Treatment VII (0.50 mg. diethylstilbestrol every other day) exhibited no initial udder development. A small amount of glandular tissue was present in both halves, but no secretion was obtained at this time. The first secretion was obtained on April 7, 1958 (2.20 gm.). It was yellowish-white in color and was slightly lumpy. Secretion was removed every other day until April 17, 1958. Thereafter the secretion was collected once a day. Secretion color was slightly yellow for the first four days. It then assumed normal

milk-like appearance in color and consistency and remained so for 16 days. The last day secretion was obtained it was becoming slightly yellowish. The consistency, however, was still similar to normal milk.

The udder appeared to enlarge for 16 days. Thereafter, until April 26, 1958, no definite change could be noticed. Peak production was reached on April 26, when 142.43 gm. were obtained.

The ewe on Treatment VIII (1.00 mg.diethylstilbestrol every other day) exhibited a very small udder at the beginning of the experiment. Glandular tissue could be detected in both halves but this too was very small. Secretion was obtained (7.04 gm.) at the onset of treatment. This was a thin, sticky fluid, and slight cloudy.

Secretion was removed every other day until April 7, 1958. Once a day milking was continued for ten days until April 17, 1958. Secretion was then removed twice a day until April 27, 1958. At this time it was decided to terminate the experiment and secretion removed thereafter was obtained once on April 28, 1958, not removed on the 29th, and once on the 30th. The last milking yielded 724.19 gm. of milk-like secretion. This was done to give some indication of the maximum capacity of this ewe's udder.

Initial secretion color has been described. For four days it rapidly became more cloudy and slightly yellowish. Thereafter it was milk-like in color and consistency and remained so throughout the trial.

The udder developed markedly. After 17 days of treatment the udder and glandular tissue area was very similar in size and appearance

to that of a normal lactating ewe. The mammary veins were well developed and clearly visible. When milk flow became quite profuse the ewe would stand and wait to be milked.

The udder of the ewe on Treatment IX (5.00 mg. diethylstilbestrol every other day) was very flabby at the beginningof the experiment. Glandular tissue was present in both halves, but was small and pliable. A small amount of clear fluid was obtained from the left half. This was not collected. No secretion was present in the right half.

The udder began developing quite rapidly after injections were started. The right half appeared to enlarge more quickly than the left half. After treatment for ten days, the udder was approaching the size and appearance of a normal lactating ewe. At this time a secondary bulge could be seen at the base of the teats; this was most likely the gland cistern distended with milk. The udder seemed to regress slightly from this time until slaughter and did not at any time attain the development reached in the ewe on Treatment VIII.

Secretion was removed every other day until April 7, 1958. Once a day milking was continued until April 17, 1958. From this time until slaughter on April 21, 1958, secretion was removed twice daily.

Secretion color was initially clear; became honey colored on the third day, and was milk-like in appearance and consistency by the fifth day. A general increase in production occurred until April 19, 1958, when 406.69 gm. of milk-like secretion were obtained. From this time until slaughter on April 21, 1958, the production decreased.

The udder of Ewe Number X (3.c.c. peanut oil every other day)

was small and flabby at the beginning of treatment. Glandular tissue was present but small. The largest amount was present in the right half. No secretion was present at this time.

From the fourth to the seventh day of treatment the glandular tissue in both halves became quite firm and appeared to enlarge slightly. However no secretion was obtained. Thereafter until slaughter on April 28, 1958, the udder and glandular tissue regressed and became increasingly more pliable.

The ewe was milked every other day until April 7, 1958, daily until April 17, 1958, then again every other day until slaughter on April 28, 1958. No secretion was obtained throughout the experimental period.

The udder of Ewe Number XI (daily manipulation) was small and flabby at the beginning of the experimental period. Glandular tissue was present in both halves and was pliable. The right half was the largest. A small amount of thick, slightly cloudy, honey-colored secretion was obtained at this time. The udder size remained the same throughout the experiment. Glandular tissue area increased slightly for eight days; thereafter no change was recorded.

Secretion was removed every other day until April 7, 1958, daily until April 17, 1958, and again every other day until slaughtered on April 28, 1958. A few drops of secretion were obtained at each milking throughout the experimental period. The color change was slight but was becoming more cloudy by April 14, 1958. There was not a sufficient amount obtained at any time to be accurately weighed or

analyzed.

Work conducted by Butcher and Van Horn (1954) showed total milk production over a 24-hour period by ewes with single or twin lambs, to range from 648 gm. to 1,244 gm. with 946 gm. being the average. It can be determined from these data that Ewe Number VII was somewhat lower in her peak daily production (142.43 gm.) than the normal animals. Ewes Number IV and IX at peak production (306.81 gm. and 406.69 gm. respectively) were approximately one third the average of that in the normal ewes. Ewe Number VIII at the peak (610.52 gm.) was approximately two thirds the average for the normals. The largest amount obtained was 724.19 gm. but this was a 36 hour accumulation in the udder.

Frequency of milking is a factor to be considered also in the total production. Research reviewed by Espe and Smith (1952) indicates increased milk production in dairy cows that were milked three times daily. It would be interesting to know just how much milk would have been produced by the ewe on Treatment VIII if she had been milked four or five times a day.

Many positive results have been reported in inducing lactation in virgin goats. In work published by Folley et al. (1941) a statement is made as follows: "Lactation in virgin goats is quite common, particularly in high milking strains." The question immediately arises in one's mind, if this is the case is there a possibility that some of the "positive results" are caused by the animal's own endocrine system?

Milk composition

Composite samples were analyzed specifically for fat, protein, ash, and total solids. Lactose was then obtained by difference. Results are presented in Table IV.

The first analyses figures were obtained from approximately a nine-day sample. Thereafter analyses were conducted on samples ranging from daily to six day composite samples.

After studying Table IV it will be noticed that the fat content shows a general rise from the first sample. This rise continued throughout the analytical period in Treatments VI and VII, but a slight drop is noticed in the last sample in Treatments IV, VIII, and IX. When these figures are compared with "normal colostrum" and "normal advanced lactation" samples, it will be noticed that the secretion from the treated ewes is consistently lower in fat. Only once in Treatments VIII and IX does it exceed 8 percent.

According to Espe and Smith (1952), short intervals between milkings is a factor that tends to give a higher fat test in the milk than would normally exist. The "normal advanced lactation" sample was taken from ewes that had lambs at side and obviously the interval between milkings would therefore be short. This could partially explain the fat content of this sample being somewhat higher than that reported by Espe and Smith (1952) and Morrison (1956).

By the same token, with the treated ewes being milked only once or twice a day the fat content may be slightly lower than it would if the secretion had been removed more frequently.

Table IV. Milk composition.

Treatment	Sample Number	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Total Solids (%)
I	1	5.1848	8.50	2.3690	.7536	16.8074
IV	4-1	4.6707	11.00	2.1160	.6484	18.4351
	4-2	7.2800	8.68	3.4486	.7728	20.1814
	4-3	7.0260	8.72	2.8097	.8727	19.4284
	4-4	7.7108	7.60	3.6055	.8255	19.7418
	4-5	7.5640	8.00	3.2748	.8148	19.6536
V	5-1	0.1753	4.45	-1.3891	.6824	3.9186
VI	6-1	3.7276	11.82	0.7342	.7216	17.0034
	6-2	4.4106	16.50	1.7299	.7735	23.4140
VII	7-1	4.4241	8.40	3.3482	.7278	16.9001
	7-2	5.3703	8.25	3.4832	.6358	17.7393
	7-3	5.8738	8.95	3.0407	.6639	18.5284
	7-4	5.9592	7.50	3.9324	.7406	18.1322
VIII	8-1	5.2338	6.90	1.9731	.7059	14.8128
	8-2	6.4096	6.70	3.6513	.7589	17.5198
	8-3	6.4062	6.50	3.4865	.8155	17.2082
	8-4	6.8165	7.40	2.7352	.8312	17.7829
	8-5	7.0015	7.10	2.9940	.7799	17.8754
	8-6	7.2537	7.40	2.4941	.8294	17.9772
	8-7	7.6132	7.10	3.4528	.8762	19.0422
	8-8	7.0784	6.70	3.9612	.9572	18.6968
	8-9	8.0219	8.10	2.6499	.9506	19.7224
	8-10	7.0514	6.90	3.2878	1.0156	18.2548
IX	9-1	5.6394	8.60	3.0187	.8651	18.1232
	9-2	8.0994	7.20	3.9630	.8616	20.1240
	9-3	7.7753	6.40	4.6634	.9043	19.7430
	9-4	7.6596	6.55	4.3891	.9676	19.5663
	9-5	7.4139	6.70	4.2486	.9577	19.3202
Normal (4 Sheep)	Colostrum	10.3720	14.70	4.0288	.9640	30.0648
Normal (10 Sheep)	Advanced Lactation	9.3764	5.10	4.2906	.8748	19.6418
Morrison (1956)		6.9	6.5	4.9	.9	19.2
Brody (1945)		6.2	5.4	4.3	.9	17.1

Protein content appears to be somewhat variable in all treatments. When comparison is made with the normal samples it is evident that the protein content generally varied slightly lower than "normal colostrum" and somewhat higher than the "normal advanced lactation" sample. Treatment VI, sample two, exceeded the colostrum sample in protein content.

The lactose value is a relative difference factor and may or may not represent the true lactose content of the milk. The negative lactose value for Treatment V is difficult to explain other than this sample was sour when the protein content was determined. This value could also be an indication as to the precision of the analytical techniques employed. Where such small amounts in chemical constituents obviously exist the analytical procedure may not be sufficiently critical to facilitate exact separation. The figures obtained, as would be expected, are variable. No definite trends are evidenced but there does seem to be a tendency toward a gradual rise from the first to last sample. When compared to "normal colostrum" and "normal advanced lactation" samples the lactose content of the milk from the treated ewes was generally lower but did approach and exceed them in Treatment IX.

The total solids analysis value is also initially lower than "normal advanced lactation". The content would gradually rise and become comparable to the "normal advanced lactation" value. At no time did the content reach that found in the "normal colostrum" sample. The second sample from Ewe Number VI did exceed 23 percent which was

the highest total solids content obtained from the treated animals.

All chemical analyses conducted on both treated and normal animals fall well within the ranges reported for sheep milk by Turner (1952).

Ash content indicates a trend in all samples to be initially low but gradually increases and reaches or exceeds that of the 'hormal colostrum' and "normal advanced lactation" samples. Ash samples from normal colostrum, advanced lactation and Treatments I, IV, and IX were composited and analyzed for phosphorus, calcium, potassium, and iron content. Results from the samples obtained in this trial plus ash analysis of cow's milk (modification of Espe and Smith 1952) are presented on the elemental basis in Table V.

Table V. Ash analysis.

Sample	P (%)	Ca (%)	K (%)	Fe (ppm.)
Treatment I	18.8	13.6	1.8	334
Treatment IV	19.6	23.6	9.3	115
Treatment IX	19.6	12.3	2.4	154
Normal colostrum	18.8	19.8	4.3	230
Normal advanced lactation	18.5	23.8	5.0	220
Cow's milk (Espe and Smith)	10.6	14.3	20.77	175

Slaughter and body weight observations

Ewe Number I was slaughtered on April 8, 1958. The secretory tissue of the udder was 2.5 inches in diameter and 6 inches in length in both halves. A large cyst was present on one ovary. It is not known if this was present at the start of the trial or was a result of

the estrone treatment. The other ovary appeared normal but was highly vascular. No corpora lutea were present. The uterine body and horns were enlarged and turgid, appearing to be in the estral state. Adrenals were removed. The right one was small with a large medulla and thin cortex but the left one appeared normal. This ewe weight 175 pounds at the beginning of the experiment and 167 pounds at the end.

Ewe Number II was not slaughtered. Her initial body weight was 157 pounds; final body weight, 170 pounds.

Ewe Number III was not slaughtered. Initial body weight was 150 pounds; final, 167 pounds.

Ewe Number IV was slaughtered on April 28, 1958. Measurement of the mammary secretory tissue gave the following: left half, 3 inches in diameter and 5 1/4 inches in length; right half glandular tissue, 1 1/2 inches in diameter and 4 1/2 inches in length. The entire reproductive tract appeared normal. No corpora lutea were present. Both adrenals appeared normal. Initial body weight was 135 pounds; final, 135 pounds.

Ewe Number V was not slaughtered. Initial body weight was 176 pounds; final, 172 pounds.

Ewe Number VI was not slaughtered. Initial weight was 146 pounds; final, 141 pounds.

Ewe Number VII was not slaughtered. Initial weight was 157 pounds; final, 160 pounds.

Ewe Number VIII was not slaughtered. Initial weight was 150 pounds; final, 131 pounds.

Ewe Number IX was slaughtered on April 21, 1958. Secretory tissue measurements were as follows: right half, 3 1/2 inches in diameter and 5 1/2 inches in length; left half, 3 inches in diameter and 6 inches in length. The entire reproductive tract appeared normal; however, both ovaries were small. No corpora lutea were present. Adrenals were normal; one had a thick cortex. Initial body weight was 170 pounds; final, 160 pounds.

Ewe Number X was slaughtered on April 28, 1958. The secretory tissue of this udder was embedded in a large amount of fatty tissue and it was difficult to obtain an accurate measurement. The gland was approximately 2 3/4 inches in diameter and 4 inches in length in both halves. The reproductive tract, as well as the adrenals appeared normal in all respects. Initial body weight was 184 pounds; final, 182 pounds.

Ewe Number XI was slaughtered on April 28, 1958. The udder was very fatty. No definite glandular area could be determined, therefore no measurements were obtained. Teats were thick and firm but hollow. The reproductive tract and the adrenals were normal. Initial body weight was 179 pounds; final, 177 pounds.

Histological studies

Histological examination was conducted on the udders of Ewes Number I, IV, IX, X, and XI.

Observation of slides from Treatment I revealed secretion present in the gland cistern. This secretion was highly vacuolated, probably the result of dissolution of fatty material during slide processing.

In addition there were numerous nucleated, vacuolated "colostrum corpuscles". Directly beneath the epithelium of the gland cistern were ducts which contained secretion similar to that previously described. The alveoli in the gland cistern region were few and scattered, lying in definite areas or "pockets". Approximately 90 percent of the tissue area was connective tissue, 7 percent alveoli, and 3 percent fatty tissue.

Sections from well within the secretory portion of the gland revealed a much smaller connective tissue area. That present was in bands running through the tissue area. Fatty tissue was present but in very small amounts. Alveoli occupied approximately 85 to 90 percent of the tissue area. "Colostrum corpuscles" were present in the secretion with lymphocytes, monocytes, and neutrophiles visible. Many vacuoles were present in the secretion. Lumen cells were columnar in the alveoli that were not secreting and cuboidal where secretion was present. At the base of the epithelium myo-epithelial cells were evident. No mitotic activity was noticed.

The udder from the ewe on Treatment IV was sectioned and slides prepared from the alveolar region of both the right and left halves. Examination of the alveoli from the left half revealed alveoli in approximately 85 to 90 percent of the total area. Most alveoli were comprised of cuboidal cells. Some (but not all) of the lumina contained a normal appearing secretion. The epithelium lining these was of a more squameous nature. The alveoli in some areas had a wall comprised of two or three layers of cuboidal epithelium. Whether or not

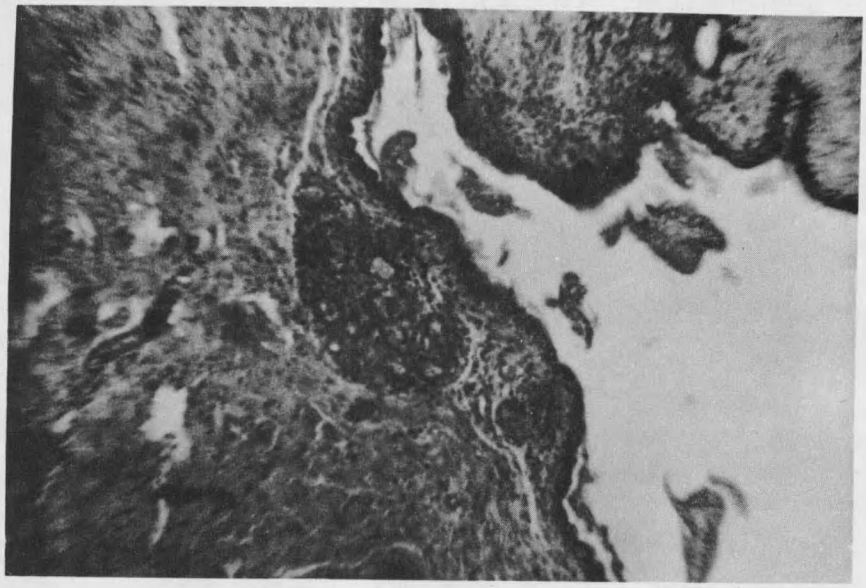

Figure 1. Treatment I: Duct Region.
(H. & E., 10μ, 100X)

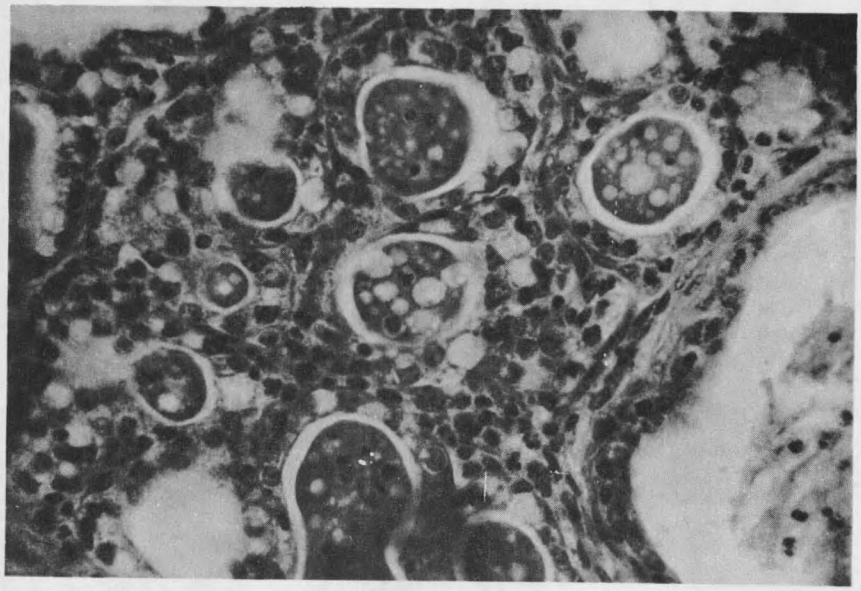

Figure 2. Treatment I: Alveoli containing "colostrum corpuscles".
(H. & E., 10μ, 440X)

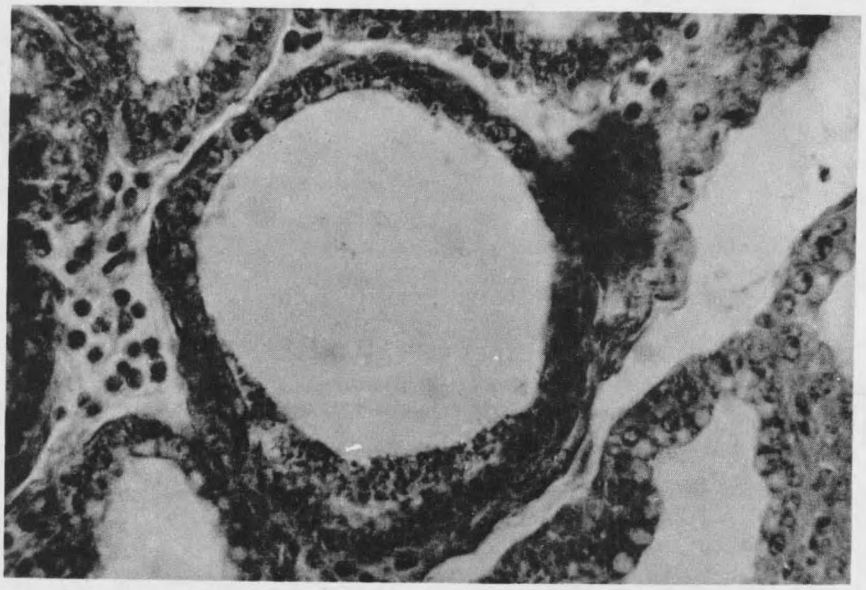

Figure 3. Treatment IV: Alveoli in left half. (H. & E., 10μ, 440X)

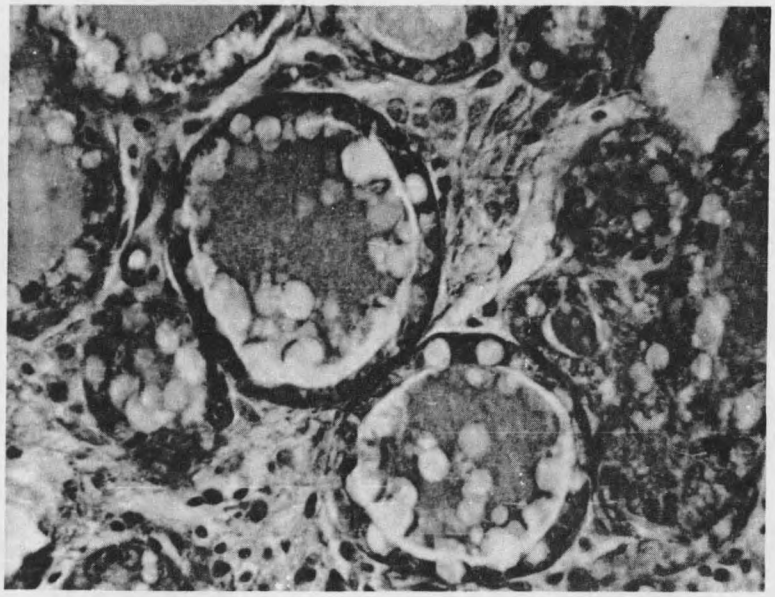

Figure 4. Treatment IV: Right half alveoli containing "colostrum corpuscles". (H. & E., 10μ, 440X)

this was developing or regressing tissue is not known. The secretion in the lumina of these alveoli appeared more fiborus than that found in the lumina of alveoli having one layer of epithelium. The epithelium lining the ducts formed many finger-like projections giving a villi-like appearance. Myo-epithelial cells were visible at the base of the alveolar epithelium. Connective tissue was present in bands that appeared to surround alveolar lobules. No fatty tissue was found and no mitotic activity could be noticed.

The alveolar cells in the right half occupied approximately 70 to 80 percent of the tissue area. The epithelial cells were low cuboidal with some approaching a squameous appearance. The cells were definitely stimulated to the secretory condition but for some reason the secretion had remained in the lumina of the alveoli. The secretion was highly vacuolated but not nucleated. These are, however, designated "colostrum corpuscles". Many vacuoles were present within the alveolus cells. Myo-epithelial cells were visible at the base of the epithelium. Connective tissue area was larger than that found in the left half. No fatty tissue was found and no mitotic activity was noticed.

Microscopic examination of slides prepared from the udder of Ewe Number IX revealed alveoli present in approximately 95 to 97 percent of the tissue area. The alveoli were very large with most of the lumen cells being of the low cuboidal to thick squameous type. The epithelial lining of the ducts contained many finger-like projections giving a villi-like appearance. The secretion state of the epithelial cells of some alveoli was interesting. The cells ranged from those

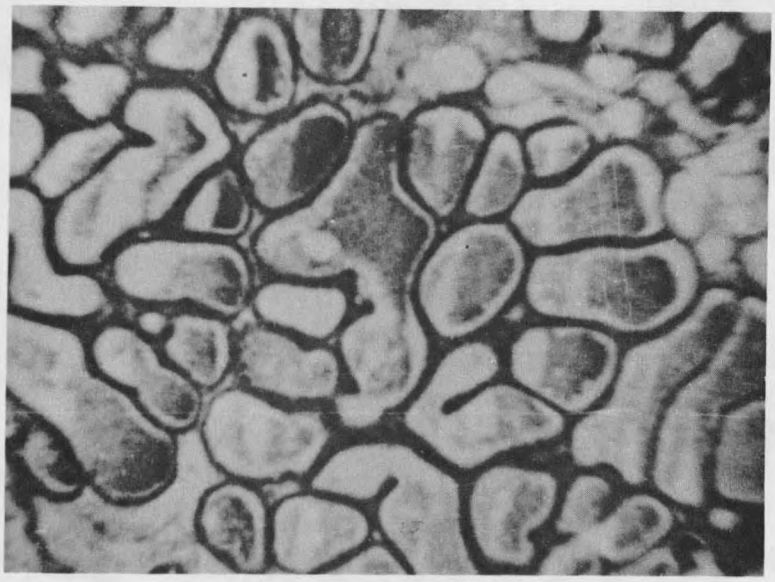

Figure 5. Treatment IX: Secretory Area.
(H. & E., 10μ, 100X)

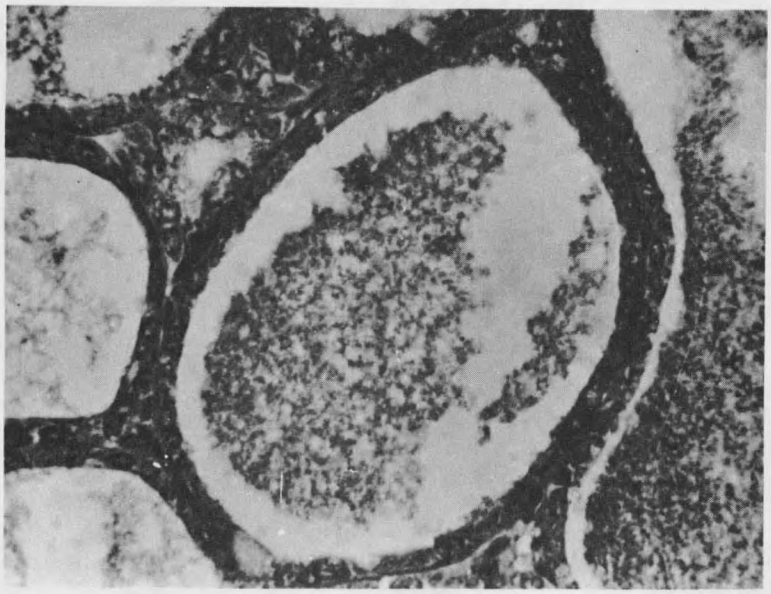

Figure 6. Treatment IX: Alveolus.
(H. & E., 10μ, 440X)

just ready to rupture into the lumen, being vacuolated at the apical end , to those of a low cuboidal type having previously ruptured. Myo-epithelial cells were plainly visible. Connective tissue was present in very thin bands traversing the slide area. The connective tissue did not appear as dense as that found in the udders of the other treated ewes. No fatty tissue or mitotic activity was found.

Histological examination of the udder of Ewe Number X revealed actual secretory tissue area occupying approximately 50 percent of the tissue area. Alveoli were comprised of a single layer of tall cuboidal epithelium. Ducts were lined with a single layer of epithelium of the same type. Alveoli showed some homogenous, vacuolated secretion within the lumina. This was again found in the ducts. Connective tissue was present in large clumps and bands throughout the slide. Large vacuolated areas indicating fatty tissue were present. Myo-epithelial cells were found at base of alveolus epithelium. No mitotic activity was found.

Microscopic examinations of the slides prepared from the udder of Ewe Number XI showed alveoli in not over 20 to 25 percent of the tissue area. Alveoli appeared to have two layers of cuboidal epithelium comprising the wall. Small amounts of homogeneous secretion products were found in the lumina of some alveoli and a few of the ducts. The secretion was not vacuolated. Very large connective tissue areas were present as well as some fatty tissue. This udder was not as fatty as that of Ewe X. Myo-epithelial cells were present at the basal end of the alveolar epithelium. No mitotic activity was found.

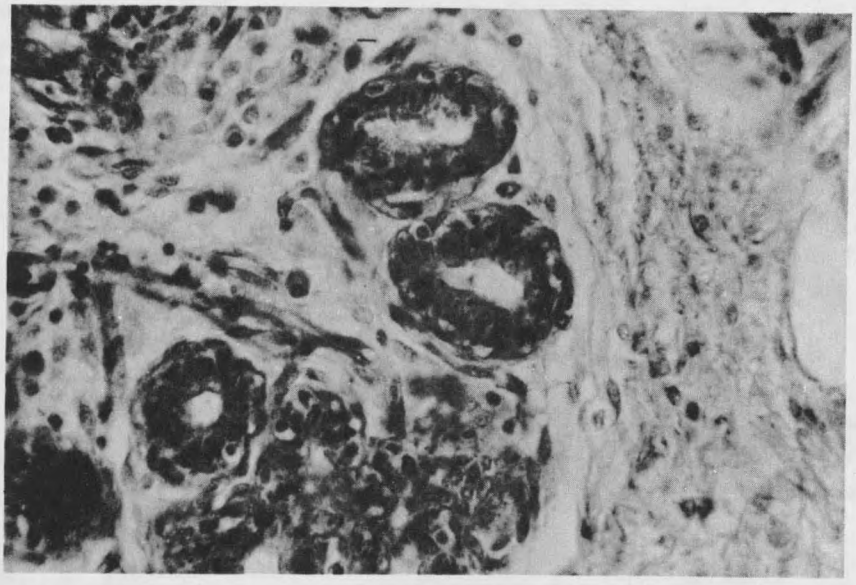

Figure 7. Treatment X: Alveoli.
(H. & E., 10μ, 440X)

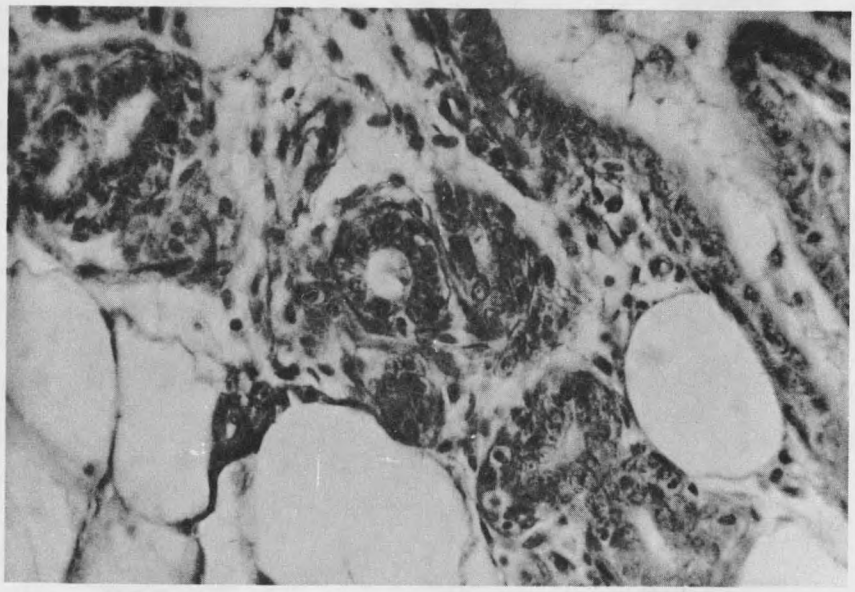

Figure 8. Treatment XI: Alveoli.
(H. & E., 10μ, 440X)

Alveolus and lumen measurements plus the percent increase over control averages for both are shown in Table VI.

Table VI. Alveolus and lumen measurements.

Treatment	Alveolus diameter (mm.)	Lumen diameter (mm.)	Percent increase over control average	
			Alveolus	Lumen
Control ewes:				
X	.0382	.0098		
XI	.0395	.0170		
	avg. .0388	avg. .0134		
Treated ewes:				
I	.0482	.0291	24.23	117.16
IV Right half	.0824	.0689	112.37	414.18
Left half	.0994	.0807	156.19	502.24
IX	.1344	.1110	246.39	728.36

Some excellent histological work has been conducted on the mammary gland of the cow and is reviewed and quoted by Turner (1952).

> "Kwong (1940) traced the development of the gland on a series of 18 animals pregnant from one to nine months. He concluded that the first three months of pregnancy comprised the period of duct proliferation. During the fourth to the seventh month of pregnancy the secretory or alveolar tissue forms. Secretion is observed in the later part of this period. In the last two months of pregnancy alveoli are much distended by secretion.
>
> "Hammond (1927) found that in the second month of pregnancy, the main ducts are sprouted out to form the minor or alveolar ducts, but the lobules themselves are not properly formed, and their outline was shadowy. The alveolar ducts are lined by a double layer of epithelial cells. By the fourth month the outline of the lobules has become more definite, and the connective tissue in this region increases in denseness by the multiplication of cells of the plasma and lamellar type. In most of the alveolar ducts which are now increasing in length rather than thickness, the

epithelium is of two layers, but here and there only one layer is seen, probably the beginning of increase in thickness. In the lumen a little secretion can be seen.

"At the fifth month the lobules have become definitely formed, although they are still small; they now stand out from the thick connective tissue bands in which they were at first produced. The granular connective tissue of the lobule has become more dense, and contains numerous blood capillaries. The growth of the nutritive plasma cells of the connective tissue and blood vessels is necessary for the proper nutrition of the developing gland. At this stage the gland has formed true alveoli, which are seen to have a single celled epithelium of rather columnar shape, while some of the cells appear clear and globular. A moderate amount of secretion is present in the lumen.

"At the sixth month the lobules have increased in size greatly, so that they are densely packed together, and are now separated more by thick bands of connective tissue than by fat cells. The proportion of alveoli to dense connective tissue in the lobule has increased by swelling of the alveoli which have now become distended with a homogeneous secretion and their single layer of epithelial cells is flattened. At this stage the honey-like secretion was being produced in large quantities.

"At the seventh month only thin strands of connective tissue separated the lobules, and in these the proportion of alveolar tissue was still further increased by swelling of the alveoli with the secretory products; the secretion was not homogeneous in all places as before, but was flaky and granular in many of the alveoli. The epithelial cells were one layer thick, and were very flattened by the distention of the alveolus with fluids; in some parts a few fairly large clear cells could be seen.

"The appearance of the gland at the eighth month in the heifer from which no fluid had been drawn off from the udder previously, was very much like that at the sixth month, but there were only thin strands of connective tissue between the lobules, and the alveoli themselves were slightly larger.

"From the preceding description it will be seen that up to the fourth or fifth months, growth of the connective tissue, vascular basis and alveolar duct elements of the lobules is taking place. About the fifth month, the character of the alveoli which have completed their growth in

> length now begin to develop in thickness, or rather, diameter; the cavity produced is filled with the honey-like secretion. Removal of this secretion apparently initiates true glandular activity and the formation of milk; when the honey-like secretion is not removed the secretory activity of the gland does not commence, but only further growth of the alveoli occurs."

In literature reviewed, no reference was found to studies of this type having been conducted on the sheep mammary gland. Therefore no statements as to the comparison of a naturally developed udder and the induced development will be made but it is evident from the histological examinations made, that sheep mammary tissue follows the same general pattern.

SUMMARY

Part I of this experiment was an exploratory trial started on April 4, 1957 with some animals being treated and observed until May 23, 1957. The experiment involved 15 non-pregnant females that had lambed and lactated normally in previous years and one castrate male sheep. Age range for the ewes was from three to six years. The wether was a yearling.

Treatments consisted of various levels of estrone, in aqueous suspension, diethylstilbestrol in aqueous suspension and an oil carrier, with and without progesterone, in an oil carrier. Prolactin was given to three ewes with one or both of the steroids. Prolactin was also given to one ewe in an attempt to stimulate milk production. One ewe was treated with oxytocin to attempt to relieve an edemic condition of the udder. The results on these two treatments were negative.

Part II was an experiment in inducing lactation started on April 3, 1958 and was continued until April 29, 1958. This trial involved 11 head of dry, six to seven year old ewes that had lambed and lactated normally in previous years. Treatment I was a graded dose of estrone; Treatment II a 1:40 estradiol to progesterone ratio; Treatments III, IV, and V were 1:40, 1:80, and 1:200 diethylstilbestrol to progesterone ratios respectively. Treatments VI, VII, VIII, and IX were 0.25 mg., 0.50 mg., 1.00 mg., and 5.00 mg. of diethylstilbestrol respectively. Treatment X was 3 c.c. of commercial peanut oil injected every other day. Treatment XI was manipulation of the udder with no injections.

All treatments were given as intramuscular injections, every other day. Peanut oil was used as the carrier.

Measurable secretion (more than 0.30 gm.) was obtained from Treatments I, IV, V, VI, VII, VIII, and IX. A small amount of honey-like secretion was obtained from Treatment XI. This, as well as the secretion obtained from Ewe Number V, was present at the beginning of the trial and was not a result of treatment. The ewe on Treatment VIII developed the largest udder and secreted the largest daily amount of milk (610.52 gm.). Treatments I, IV, VI, VII, and IX's peak daily production was 29.90 gm., 306.81 gm., 5.50 gm., 142.43 gm., and 406.69 gm. respectively. The daily production data are shown in Table III.

Secretion was also collected from four normal ewes that had just lambed for a colostrum sample and from ten normal ewes that had lambed four to six weeks previous for an advanced lactation sample.

Chemical analyses for fat, protein, lactose, ash, and total solids conducted on all samples showed the secretion from the treated ewes to be lower than normal colostrum in all components. As lactation proceeded, the secretion rapidly assumed the composition of normal milk in both composition and consistency. Analytical results are shown in Table IV.

Mineral content of the ash from Treatments I, IV, IX, normal colostrum, and normal advanced lactation was determined. Phosphorus content ranged from 18.5 to 19.6 precent; calcium ranged from 12.3 to 23.8 percent; potassium from 1.8 to 9.3 percent; iron from 115 to 334 parts per million. Ash analyses data are shown in Table V.

Routine histological examintations were conducted on Ewes Number I, IV, IX, X, and XI. Ocular micrometer measurements of alveoli and lumina showed the percent increase over the control average alveoli diameter to be 24.23 percent, 112.37 percent, 156.19 percent, and 246.39 percent for Treatments I, IV (right and left half), and IX respectively. Percent increase over the control average lumina diameters were 117.16 percent, 414.18 percent, 502.24 percent, and 728.38 percent for Treatments I, IV (right and left half), and IX respectively. Histological measurement data are shown in Table VI.

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APPENDIX

Appendix Table I. Treatment Observations for 1957.

Treatment	Date	Hormone	Hormone Amount	Observations
I (Rambouillet)	4/4/57	Estrone	12.5 mg.	No initial udder development.
	4/5/57			No change.
	4/6/57			No change.
	4/8/57			Slight udder development; rather solid glandular tissue. Right half slightly larger than left.
	4/10/57			Udder larger. Milk-like secretion from right half. Left half enlarging.
	4/11/57	Estrone	12.5 mg.	Udder enlarging. No secretion.
	4/15/57			Udder same. No secretion. Marked by ram.
	4/19/57			Udder shows more development. Glandular tissue in both halves.
	4/23/57			Udder slightly larger. Colostrum-like secretion from both halves. More from right.
	4/24/57			Udder size same. Milk-like secretion from right half. Colostrum-like from left half.
	4/25/57	Estrone	12.5 mg.	Milky secretion still in both halves but smaller amount. Secretion from left of a colostrum consistency.
	4/26/57			Udder more flabby. Secretion thicker in both halves. Fluid from left half yellowish.

Treatment	Date	Hormone	Hormone Amount	Observations
I contd.				More firm tissue present. Fluid from right half more clear. Yellowish fluid from left half.
	4/28/57			No change.
	5/2/57			Udder quite large. Fairly large amount of milk-like secretion from right half. Yellowish fluid from left half.
	5/6/57			No change. Secretion amount smaller.
	5/12/57			Udder smaller and more flabby. Secretion amount small.
II (Rambouillet)	4/4/57	Estrone	25.0 mg.	No initial uddder development.
	4/5/57	None		No change.
	4/6/57	None		Very little udder development. Milk-like secretion from both halves.
	4/8/57	None		Udder slightly larger. Milk-like secretion from both halves. Larger amount from left.
	4/10/57	None		Udder same. Milk-like secretion present in both halves. Larger amount from right.
	4/11/57	Estrone	25.0 mg.	Udder slightly larger. Glandular tissue in right not as firm. Milk-like secretion present in both halves; smaller amount.

Treatment	Date	Hormone	Hormone Amount	Observations
II contd.	4/15/57	None		Udder soft and flabby. No secretion.
	4/19/57	None		Udder more firm. No secretion.
III (Wether)	4/6/57	Estrone	12.5 mg.	No udder. Teats short.
(Columbia)	4/8/57	None		No change.
	4/10/57	Estrone	12.5 mg.	No change.
	4/11/57	None		No change.
	4/15/57	None		No change.
	4/19/57	None		No change. Teats still short.
IV	4/4/57	Diethyl-stilbestrol (oil)	50.0 mg.	No initial udder development.
	4/5/57	None		No change.
	4/6/57	None		Questionable - may be slight udder development.
	4/8/57	None		Very slight udder development, flabby tissue.
	4/10/57	None		No change.
	4/11/57	Diethyl-stilbestrol (oil)	50.0 mg.	No change.
	4/15/57	None		No change.
	4/19/57	None		Udder smaller; soft and flabby.

Treatment	Date	Hormone	Hormone Amount	Observations
V (Rambouillet)	4/8/57	Diethyl-stilbestrol (aqueous)	50.0 mg.	Slight initial udder development. Approximately 10 c.c. milky secretion from each half. Dry on injection.
	4/10/57	None		Udder same. Milky secretion again present.
	4/11/57	None		Udder same. Milky secretion present but very small amount.
	4/15/57	None		Udder and secretion same as 4/11.
	4/19/57	None		Udder soft and flabby. Small amount milky secretion from both halves. Most from right.
VI (Rambouillet)	4/4/57	Diethyl-stilbestrol	25.0 mg.	No initial udder development.
	4/5/57	None		No change.
	4/6/57	None		No change.
	4/8/57	None		Slight udder development. Glandular tissue beginning to develop.
	4/10/57	None		No change.
	4/11/57	Diethyl-stilbestrol	25.0 mg.	Udder slightly larger. Firm tissue beginning to soften.
	4/15/57	None		Udder becoming smaller.
	4/19/57	None		Udder soft and flabby. No secretion.
VII (Rambouillet)	4/8/57	Diethyl-stilbestrol	25.0 mg.	Some initial udder development.

Treatment	Date	Hormone	Hormone Amount	Observations
VII contd.	4/10/57	None		No change.
	4/11/57	None		Udder about same. Left half slightly larger and small amount of milky fluid present.
	4/15/57	None		No change. Very small amount of milky fluid from left half.
	4/19/57	None		Udder same. Very small amount cloudy fluid from both halves.
VIII (Rambouillet)	4/4/57	Diethyl-stilbestrol plus implant	25.0 mg. 15.0 mg.	Slight initial udder development.
	4/5/57	None		No change.
	4/6/57	None		Udder slightly larger. Clear, water-like secretion from left half.
	4/8/57	None		Udder increasing in size. Larger amount of secretion from left half; water-like.
	4/10/57	None		Udder same. Watery secretion from left half; milky secretion from right.
	4/11/57	None		Udder same. Secretion same as 4/10. Fiborus plug removed from right teat.
	4/15/57	None		Udder same. Water-like fluid present in both halves.
	4/19/57	None		Udder soft and flabby. Watery secretion present in left half. None in right.

Treatment	Date	Hormone	Hormone Amount	Observations
IX (Rambouillet)	5/9/57	Diethyl-stilbestrol	5.0 mg.	No initial udder development.
	5/10/57	Diethyl-stilbestrol	5.0 mg.	Udder about same. One drop of fluid from right half.
	5/11/57	Diethyl-stilbestrol	5.0 mg.	No change.
	5/12/57	Diethyl-stilbestrol	5.0 mg.	No change.
	5/23/57	None		Very little udder development. Medium amount milk-like secretion from both halves.
X (Rambouillet)	4/23/57	Estrone	5.0 mg.	No initial udder development.
	4/24/57	Estrone	5.0 mg.	No change.
	4/25/57	Estrone	5.0 mg.	Firm glandular tissue beginning to appear.
	4/26/57	Estrone	5.0 mg.	More glandular tissue present; milk-like secretion from both halves - more from right half.
	4/27/57	Estrone	5.0 mg.	More udder development. Fairly large amount of milk-like secretion from both halves.
	4/28/57	Progesterone	5.0 mg.	Udder becoming more flabby. Secretion amount about same.
	4/29/57	Progesterone	5.0 mg.	Udder and secretion same as 4/28.
	4/30/57	Progesterone	5.0 mg.	Udder may be more firm. Secretion same as 4/28.
	5/1/57	Progesterone	5.0 mg.	Udder flabby. Secretion becoming clear.

Treatment	Date	Hormone	Hormone Amount	Observations
X contd.	5/2/57	Progesterone	5.0 mg.	Udder flabby. Secretion becoming clear.
	5/3/57	Prolactin	200.0 I.U.	Udder same as 5/2. Secretion present in fair amount but rather clear.
	5/4/57	Prolactin	200.0 I.U.	Milk-like secretion present in left half in rather large amount. Some in right half.
	5/5/57	None		Can notice no change in udder size. Secretion amount smaller.
	5/23/57	None		Udder very small. Secretion amount small and quite clear.
XI (Rambouillet)	4/23/57	Estrone	5.0 mg.	No initial udder development.
	4/24/57	Estrone	5.0 mg.	No change.
	4/25/57	Estrone	5.0 mg.	Slight udder development. Small amount of milk-like secretion from both halves.
	4/26/57	Estrone	5.0 mg.	Udder flabby but fair development. Fairly large amount of milk-like secretion from both halves.
	4/27/57	Estrone	5.0 mg.	Udder more flabby; less firm tissue. Secretion similar to 4/26 in amount and appearance.
	4/28/57	Estrone	5.0 mg.	Udder about same. Secretion decreasing but present in both halves and left rudimentary teat.
	4/29/57	Estrone	5.0 mg.	Udder more flabby. Small amount of milk-like secretion present.

Treatment	Date	Hormone	Hormone Amount	Observations
XI contd.	4/30/57	Estrone	5.0 mg.	Udder same as 4/29. Secretion in both halves becoming colostrum-like.
	5/1/57	Estrone	5.0 mg.	No change.
	5/2/57	Estrone	5.0 mg.	Udder same. Small amount of secretion; thick and yellowish from right half, milk-like from left.
	5/3/57	Progesterone	5.0 mg.	Udder same. Thick fluid from left half only.
	5/4/57	Progesterone	5.0 mg.	Udder and secretion same as 5/3.
	5/5/57	Progesterone	5.0 mg.	No change.
	5/6/57	Progesterone	5.0 mg.	Secretion yellowish.
	5/7/57	Progesterone	5.0 mg.	No change.
	5/9/57	Progesterone	10.0 mg.	No change.
	5/10/57	Progesterone	10.0 mg.	No change.
	5/11/57	Progesterone	10.0 mg.	Udder may be slightly larger.
	5/12/57	Progesterone	10.0 mg.	No change. Fluid still yellow.
	5/23/57	None		Udder small and flabby. Small amount of clear, watery fluid from both halves.
XII (Rambouillet)	4/23/57	Estrone	5.0 mg.	No initial udder development. Small amount of brownish fluid from left half.
	4/24/57	Estrone	5.0 mg.	Udder same. Brownish fluid from both halves.

Treatment	Date	Hormone	Hormone Amount	Observations
XII contd.	4/25/57	Estrone	5.0 mg.	Udder same. Brownish fluid still present.
	4/26/57	Estrone	5.0 mg.	Glandular tissue beginning to appear in both halves. Brownish fluid still present.
	4/27/57	Estrone	5.0 mg.	Glandular tissue about same. Brownish fluid still present but becoming more clear.
	4/28/57	Estrone	5.0 mg.	Udder soft and flabby; seems to be decreasing in size. Brownish fluid not present.
	4/29/57	Estrone	5.0 mg.	Udder flabby. Few drops brownish fluid from left half.
	4/30/57	Estrone	5.0 mg.	Udder smaller. No fluid present.
	5/1/57	Estrone	5.0 mg.	Udder smaller. No fluid present.
	5/2/57	Estrone	5.0 mg.	Udder same as 5/1.
	5/3/57	Progesterone	5.0 mg.	No change.
	5/4/57	Progesterone	5.0 mg.	No change.
	5/5/57	Progesterone	5.0 mg.	No change.
	5/6/57	Progesterone	5.0 mg.	No change.
	5/7/57	Progesterone	5.0 mg.	No change.
	5/8/57	Progesterone	5.0 mg.	No change.
	5/9/57	Progesterone	10.0 mg.	No change. Udder very small.
	5/10/57	Progesterone	10.0 mg.	No change.

Treatment	Date	Hormone	Hormone Amount	Observations
XII contd.	5/11/57	Progesterone	10.0 mg.	No change.
	5/12/57	Progesterone	10.0 mg.	No change.
	5/23/57	None		
XIII (Rambouillet)	4/23/57	Estrone	1.0 mg.	Small amount of flabby tissue present.
	4/24/57	Estrone	2.0 mg.	No change.
	4/25/57	Estrone	3.0 mg.	Slight udder development. Left half larger. Small amount of milk-like secretion from left half.
	4/26/57	Estrone	4.0 mg.	Right half increasing in size. Left no change. Milk-like secretion from both halves; largest amount from left.
	4/27/57	Estrone	5.0 mg.	Udder flabby. Milk-like secretion present in left half. Secretion from right yellowish and cloudy.
	4/28/57	Progesterone	5.0 mg.	Udder flabby. No secretion.
	4/29/57	Progesterone	5.0 mg.	Udder flabby. No secretion.
	4/30/57	Progesterone	5.0 mg.	No change.
	5/1/57	Progesterone	5.0 mg.	No change.
	5/2/57	Progesterone	5.0 mg.	Udder may be more firm.
	5/3/57	Prolactin	200 I.U.	No change.
	5/4/57	Prolactin	200 I.U.	No change. Udder flabby.
	5/5/57	None		No change.
	5/6/57	None		No change.

Treatment	Date	Hormone	Hormone Amount	Observations
XIII contd.	5/12/57	None		No change.
	5/23/57	None		Udder flabby. Clear, watery fluid from both halves.
XIV (Rambouillet)	4/23/57	Estrone	1.0 mg.	Some initial udder development.
	4/24/57	Estrone	2.0 mg.	No change.
	4/25/57	Estrone	3.0 mg.	Glandular tissue beginning to appear in both halves.
	4/26/57	Estrone	4.0 mg.	Glandular tissue increasing. No secretion.
	4/27/57	Estrone	5.0 mg.	Glandular tissue larger. Milk-like secretion from both halves.
	4/28/57	Progesterone Estrone	5.0 mg. 3.0 mg.	Udder larger and large amount of milk-like secretion from both halves.
	4/29/57	Progesterone Estrone	5.0 mg. 3.0 mg.	Udder quite large. Large amount of milk-like secretion from both halves.
	4/30/57	Progesterone Estrone	5.0 mg. 3.0 mg.	Udder smaller and more flabby. Fairly large amount of milk-like secretion.
	5/1/57	Progesterone Estrone	5.0 mg. 3.0 mg.	Udder same. Secretion same, still large amount.
	5/2/57	Progesterone Estrone	5.0 mg. 3.0 mg.	Udder same. Secretion amount smaller but still milk-like.
	5/3/57	Prolactin	200 I.U.	Udder same. Milk-like secretion still present.
	5/4/57	Prolactin	200 I.U.	Udder very flabby. No secretion.

Treatment	Date	Hormone	Hormone Amount	Observations
XIV contd.	5/5/57	None		No change.
	5/6/57	None		No change.
	5/12/57	None		No change.
	5/23/57	None		No change.
XV (Targhee)	4/26/57	Prolactin	200 I.U.	Ewe's udder well developed, but amount of milk produced was small. One twin average size; second very thin from lack of milk. No lack of "mothering".
	4/27/57	Prolactin	200 I.U.	
	4/29/57	Prolactin	200 I.U.	
	5/4/57	None		Could tell no difference in milk amount but smaller lamb looked better.
XVI (Targhee)	5/9/57	Oxytocin	0.75 c.c.	Udder hard and small amount of milk.
	5/10/57	Estrone	2.0 mg.	No change.
	5/12/57	None		No change.
	5/23/57	None		Udder still quite hard. Lamb looking better.

Appendix Table II. Treatment observations for 1958.

Treatment	Date	Hormone	Hormone Amount	Observations
I (Columbia)	4/3/58	Estrone	1.000 mg.	Slight initial udder development. Flabby. Glandular tissue larger in right half; some in left. 0.77 gm. thick, yellowish-white fluid obtained from both halves. Initial weight 175 pounds.
	4/5/58	Estrone	2.000 mg.	Glandular tissue larger in both halves. 2.84 gm. secretion obtained; becoming more white.
	4/6/58	Estrone	3.000 mg.	Udder larger. Glandular tissue 2 inches in diameter right half, 1 1/2 inches in left half. 29.90 gm. milk-like secretion.
	4/7/58	Estrone	4.000 mg.	Udder same. 29.00 gm. milk-like secretion; larger amount from left half.
	4/8/58	None		Udder same. 11.56 gm. milk-like secretion obtained; larger amount from left half.
	Slaughtered			On slaughtering this animal, the mammary secretory tissue was found to be 2 1/2 inches in diameter and 6 inches in length in both halves. Ovaries - one was cystic; contained large amount of fluid. Other ovary was of normal size but highly vascular. Uterus and horns turgid, appearing to be in the estral state. Adrenals - right, small; large medulla, thin cortex. Left - appeared normal. Final weight 167 pounds.

Treatment	Date	Hormone	Hormone Amount	Observations
II 1:40 estradiol: progesterone ratio. (Rambouillet)	4/3/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No initial udder development. Very flabby. No secretion. Initial weight 157 pounds.
	4/5/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/7/58	Estradiol Progesterone	.583 mg. 23.333 mg.	Udder still flabby. Small amount firm tissue appearing in both halves. No secretion.
	4/9/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/11/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/13/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/15/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/17/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/19/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/21/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/23/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/25/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change.
	4/27/58	Estradiol Progesterone	.583 mg. 23.333 mg.	No change. Final weight 170 pounds.
Additional treatment				
	5/1/58	Diethyl-		No initial udder develop-

Treatment	Date	Hormone	Hormone Amount	Observations
II contd.		stilbestrol	5.000 mg.	ment.
	5/3/58	Diethyl-stilbestrol	5.000 mg.	Udder same. Few drops cloudy secretion. Not collected.
	5/5/58	None		Udder slightly larger. Milk-like secretion from both halves. Not collected.
	5/9/58	None		Udder larger. Milk-like secretion obtained from both halves. Not collected.
III 1:40 diethyl-stilbestrol: progesterone ratio. (Rambouillet)	4/3/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	No initial udder development. Glandular tissue soft. Few drops clear, golden secretion. Initial weight 150 pounds.
	4/5/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Udder no change. Secretion same.
	4/7/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Udder small and flabby. Firm tissue may be slightly larger; approximately 1 inch in diameter in right half. Few drops secretion present. Becoming slightly cloudy. String of white material obtained from right teat.
	4/8/58	None		No change. Secretion slightly cloudy. No ropy material from right teat.
	4/9/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Firm tissue slightly larger. Drop clear secretion from both halves.
	4/10/58	None		Firm tissue may be lengthening. Secretion again

Treatment	Date	Hormone	Hormone Amount	Observations
III contd.				cloudy. Ropy material again obtained from right teat.
	4/11/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	No change. No secretion.
	4/12/58	None		Udder very flabby; firm tissue same. One drop secretion from right half.
	4/13/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Udder same. No secretion.
	4/14/58	None		No change.
	4/15/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Firm tissue seems some larger in right half. Slightly larger in left. No secretion.
	4/16/58	None		Firm tissue more pliable. No secretion. Ewe shows evidence of estrus.
	4/17/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	No change. No secretion.
	4/19/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	No change.
	4/21/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	No change.
	4/23/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	No change.
	4/25/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Udder still flabby. Few drops yellowish-clear secretion from both halves.
	4/27/58	Stilbestrol Progesterone	.583 mg. 23.333 mg.	Udder very flabby. Glandular tissue quite mushy. Few drops secretion from right half. Final weight 167 pounds.

Treatment	Date	Hormone	Hormone Amount	Observations
III Additional treatment contd.				
	5/1/58	Diethyl-stilbestrol	5.000 mg.	Udder same as 4/27. No secretion.
	5/3/58	Diethyl-stilbestrol	5.000 mg.	Udder same. Few drops clear (slightly cloudy) secretion.
	5/5/58	None		Udder larger. Milk-like secretion from both halves. Not collected.
	5/9 58	None		Udder larger. Milk-like secretion obtained from both halves. Not collected.
IV 1:80 diethyl-stilbestrol to progesterone ratio.	4/3/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder small; mostly firm. 3.14 gm. cloudy secretion from left half. Initial weight 135 pounds.
(Columbia)	4/5/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder size same. Firm tissue largest in right half. 0.36 gm. milky secretion from left half.
	4/7/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder larger. Firm tissue larger - approximately 2 inches in diameter in right half; 1 inch in left half. 0.32 gm. milky fluid from left half.
	4/8/58	None		Udder same. 1.10 gm. milky secretion from left half; still none from right.
	4/9/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder same. 1.27 gm. milky secretion.
	4/10/58	None		Udder same. 0.82 gm. milky secretion.
	4/11/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder slightly larger. Firm tissue approximately

Treatment	Date	Hormone	Hormone Amount	Observations
IV contd.				1 1/2 inches in diameter in left half; right same. 0.93 gm. milky secretion from left half.
	4/12/58	None		Firm tissue 2 1/4 inches in diameter in right half; 1 3/4 inches in left half. 1.04 gm. milky secretion from left half.
	4/13/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder same. 1.32 gm. cloudy secretion; still none from left.
	4/14/58	None		Udder slightly larger. Firm tissue enlarging. 3.66 gm. milky secretion from left half.
	4/15/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Glandular tissue developing some. 8.44 gm. milky secretion, more from right half.
	4/16/58	None		Large amount firm tissue in both halves. 15.42 gm. milky secretion from right half.
	4/17/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Firm tissue quite large in both halves. Not quite as hard in left half. 25.82 gm. milky secretion from left half; few drops from right.
	4/18/58	None		Glandular tissue definitely larger in both halves. 44.96 gm. milk-like secretion from left half. Few drops from right.
	4/19/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Udder larger in both halves, very firm. 52.03 gm. milk-like secretion - all from left.

Treatment	Date	Hormone	Hormone Amount	Observations
IV contd.	4/20/58	None		Secretory tissue quite large in left half. 94.45 gm. milk-like secretion from left half. Few drops from right.
	4/21/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Secretory tissue slightly larger in left half. 81.16 gm. milk-like secretion from left.
	4/22/58	None		Left half large and 114.90 gm. milk-like secretion obtained. Right half smaller.
	4/23/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	No change in left half; right still smaller. 124.23 gm. milk-like secretion from left half.
	4/24/58	None		Left half slightly larger. 126.80 gm. milk-like secretion from left; none from right.
	4/25/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Left half larger; 210.90 gm. secretion. Right half enlarging some; few drops milky secretion.
	4/26/58	None		Left half same. Right half - glandular tissue feels lumpy. Milked twice (a.m. and p.m.) 306.81 gm. milk-like secretion total.
	4/27/58	Stilbestrol Progesterone	.292 mg. 23.333 mg.	Left half same. Right slightly larger; still only few drops milk-like secretion from left.
	4/28/58	None Slaughtered		Udder same. 249.09 gm. milk-like secretion from left half. Few drops from right. Final weight 135 pounds.

Treatment	Date	Hormone	Hormone Amount	Observations
IV contd.				Slaughter observations: Entire reproductive tract normal. Adrenals appeared normal. Secretory tissue dimensions - left half: 3" x 5 1/4"; right: 1 1/2" x 4 1/2".
V 1:200 diethyl-stilbestrol to progesterone ratio. (Rambouillet)	4/3/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder shows some initial development. Flabby. Glandular tissue larger in right half. 29.70 gm. yellowish secretion obtained. Initial weight 176 pounds.
	4/5/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder about same. 17.97 gm. secretion obtained; still yellowish (similar to blood plasma).
	4/7/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder slightly larger. Glandular tissue 2 1/2 inches in diameter in right half; 1 1/2 inches in left half. 8.70 gm. secretion; becoming cloudy.
	4/8/58	None		Udder same. 5.00 gm. secretion; cloudy.
	4/9/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder same. 2.98 gm. cloudy secretion.
	4/10/58	None		Udder same. 2.72 gm. cloudy secretion.
	4/11/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder same. 0.94 gm. cloudy secretion.
	4/12/58	None		Udder same. Small amount cloudy secretion.
	4/13/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder slightly smaller. Secretion amount smaller; still cloudy.

Treatment	Date	Hormone	Hormone Amount	Observations
V contd.	4/14/58	None		Glandular tissue longer in both halves. Small amount cloudy secretion.
	4/15/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder same. Small amount cloudy secretion - all from left half.
	4/16/58	None		Glandular tissue seems to be larger in both halves. Small amount cloudy secretion.
	4/17/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Glandular tissue larger. Few drops cloudy secretion.
	4/19/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	Udder seems smaller and very flabby.
	4/21/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	No change. Few drops cloudy secretion.
	4/23/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	No change.
	4/25/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	No change.
	4/27/58	Stilbestrol Progesterone	.117 mg. 23.333 mg.	No change. Final weight 172 pounds.
VI 0.25 mg. diethyl-stilbestrol in oil. (Rambouillet)	4/3/58	Diethyl-stilbestrol	.250 mg.	Udder small and flabby. Glandular tissue quite firm in both halves. Small amount cloudy secretion present; not collected. Initial weight 146 pounds.
	4/5/58	Diethyl-stilbestrol	.250 mg.	Udder same. 3.55 gm. yellowish-white secretion.
	4/7/58	Diethyl-stilbestrol	.250 mg.	Glandular tissue slightly larger in both halves. 3.88 gm. secretion similar to 4/5.

Treatment	Date	Hormone	Hormone Amount	Observations
VI contd.	4/8/58	None		Glandular tissue approximately 3/4 inch in diameter in both halves. 3.94 gm. yellowish-white secretion.
	4/9/58	Diethyl-stilbestrol	.250 mg.	Glandular tissue slightly larger. 2.33 gm. secretion.
	4/10/58	None		Glandular tissue approximately 1 inch in diameter in both halves. 2.28 gm. yellowish-white secretion.
	4/11/58	Diethyl-stilbestrol	.250 mg.	Glandular tissue 1 3/4 inches in diameter in right; 1 1/2 inches in left. 0.78 gm. yellowish-white secretion.
	4/12/58	None		Udder same. 1.16 gm. yellowish-white secretion.
	4/13/58	Diethyl-stilbestrol	.250 mg.	Udder same. 1.24 gm. yellowish-white secretion.
	4/14/58	None		Gland slightly larger in both halves. 2.60 gm. yellowish-white secretion.
	4/15/58	Diethyl-stilbestrol	.250 mg.	Glandular tissue slightly smaller. 3.43 gm. yellowish-white secretion.
	4/16/58	None		Udder aboute same; quite firm. 1.62 gm. yellowish-white secretion.
	4/17/58	Diethyl-stilbestrol	.250 mg.	Udder same. 1.94 gm. milk-like secretion.
	4/19/58	Diethyl-stilbestrol	.250 mg.	Udder same. 4.72 gm. yellowish-white secretion.

Treatment	Date	Hormone	Hormone Amount	Observations
VI contd.	4/21/58	Diethyl-stilbestrol	.250 mg.	Udder smaller; teats shorter. 5.50 gm. yellowish-white secretion (becoming more yellow).
	4/23/58	Diethyl-stilbestrol	.250 mg.	Udder smaller; glandular tissue smaller. 4.73 gm. secretion; quite yellow.
	4/25/58	Diethyl-stilbestrol	.250 mg.	Udder smaller; flabby. 3.90 gm. secretion; colostrum-like, (thick).
	4/27/58	Diethyl-stilbestrol	.250 mg.	Udder small and flabby. 3.14 gm. colostrum-like secretion. Final weight 141 pounds.
VII 0.50 mg. diethyl-stilbestrol in oil. (Columbia)	4/3/58	Diethyl-stilbestrol	.500 mg.	Udder small and flabby. Small amount glandular tissue in both halves. No secretion. Initial weight 157 pounds.
	4/5/58	Diethyl-stilbestrol	.500 mg.	Glandular tissue slightly larger. No secretion.
	4/7/58	Diethyl-stilbestrol	.500 mg.	Glandular tissue more firm; diameters: right half, 1 3/4 inches; left half, 3/4 inch. 2.20 gm. yellowish-white, lumpy secretion.
	4/8/58	None		Udder same. 5.52 gm. secretion; not lumpy.
	4/9/58	Diethyl-stilbestrol	.500 mg.	Udder same. 12.06 gm. secretion; more milk-like.
	4/10/58	None		Udder same. 11.88 gm. slightly yellow secretion.
	4/11/58	Diethyl-stilbestrol	.500 mg.	Glandular tissue slightly larger. 16.08 gm. milk-like secretion.

Treatment	Date	Hormone	Hormone Amount	Observations
VII contd.	4/12/58	None		Glandular tissue diameters: right half, 1 1/2 inch; left, 1 1/4 inch. 23.36 gm. milk-like secretion.
	4/13/58	Diethyl-stilbestrol	.500 mg.	Udder larger, more pliable. 26.94 gm. milk-like secretion.
	4/14/58	None		Udder about same. 37.39 gm. milk-like secretion.
	4/15/58	Diethyl-stilbestrol	.500 mg.	Udder about same. 35.83 gm. milk-like secretion.
	4/16/58	None		Glandular tissue large and pliable in both halves. 38.16 gm. milk-like secretion.
	4/17/58	Diethyl-stilbestrol	.500 mg.	Udder same. 62.64 gm. milk-like secretion.
	4/18/58	None		Udder slightly larger. 54.38 gm. milk-like secretion.
	4/19/58	Diethyl-stilbestrol	.500 mg.	Udder slightly larger; very pliable. 55.11 gm. milk-like secretion.
	4/20/58	None		Udder same. 72.78 gm. milk-like secretion.
	4/21/58	Diethyl-stilbestrol	.500 mg.	Udder same. 67.34 gm. milk-like secretion.
	4/22/58	None		Udder may be slightly smaller. 78.54 gm. milk-like secretion.
	4/23/58	Diethyl-stilbestrol	.500 mg.	Udder same. 87.07 gm. milk-like secretion.
	4/24/58	None		Udder same. 85.66 gm. milk-like secretion.

Treatment	Date	Hormone	Hormone Amount	Observations
VII contd.	4/25/58	Diethyl-stilbestrol	.500 mg.	Udder slightly larger. 104.74 gm. milk-like secretion.
	4/26/58	None		Udder same. 142.43 gm. milk-like secretion.
	4/27/58	Diethyl-stilbestrol	.500 mg.	Udder slightly smaller. 98.48 gm. milk-like (slightly yellowish) secretion. Final weight 160 pounds.
VIII 1 mg. diethyl-stilbestrol in oil. (Columbia)	4/3/58	Diethyl-stilbestrol	1.000 mg.	Udder very small. Glandular tissue small in both halves. 7.04 gm. secretion resembling cloudy blood plasma. Initial weight 150 pounds.
	4/5/58	Diethyl-stilbestrol	1.000 mg.	Glandular tissue slightly larger in right half; left half about same. 3.17 gm. secretion more cloudy than 4/3.
	4/7/58	Diethyl-stilbestrol	1.000 mg.	Udder larger. Glandular tissue diameters: right half, 1 1/2 inches; left, 3/4 inch. 29.02 gm. milk-like (yellowish) secretion.
	4/8/58	None		Glandular tissue in left half larger; right half increasing some. 26.92 gm. milk-like (yellowish tint) secretion.
	4/9/58	Diethyl-stilbestrol	1.000 mg.	Glandular tissue enlarging in both halves. 30.30 gm. milk-like secretion (white).

Treatment	Date	Hormone	Hormone Amount	Observations
VIII contd.	4/10/58	None		Entire udder quite firm. 20.90 gm. milk-like secretion.
	4/11/58	Diethyl-stilbestrol	1.000 mg.	Udder same size, becoming more pliable. 57.52 gm. milk-like secretion.
	4/12/58	None		Udder same. 71.48 gm. milk-like secretion.
	4/13/58	Diethyl-stilbestrol	1.000 mg.	Glandular tissue larger and more pliable. 93.71 gm. milk-like secretion.
	4/14/58	None		Udder about same size; very pliable. 151.02 gm. milk-like secretion.
	4/15/58	Diethyl-stilbestrol	1.000 mg.	Udder same. 170.42 gm. milk-like secretion.
	4/16/58	None		Left half slightly larger, glandular tissue more firm. Right, same as 4/15. 209.16 gm. milk-like secretion.
	4/17/58	Diethyl-stilbestrol	1.000 mg.	Udder same. Milked a.m. and p.m. 246.72 gm. milk-like secretion, total for both milkings.
	4/18/58	None		Glandular tissue larger in both halves. Milked twice; 278.45 gm. milk-like secretion, total.
	4/19/58	Diethyl-stilbestrol	1.000 mg.	Udder quite firm; larger. Milked twice; 308.45 gm. milk-like secretion, total.
	4/20/58	None		Udder size approaching that of normal lactating ewe. Mammary veins clear-

Treatment	Date	Hormone	Hormone Amount	Observations
VIII contd.				ly visible. Milked twice; 399.22 gm. milk-like secretion, total.
	4/21/58	Diethyl-stilbestrol	1.000 mg.	Udder slightly larger; quite firm. Milked twice; 348.92 gm. milk-like secretion, total.
	4/22/58	None		Udder about same, not as firm. Milked twice; 419.53 gm. milk-like secretion, total.
	4/23/58	Diethyl-stilbestrol	1.000 mg.	Udder more firm again. Milked twice; 468.16 gm. milk-like secretion, total.
	4/24/58	None		Udder same size; quite pliable. Milked twice; 456.90 gm. milk-like secretion, total.
	4/25/58	Diethyl-stilbestrol	1.000 mg.	Udder same. Milked twice; 514.44 gm. milk-like secretion, total.
	4/26/58	None		Udder same. Milked twice; 610.52 gm. milk-like secretion, total.
	4/27/58	Diethyl-stilbestrol	1.000 mg.	Udder same. Milked once; 538.22 gm. milk-like secretion, total.
	4/28/58	None		Udder slightly smaller. Milked twice; 505.42 gm. milk-like secretion, total.
	4/30/58	None		Udder very large before milking but slightly smaller than on 4/28 after milking. 724.19 gm. milk-like secretion obtained. Final weight 131 pounds.

Treatment	Date	Hormone	Hormone Amount	Observations
IX 5 mg. diethyl-stilbestrol in oil. (Rambouillet)	4/3/58	Diethyl-stilbestrol	5.000 mg.	Udder flabby. Glandular tissue small and mushy. Clear fluid present in left, small amount, not collected. Initial weight 170 pounds.
	4/5/58	Diethyl-stilbestrol	5.000 mg.	Udder less flabby. Glandular tissue larger and more firm in both halves. 3.10 gm. honey-colored secretion.
	4/7/58	Diethyl-stilbestrol	5.000 mg.	Udder larger. Glandular tissue increasing in size. Diameters: right half, 2 1/2 inches; left, 1 1/4 inches. 45.18 gm. milky secretion.
	4/8/58	None		Right half slightly larger; left half same. 50.58 gm. milk-like secretion.
	4/9/58	Diethyl-stilbestrol	5.000 mg.	Right half larger; left half slightly larger. 50.80 gm. milk-like secretion.
	4/10/58	None		Glandular tissue larger in both halves, quite pliable. 62.72 gm. milk-like secretion.
	4/11/58	Diethyl-stilbestrol	5.000 mg.	Glandular tissue slightly larger in both halves. 70.45 gm. milk-like secretion.
	4/12/58	None		Udder approaching size and appearance of normal lactating ewe. Rather large secondary bulge appearing at base of teats, probably gland cistern. 105.43 gm. milk-like secretion.

Treatment	Date	Hormone	Hormone Amount	Observations
IX contd.	4/13/58	Diethyl-stilbestrol	5.000 mg.	Udder same. 101.63 gm. milk-like secretion.
	4/14/58	None		Udder very pliable. 157.20 gm. milk-like secretion.
	4/15/58	Diethyl-stilbestrol	5.000 mg.	Udder same. 178.51 gm. milk-like secretion.
	4/16/58	None		Udder same. 277.50 gm. milk-like secretion.
	4/17/58	Diethyl-stilbestrol	5.000 mg.	Udder same. Milked twice; 236.28 gm. milk-like secretion.
	4/18/58	None		Udder may be smaller. Milked twice; 228.59 gm. milk-like secretion.
	4/19/58	Diethyl-stilbestrol	5.000 mg.	Udder quite large before milking; after milk removed udder seems smaller than on 4/18. Milked twice; 406.69 gm. milk-like secretion, total.
	4/20/58	None		Udder slightly smaller. 200.06 gm. milk-like secretion.
	4/21/58	Diethyl-stilbestrol	5.000 mg.	Udder same. Milked twice; 124.06 gm. milk-like secretion, total.
	Slaughtered			Appeared normal in all respects. Ovaries rather small - no corpora lutea. Adrenals normal; one with thick cortex. Mammary gland secretory tissue quite large. Dimensions: right half, 3 1/2" x 5 1/2"; left half, 3" x 6". Final weight 160 pounds.

Treatment	Date	Hormone	Hormone Amount	Observations
X Control Peanut oil injections. (Rambouillet)	4/3/58	Peanut oil	3 c.c.	Udder small, flabby. Glandular tissue small; largest in right half. No initial secretion. Initial body weight 184 pounds.
	4/5/58	Peanut oil	3 c.c.	No change.
	4/7/58	Peanut oil	3 c.c.	Udder same. Glandular tissue quite firm in right half - 2 inches in diameter; very small in left half. No secretion.
	4/8/58	None		Right half same. Small amount glandular tissue in left half. No secretion.
	4/9/58	Peanut oil	3 c.c.	Glandular tissue 2 1/4 inches in diameter in right half; 1/2 inch left half. No secretion.
	4/10/58	None		Right half same. Glandular tissue 1 1/4 inches in diameter in left half. No secretion.
	4/11/58	Peanut oil	3 c.c.	Glandular tissue same size but very solid. No secretion.
	4/12/58	None		Glandular tissue same size; more pliable than 4/11. No secretion.
	4/13/58	Peanut oil	3 c.c.	No change. No secretion.
	4/14/58	None		No change. No secretion.
	4/15/58	Peanut oil	3 c.c.	No change. No secretion.
	4/16/58	None		Udder more pliable. No secretion.

Treatment	Date	Hormone	Hormone Amount	Observations
X contd.	4/17/58	Peanut oil	3 c.c.	Glandular tissue smaller. No secretion.
	4/19/58	Peanut oil	3 c.c.	No change. No secretion.
	4/21/58	Peanut oil	3 c.c.	Udder smaller; teats shorter. No secretion.
	4/23/58	Peanut oil	3 c.c.	No change. No secretion.
	4/25/58	Peanut oil	3 c.c.	No change. No secretion.
	4/27/58	Peanut oil	3 c.c.	No change. No secretion. Final weight 182 pounds.
	4/28/58	Slaughtered		Appeared normal in all respects. Reproductive tract small. Adrenals - normal. Mammary gland - small; fatty. Glandular area difficult to obtain accurate measurements. Approximately 2 3/4" x 4" in both halves.
XI Control Manipulation of udder. (Rambouillet)	4/3/50	Manipulated		Udder flabby. Glandular tissue pliable; largest in right half. Small amount honey-colored (slightly cloudy), thick secretion. Initial weight 179 pounds.
	4/5/58	Manipulated		Udder same. Few drops secretion, similar to that of 4/3.
	4/7/58	Manipulated		Udder same. Secretion amount same; more cloudy.
	4/8/58	Manipulated		Udder and secretion same as 4/7.
	4/9/58	Manipulated		Glandular tissue larger in both halves. Secretion same as 4/7.

Treatment	Date	Hormone	Hormone Amount	Observations
XI contd.	4/10/58	Manipulated		Udder and secretion same as 4/7.
	4/11/58	Manipulated		Glandular tissue diameters: right half, 2 inches; left half, 1/2 inch. No change in secretion.
	4/12/58	Manipulated		Right half no change. Left half 1 inch in diameter. No change in secretion.
	4/13/58	Manipulated		Udder same. No change in secretion.
	4/14/58	Manipulated		Udder same. Secretion more cloudy.
	4/15/58	Manipulated		Udder same. Secretion cloudy.
	4/16/58	Manipulated		No change.
	4/17/58	Manipulated		Udder same. Still only few drops secretion.
	4/19/58	Manipulated		Udder and secretion no change.
	4/21/58	Manipulated		No change.
	4/23/58	Manipulated		Glandular tissue more pliable. No change in secretion.
	4/25/58	Manipulated		No change.
	4/27/58	Manipulated		No change. Final weight 177 pounds.
	4/28/58	Slaughtered		Appeared normal in all respects. Reproductive tract and adrenals normal. Mammary gland fatty; teats firm but hollow.