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Effects of oxytocin administered in the dry period on subsequent lactation in cows

George Martin Gorman

To the Graduate Council:

I am submitting herewith a thesis written by George Martin Gorman entitled "Effects of oxytocin administered in the dry period on subsequent lactation in cows." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Eric W. Swanson, Major Professor

We have read this thesis and recommend its acceptance:

R.L. Murphree, D.O. Richardson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 30, 1966

To the Graduate Council:

I am submitting herewith a thesis written by George Martin Gorman entitled "Effects of Oxytocin Administered in the Dry Period on Subsequent Lactation in Cows." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

Eric W. Swanson
Major Professor

We have read this thesis and recommend its acceptance:

R. L. Murphree

Don O. Richardson

J. T. Miles

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

EFFECTS OF OXYTOCIN ADMINISTERED IN THE DRY PERIOD ON SUBSEQUENT LACTATION IN COWS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

George Martin Gorman

December 1966

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INTRODUCTION

The need for a dry period in dairy cows has been established for more than a century, but the optimum length of the period and reason for the benefit derived from the pause in lactation has been studied only recently. On the basis of survey studies a dry period of 60-90 days has been shown to result in maximum production. Until a few years ago the stimulus of a dry period to lactation was thought to be due solely to a rebuilding of the nutrient stores in the animal. More recently the involvement of hormonal factors such as an interaction of oxytocin with hormones necessary for maximum lactation has been postulated. Injections of oxytocin during the dry period could simulate the response to milking without evacuation of the gland or loss of nutrients due to lactation. Such treatment would be expected to delay mammary "involution" and might produce an effect in "dry" cows similar to those which were not dried off between lactations.

CHAPTER II

REVIEW OF LITERATURE

Effect of dry periods on next lactation. The need for a pause in the lactation of cows has long been recognized. This pause, or dry period, was first developed, as reported by Arnold and Becker (3) in the late 1700's and early 1800's from practical experience of farmers and dairymen. The optimum duration of the dry period became a matter of debate and was first studied by survey methods. In 1913 Carroll (11) reported that, on the basis of two years of records from the Utah Cow Testing Association, a two-month dry period resulted in a greater amount of butterfat and profit than a dry period of one month. He also concluded that two months was about the time needed for the average cow to obtain sufficient rest for her highest production, and a dry period of longer than two months did not appear to benefit the next lactation. Hammond and Sanders (29) concluded that "yield is considerably lowered by a very short dry period but not greatly increased by a very long one". They found from a study of 408 cows that those with a dry period of 80 to 119 days gave 14.2 per cent more milk and those dry 40-79 days gave 10.9 per cent more milk than those dry 39 days or less. Copeland (13) summarized Jersey herd test records and found that the average test cow was dry 15.39 per cent of the time which is a dry period of 56 days per year. Arnold and Becker (3) also found that the maximum daily yield was highest when the preceeding dry period was 31-60 days.

Klein and Woodward (39) studied the effect of dry period in 15,442 Dairy Herd Improvement lactations. Within this group there were 1,139 comparisons of cows with varying dry period lengths in successive lactations. There was a 9.2 per cent increase in production of cows having a one- to two-month dry period over that when they were dry zero to one month, 4.3 per cent increase when dry two to three months compared to a dry period of one to two months and a 1.4 per cent increase for three to four months compared to two to three months. Total milk yield was reduced with a dry period longer or shorter than 55 days for cows with a 365-day calving interval.

Morrow, Keener, and Hall (55) found the high point in 2,631 production records to coincide with a dry period of 65 days "with very little difference occurring between 45 and 85 days". Makhnyuk (42), upon examination of the records of 500 cows divided into four groups with dry periods of 45, 60, 60-90, and more than 90 days, found no significant difference in yields of the first three groups. He recommended that the dry period be reduced from 60 to 45 days.

The reason for lactation stimulus by dry periods. The question is why do cows produce more milk after a dry period? One of the possible reasons brought out by Hammond and Sanders (29) is to allow for growth of the mammary tissue. Pardue and Swanson (60) examined the desoxyribonucleic acid (DNA) content of udders in which the left halves were dried off 60 days prepartum and the right halves dried off seven days before slaughter which was a few days prepartum. They concluded that lack of a dry period did not inhibit cellular development.

The average DNA content was 56.8 g for the left halves and 57.4 g for the right. Pardue and Swanson (60) also found that involution occurred most rapidly 45 days after milking ceased. The ratio of DNA between the left and right halves dropped from 84.8 per cent to 83.8 per cent to 63.4 per cent for 30, 45, and 60 days dry, respectively. This would tend to substantiate the conclusion of Sanders (67) who stated that "inaction for a period of 40 days, and no more, is necessary for full regeneration and development of the mammary gland, and that the benefit of further rest lies in its allowing of the accumulation of food reserves...".

Dawson and Underwood (15), maintain that the dry period is "required to replenish the losses and to store adequate reserves for the next lactation". Dickerson and Chapman (20) after studying 1,574 records from 274 cows in 41 Holstein herds stratified according to feeding conditions agreed that "the importance of the length of the 'rest period' preceding a lactation would become greater the lower the plane of nutrition...". Brody et al. (9) also explained that the initial rise in milk secretion was due to improved condition and increasing food consumption of the animal prepartum. A number of other workers also reached similar conclusions (3, 29, 66, 68).

Swanson (73) compared the milk production of identical twins which were fed so that the one of each pair which was milked continuously was in as high or higher state of nutrition than its mate who received a 60-day dry period. The animals which were milked continuously produced 75 per cent and 62 per cent as much as their control mates with normal

dry period, in the second and third lactations, respectively. He concluded that the dry period was necessary for factors other than nutrition. Smith, Wheelock, and Dodd (71) simulated this study (73) but compared different quarters of the same cow. The right-fore and left-hind quarters were dried off ten weeks prior to calving while the left-fore and right-hind quarters were milked throughout pregnancy. Their results were similar to Swanson's (73); the milked quarters yielded 56 per cent of the control quarters in one animal and 62 per cent in the other animal. Since the environment was alike for all quarters, the effect could not have been due to nutrition or a direct hormone action. They suggested that continuous removal of secretion prevented regeneration of the epithelium in the milked quarters.

Oxytocin and milk ejection. In addition to the anabolic processes of the udder and the presence of nutritive stores in the animal, several hormones are essential to maximum milk production. One of the essential hormones is oxytocin which is necessary for normal milk ejection in the bovine and other species.

The role of oxytocin in stimulating the ejection of milk was established by Ely and Petersen (23). They brought together two isolated facts: (1) that denervation of the udder had no effect on the milk ejection response, and (2) that injections of oxytocin simulated natural ejection response. They concluded that the stimulus of suckling or milking releases oxytocin from the posterior pituitary into the blood from which it acts on the mammary gland to cause milk ejection. Martinet and Denamur (46) found the same to be true in goats and ewes. Syntocinon,

a synthetic oxytocin, was also used to initiate normal milk let-down in women by Rainer (63).

Yokoyama and Ota (76) found that hypothalamic lesions which prevented oxytocin release in rats prevented their pups from growing even though the mammary glands were filled with milk. Denamur and Martinet (19) observed a linear relationship between milk yield and the amount of oxytocin injected into ewes. Manunta and Marongiu (43) found a similar response by measuring the intramammary pressure of ewes given varying levels of oxytocin. The milk ejection response of oxytocin has been used in experiments to remove residual milk (40, 62, 69).

Extramammary stimulation of oxytocin. Hays and Van Demark (35) stated that Neusch in 1904 suggested a relationship between stimulation of the reproductive organs and milk ejection. Neusch mentioned that a publication in 1727 reported the Hottentots blew air into the vagina to bring about milk ejection in the absence of a calf. The Skyths of India milked mares in a similar manner. Neusch also mentioned that milk ejection could be brought about by rinsing the vagina or uterus or by manual removal of the afterbirth. Debachere and Peeters (16) found that milk ejection started when the vagina of lactating cows was distended by inflation of a rubber balloon.

Hays and Van Demark (35) found that blood taken from a cow before stimulation of the vulva and cervix had no effect on activity when perfused into an excised uterus. However, samples taken after stimulation caused a series of contractions which were similar to those caused

by injected oxytocin. They (34) also found that the uterine contraction patterns resulting from handling the uterus or ovaries were very similar to the patterns at the time of mating.

Van Demark and Hays (74) using a balloon inserted in the uterus found that in the estrus cow uterine contractions increased progressively as a bull was brought into her sight, allowed to nuzzle the vulva and hind quarters, permitted to mount but not copulate, mount and copulate, and finally to ejaculate. These same cows in the post-estrus stage showed similar but less pronounced reactions.

Hammond (28) noted that in lactating mares milk often flows freely during mating. Hays and Van Demark (33) measured the intramammary pressure created by artificial insemination techniques and found a 47.4 per cent average increase in pressure during the time of massage and the insertion of the inseminating tube in 86 per cent of the cows which gave positive responses. In a later study (34) they found nothing to indicate that this was purely a nervous reflex. Other workers (23, 24) have shown that cutting the nerve supply to the mammary gland had no effect on milk ejection.

Van Demark and Hays (75) stated that Alexander in 1945 found uterine strips of cows and ewes underwent strong contractions when added to an oxytocin bath. That the contractions remained tonic while under the influence of pitocin at all stages of the estrous cycle was noted by Cupps and Asdell (14). Evans and Miller (25), using the balloon technique, reported that in the intact cow the uterine reaction to pituitrin was greatest at the time of estrus after which it declined

until the sixteenth day of the cycle when it began to increase. Hays and Van Demark (34) using the same technique found that "oxytocin increased the activity in each of 28 observations on the intact animals". Ten to 12 seconds after the injections a strong contraction occurred and then tapered off. No difference in reaction was found in the intact animal or perfused section. They later concluded (35) that the relationship between the reproductive organs and the mammary gland is by sensory nerve impulses along with oxytocin.

Gonadotrophic hormone release from oxytocin. Recently oxytocin has been shown to be involved with the release of other hormones. Marion et al. (45) observed that when heifers were serviced by a vasectomized bull the time of ovulation was 7.7 hours compared to 9.9 hours for their controls, which suggested an earlier release of gonadotrophin as a result of increased secretion of oxytocin due to service.

Hansel and coworkers (30) found that oxytocin administered at the beginning of estrus shortened the interval between estrus and ovulation. Black and Duby (8) also found a shortened cycle in heifers after oxytocin administration and suggested as a possible cause that oxytocin stimulated the release of luteinizing hormone (LH) from the pituitary causing maturation of the follicle which in turn caused regression of the old corpus luteum. Armstrong and Hansel (2) used the effect of a shortened estrous cycle to investigate the role of oxytocin in regulating the secretion of gonadotrophic hormones. They found oxytocin reduced normal luteal activity resulting in a deficiency of

progesterone secretion.

Martini et al. (47) assayed rabbit urine after injections of different preparations of posterior pituitary hormones and found they all caused a significant rise in the release of gonadotrophins. The injection of oxytocin daily from the day of estrus to the sixth day of the cycle produced a smaller corpus luteum and a reduced concentration of progesterone in studies by Labhsetwar (41). He also found the oxytocin treatment had no significant effect on the hypophyseal levels of follicle stimulating hormone (FSH) or LH. Mares and Casida (44) found oxytocin increased the synthesis of progesterone by the corpus luteum in vivo but decreased its ability to synthesize progesterone in vitro.

Hansel and Wagner (31) reported that oxytocin injections in heifers from the third through the sixth day of the estrous cycle shortened the cycle eight to twelve days. Shortened cycles occurred only when oxytocin injections were given during the first half of the cycle, when the corpus luteum was being formed. Cameron and Fosgate (10) in a similar study found no shortening of the cycle when oxytocin was injected from the day of estrus through the third day of the estrous cycle. They concluded that oxytocin exerts its influence by inhibiting gonadotrophic secretions from the pituitary complex. After a study of oxytocin induced luteal inhibition, Donaldson and coworkers (21) suggested that oxytocin caused a release of gonadotrophins rather than a decreased synthesis since pituitary gonadotrophins were depleted by injections of oxytocin.

The ability of oxytocin injections to inhibit growth of the corpus luteum has been used as a tool in experiments (70, 72). Deis (17) studied the influence of sex hormones on the posterior pituitary oxytocin concentration in rats. He found that castration in both sexes increased the amount of oxytocin and that replacement of normal amounts of testosterone and estrogen decreased the oxytocin content in castrate animals. Progesterone had no effect. He also noted that males had lower levels of oxytocin than females.

Melin and Kihlstrom (54) noted an increase in sexual drive after injections of oxytocin in male rabbits. However, they attributed this to a stiumlation of muscular contractions in the genital organs. Armstrong and Hansel (1), also working with rabbits, found that oxytocin stimulated development of the seminiferous tubules, interstitial cells of Leydig, and enhanced development of the prostate gland. They suggested that oxytocin stimulation resulted in an increased secretion of an interstitial cell stimulating hormone, possibly prolactin.

Release of prolactin by oxytocin. Petersen (61) was the first to suggest that the secretory activity of the posterior and anterior pituitary were related. Noting a decline in pituitary prolactin after suckling, he postulated that the posterior pituitary may be responsible. Benson and Folley (6) suggested that oxytocin may induce the release of prolactin after finding that mammary involution was retarded in rats when oxytocin injections were given daily after removal of the pups on the fourth day of lactation. The retardation of mammary involution by oxytocin has since been confirmed (5, 7, 36,

48, 49, 51, 57). Increased growth of pigeon crop glands has been used as an assay for prolactin. Chaudhury and Chaudhury (12) found injections of Syntocinon intradermally over the crop gland of pigeons increased the gland's weight. They suggested that oxytocin may cause a release of prolactin in pigeons, but that it may also have a direct action on the gland. Armstrong and Hansel (1) also suggested prolactin may be involved in testes development in male rabbits.

The thesis of prolactin release by oxytocin was tested by Grosvenor and Turner (27) who found no difference in pituitary lactogen between control and oxytocin injected anesthetized rats. In a later study Griffith and Turner (26) found from measuring the DNA content of rat mammary glands that lactogen and hydrocortisone acetate retarded cellular involution but oxytocin did not. Ota and coworkers (59) found that oxytocin retarded the decrease of DNA but the ribonucleic acid (RNA):DNA ratio was similar to that of weaned controls. In a later study (58) they noted oxytocin preserved only the structure of the mammary gland as reflected in the DNA content and alveolar structure. Prolactin, however, not only preserved the structure but also the functional activity of the gland. They concluded that the inhibitory effect of oxytocin on mammary involution was not due to stimulation of prolactin secretion. Johnson and Meites (37) reported that although injections of oxytocin and prolactin in rats increased the growth rate of their litters over the controls, histological examination of the mammary glands after 28 days of lactation showed that prolactin markedly retarded involution while oxytocin had no effect. Nicoll (56) also found

oxytocin did not effect prolactin secretion of anterior pituitary explants in vitro. Riesen (64), in a recent study of pituitaries from Holstein cows, found the "mean prolactin activity was greater for nonsuckled groups than for the corresponding suckled groups", as measured by pigeon crop gland weights. Kanazawa et al. (38) failed to demonstrate a prolactin release by oxytocin in pre-estrous or estrous rats. Oxytocin had no effect on the estrous cycle, or on uterine trauma, or on deciduomata in lactating rats deprived of their litters in a study by Rothchild and Quilligan (65). Denamur (18) concluded that oxytocin had no direct or indirect action on prolactin secretion after he found no significant differences in the acid soluble RNA of control, prolactin injected, or oxytocin injected rabbits.

Meites, Talwalker, and Nicoll (53) reported that prolactin initiated milk production in pseudopregnant rabbits but injections of oxytocin failed to achieve this result. Haun (32), however, reported lactogenesis in four of five rabbits injected with oxytocin.

To investigate any effect oxytocin may have on prolactin in the intact rat, Meites and Hopkins (50) studied a group of rats which were hypophysectomized on the fourth day of lactation after removal of their litters. Neither saline nor oxytocin injections maintained mammary function. By injecting prolactin and adrenocorticotrophic hormone (ACTH) together, mammary involution was partially inhibited; and with the addition of oxytocin, secretion was maintained and the lobular-alevolar involution was more efficiently retarded. They concluded that oxytocin did not act through the anterior pituitary, but that oxytocin

acted directly on the gland, which agreed with a previous study (51) on intact rats.

Meites, Nicoll, and Talwalker (52) found that about ten minutes after oxytocin injections, the ducts of the mammary gland were filled with secretion while the alveoli were shrunken and did not become filled for about eight hours. They suggested that oxytocin relieved the pressure in the alveoli by forcing milk into the ducts, thus allowing prolactin and the other hormones to initiate further secretion. "Milk may be reabsorbed from the ducts and some may flow back into the alveoli." Ota et al. (58) agreed saying "...resorption of accumulated milk is accelerated by oxytocin injections...".

CHAPTER III

EXPERIMENTAL METHODS

Two experiments were performed. The first was a preliminary study with rats, and the second utilized dairy cows.

Experiment I

Management of animals. Thirty female Sprague-Dawley rats of various ages were exposed to 15 males for five days to concentrate littering dates and facilitate equalizing litter size. On the fifteenth day after the males were first introduced, pregnancy checks were made by abdominal palpation. Those diagnosed non-pregnant were checked again four days later. Eighteen of the 21 pregnant rats were divided equally into control and treated groups according to their body weight prior to breeding. The average weights for the control and treated groups were 233.9 grams and 234.8 grams, respectively. The remaining three animals were used as donors to maintain a large litter size on the lactating rats.

Injection procedure. Oxytocin administration was started 15 days after the males were first introduced (approximately the last week of gestation), a time which was expected to be comparable to the prepartum dry period of a cow. Oxytocin was injected subcutaneously three times daily for seven days at the rate of one United States Pharmacopoeia (USP)

unit per injection or three USP units per day. Pitman-Moore's "Posterior Pituitary Injection" was used for the first four days until the supply was exhausted. Veterinary Laboratories' "Oxytocin Injection" was used for the remainder of the treatment. All animals were handled the same except for the injections. The control rats were not given any injections. Adequate amounts of normal feed rations and water were always available.

Data collected. Littering began one day after the last injection and spread over a six day period. The litters were weighed, counted, and sexed after parturition. On the day after littering an attempt was made to obtain a constant weight for each litter and the litter size was adjusted to ten pups per litter. The donor animals were used to replace any dead or eaten pups. The litter size was held at ten pups per litter throughout the lactation period of 22 days. No replacement pups were needed after the fourth day of lactation. Litter weights were recorded daily. Litter weight gains were used as a measure of milk production of the dam.

Experiment II

Selection of animals. The experiment was conducted over a period of nine months from August, 1965, through May, 1966. Eight pairs of cows were selected from the University of Tennessee herd at Knoxville, Tennessee. They were paired on the basis of previous lactation, age, calving dates, and other factors which might affect their next lactation (Table I). Two pairs of Holsteins were eliminated during the experiment.

TABLE I

INFORMATION USED FOR PAIRING COWS IN A STUDY OF EFFECT OF OXYTOCIN INJECTIONS IN THE DRY PERIOD

Pair	Group[a]	Cow Number	Breed	Birth Date	Milk[b] Yield	Calving Date	Length of Dry Period
					(kg)		(days)
2	C	192	Holstein	7-62	2,825	9- 8-65	54
	T	177		1-62	3,243	10-10-65	54
3	C	T196	Jersey	12-61	1,842	11- 8-65	62
	T	T192		11-61	1,926	10-18-65	60
5	C	220	Holstein	9-62	3,443	10-11-65	60
	T	175		1-62	3,404	11-12-65	52
6	C	143	Holstein	10-61	4,523	11-15-65	58
	T	125		8-61	4,308	11-22-65	42
7	C	L39	Jersey	7-60	2,695	12-23-65	175
	T	L34		6-60	2,621	12- 4-65	63

TABLE I (Continued)

Pair	Group[a]	Cow Number	Breed	Birth Date	Milk[b] Yield	Calving Date	Length of Dry Period
					(kg)		(days)
8	C	195	Holstein	7-62	3,320	10-29-65	48
	T	198		7-62	3,368	12-22-65	48
Average							
Control					3,109		76[c]
Treated					3,145		54

[a] C, Control; T, Treated

[b] Actual milk for the first 22 weeks of 1964-1965 lactation.

[c] Statistically not significant.

In one pair the treated animal developed metritis and died ten days after calving. The control animal of another pair was sold after 14 weeks of lactation and an acute mastitis attack during the ninth week of lactation rendered the treated mate unsuitable for comparable time comparisons. The remaining six pairs, four Holsteins and two Jerseys, completed the study as planned. Figure 1 shows the average milk production of the control and treated groups for the first 22 weeks of the lactation prior to the test period.

Treatments. One of each pair was assigned randomly to the treated group. The treated group received intravenous injections of five International Units (IU) oxytocin twice daily at about the time of morning and afternoon milkings. Attempts were made to minimize the excitment of the treated animals. A squeeze chute was used to secure the animals and injections were made with a two inch, 20 gauge hypodermic needle into the jugular vein. Injection sites were varied along both sides of the neck to reduce undue irritation. Contrary to expectations, all animals became accustomed to the injection procedure and after about two weeks, they offered no resistance. This would appear to alleviate the possibility of adrenalin overriding the effect of oxytocin. The injections began the afternoon or morning after the animal was last milked and continued until she calved. Sandoz Pharmaceuticals' "Syntocinon" was used for the first 400 injections, and then Veterinary Laboratories' "Oxytocin Injection" was used for the remainder of the experiment. The dry periods averaged 76 and 54 days for the control and treated groups, respectively (Table 1, page 16). Feeding and manage-

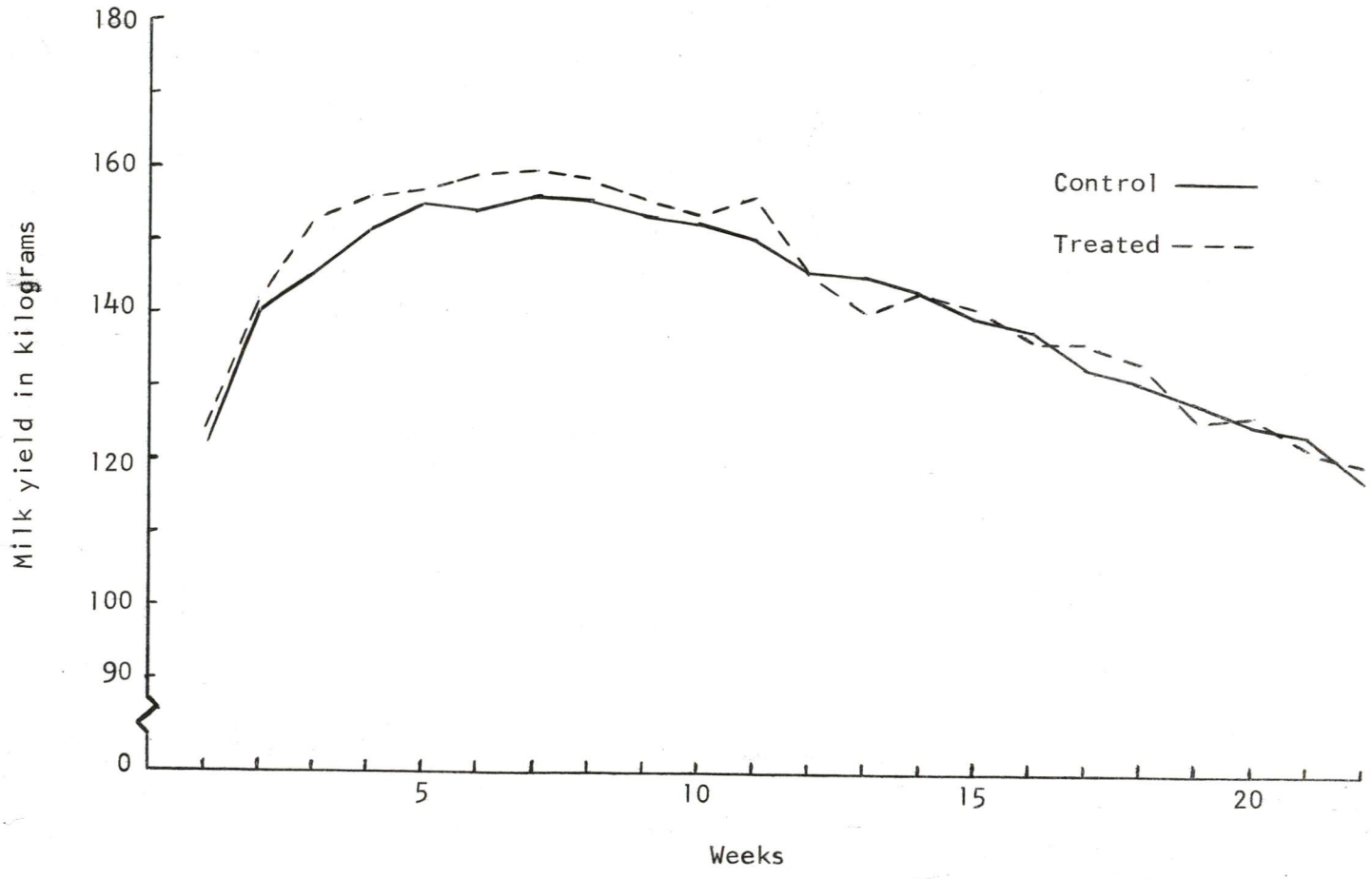


FIGURE I

AVERAGE MILK PRODUCTION OF THE COWS USED
PRELIMINARY PERIOD 1964-1965

ment of all of the cows were identical during the prepartum period. Roughage was fed ad libitum and grain was fed to all animals prior to calving. After calving roughages were fed ad libitum in the usual manner to cows housed in a loose housing system. Concentrates were fed at a ratio of about 1:3 according to milk production.

Data collected. Complete records of health and other observations were kept throughout the experiment. After the first week of injections a visible udder difference was noted between the treated and control cows; so weekly photographs of the udders were taken of all animals until parturition. After calving, colostrum and weekly milk samples were obtained. Daily milk weights were recorded from Alfa Laval spring scales and read to about one-third pound for the first 22 weeks of lactation.

Milk analysis. Composite afternoon and morning milk samples were obtained once each week and analyzed for milk fat and total solids. Colostrum samples were obtained at the first milking and stored under refrigeration until analyzed for protein, milk fat, and total solids.

Testing for total solids consisted of accurately weighing two milliliters of milk into aluminum cups and allowing it to dry in a cabinet at room temperature over night. Most of the water was evaporated by the next day. The pans were put in a drying oven at 103 C for approximately two hours to evaporate the remaining moisture. The samples were then weighed and calculations made for total solids. The Babcock method was used to test for fat. Solids-not-fat (SNF)

values were arrived at by subtracting the milk fat from the total solids. To determine protein two milliliters of the milk sample was put in a Kjeldahl flask with a catalyst and analyzed according to the Association of Agricultural Chemists' Kjeldahl method (4).

CHAPTER IV

RESULTS

Experiment I

General. The oxytocin treatment appeared to have had little effect on the length of gestation, litter size, or sex ratio at birth (Table II). There were, however, more pups born dead and a lower average litter weight in the oxytocin treated group.

Maintenance of litter size. The day after littering (Day One) all litters were equalized, by addition or removal of pups, to contain ten pups per litter. Dead or eaten pups were replaced to maintain a litter size of ten pups throughout the 21 days of lactation. After the litters were equalized eight pups were replaced; two in the control group and six in the treated group. On Day Two one pup was replaced in a control litter and four pups in as many litters in the treated group. Two pups were replaced in separate litters in the treated group on Day Three. On Day Four one pup was replaced in a control litter.

Growth of the litter. The average initial litter weight of the treated group was lower than the control group because of the lighter litters at birth, and remained lower than the controls throughout the 21 day lactation period (Figure 2). This would indicate that the milk production was similar for both groups. Examination of the average daily

TABLE II

LITTER RECORD FOR CONTROL AND OXYTOCIN TREATED PREGNANT RATS

Animal number	Litter Size				Litter weight
	Male	Female	Born dead	Total alive	(grams)
Control					
2	5	7	0	12	71
3	3	7	0	10	59
4	9	3	0	12	70
5	7	4	0	11	62
9	6	1	0	7	40
10	8	5	1	13	76
11	8	6	1	14	80
12	5	9	0	14	77
14	7	5	1	12	77
Average	6.4	5.2	.33	11.6	68.0
Treated					
16	9	4	1	13	73
18	5	7	0	12	63
19	6	6	1	12	64
20	8	3	3	11	61
22	7	2	1	9	50
25	10	2	0	12	66
26	2	4	0	6	20
28	8	3	1	11	60
29	10	6	0	16	80
Average	7.2	4.1	.78	11.3	59.7

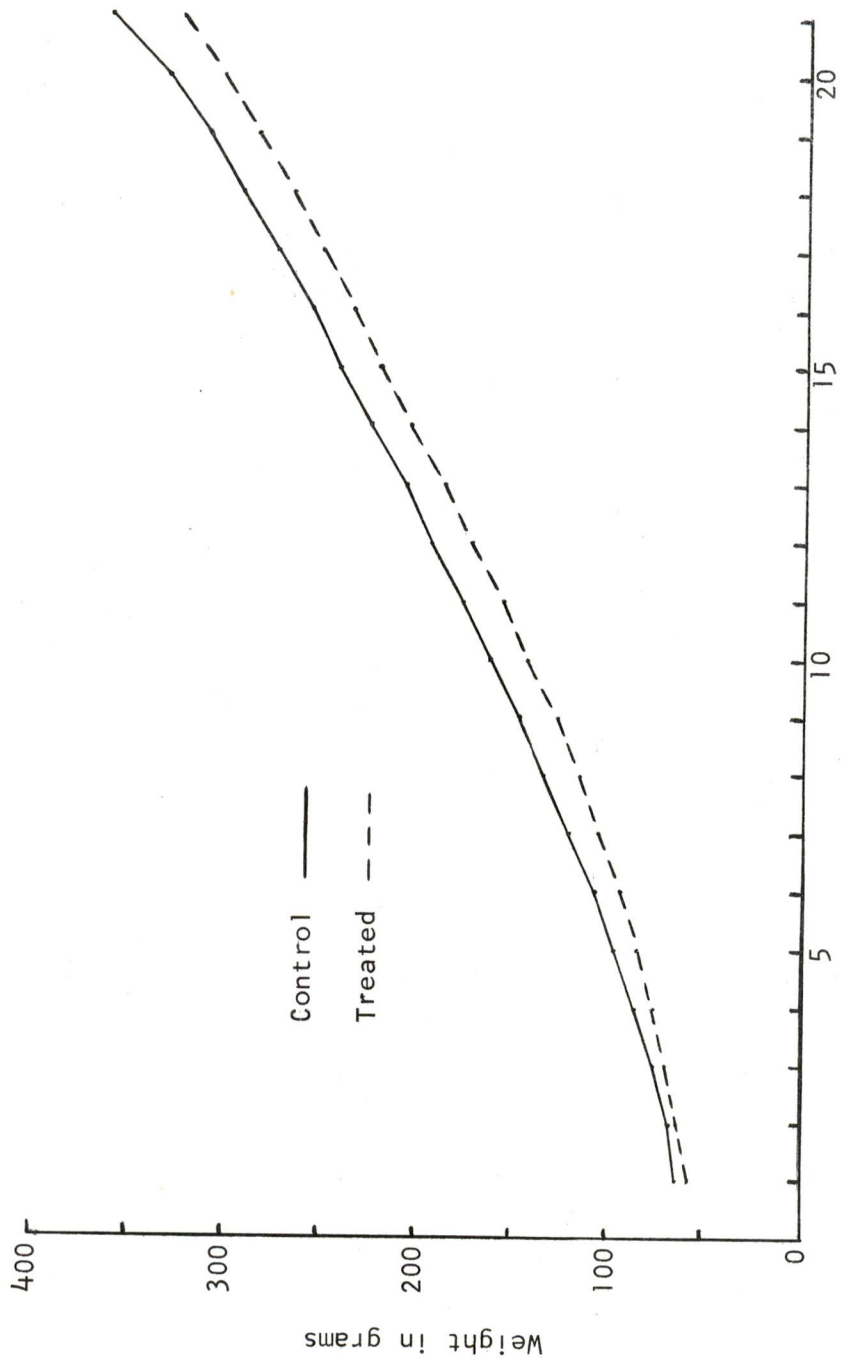


FIGURE 2

AVERAGE DAILY LITTER WEIGHT FOR CONTROL
AND OXYTOCIN TREATED GROUPS

gain per litter (Figure 3) revealed that for the first ten days of lactation not only was the average litter weight in the treated group below the control but the rate of gain was also lower. An analysis of variance showed the treatment effect to be significant ($P < .01$).

Since the pups began eating solid feed about the eleventh or twelfth day, the gains thereafter could not be attributed solely to milk production by the dam. This effect is indicated by the irregular pattern of gains after the tenth day.

Experiment II

Effect on udder. After about a week of injections, the appearance of the udder indicated that involution was not proceeding normally in the treated animals. The udder remained distended and in some cases increased in size for a period which varied among individuals from two to five weeks. The udders of all control animals decreased in size and turgidity as usual. Photographs in Figures 4, 5, and 6 illustrate this effect. Cow T192, a treated animal, showed the most dramatic effect as seen in Figure 4 with her control mate, T196. Figures 4A and 4B on the fourteenth day after last milking show the relative udder distention. On the thirty-fifth day of the dry period the udder of Cow T196, the control, has regressed as would be expected (Figure 4C). The treated mate, Figure 4D, however, still retained a distended udder. It is interesting to note that this animal exhibited the largest depression of milk production, producing only 31.7 per cent as much as her control mate. The same effect is visible in Figure 5 but not to as great an extent.

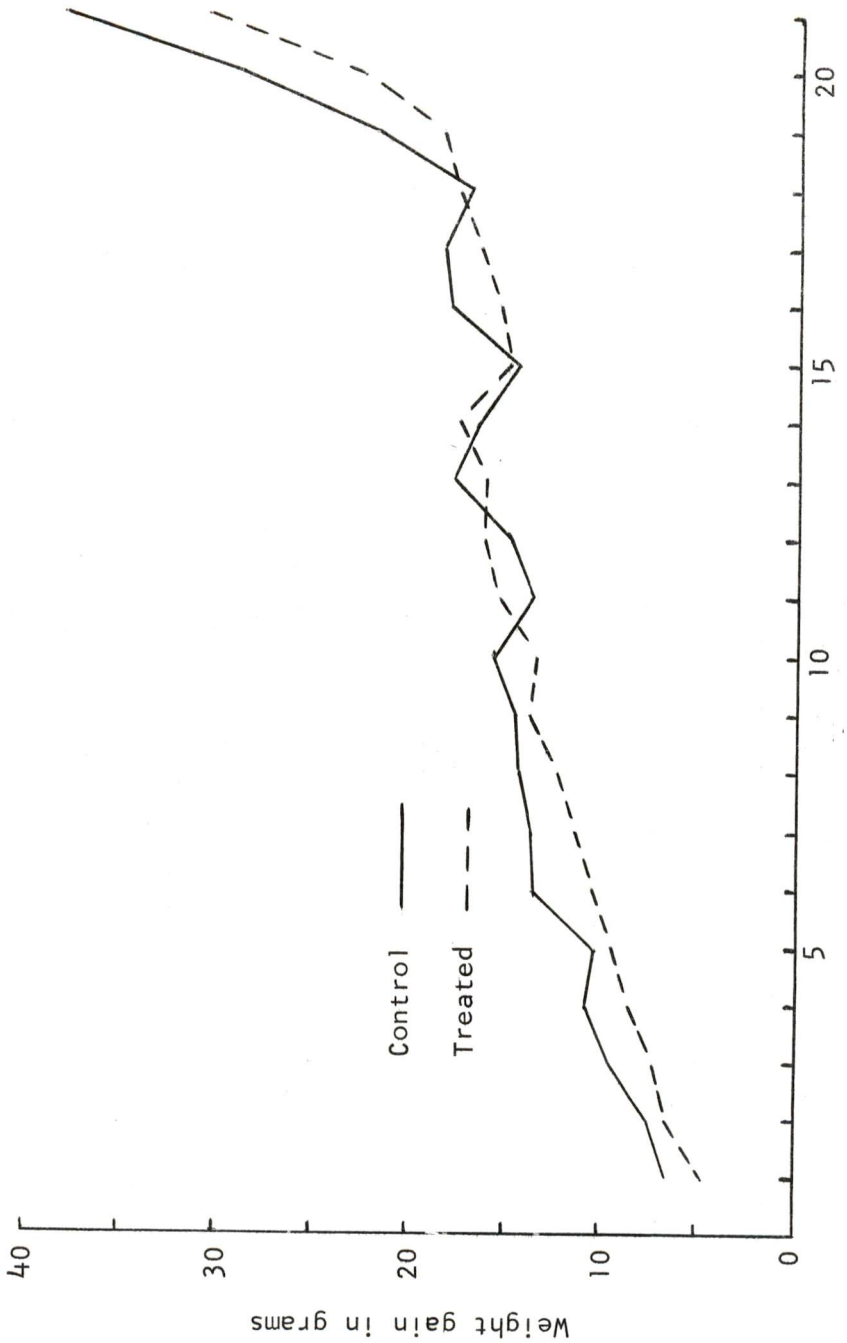
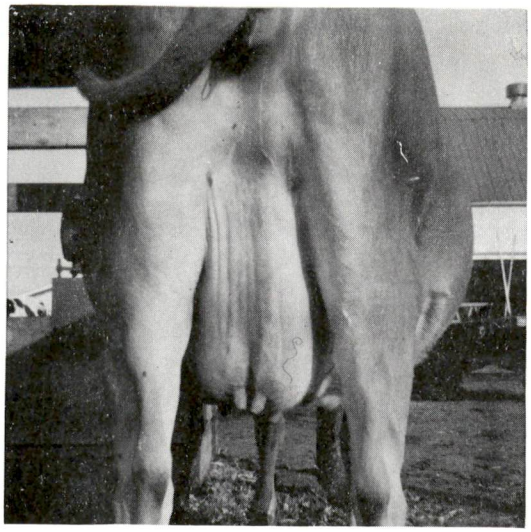


FIGURE 3

AVERAGE DAILY WEIGHT GAIN OF CONTROL AND OXYTOCIN TREATED GROUPS



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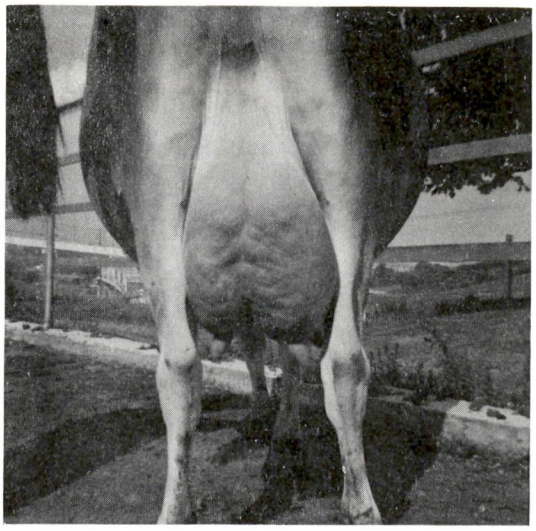

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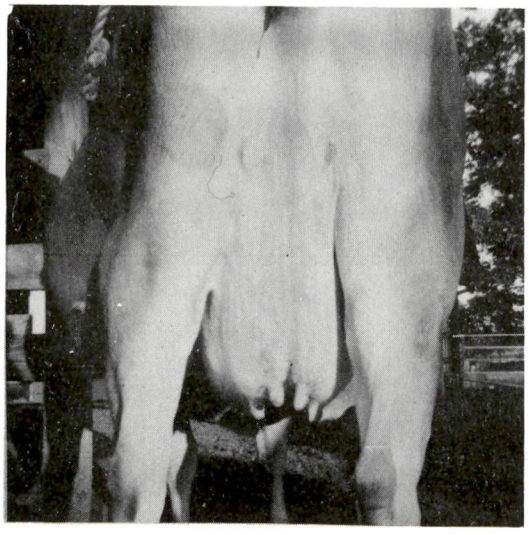

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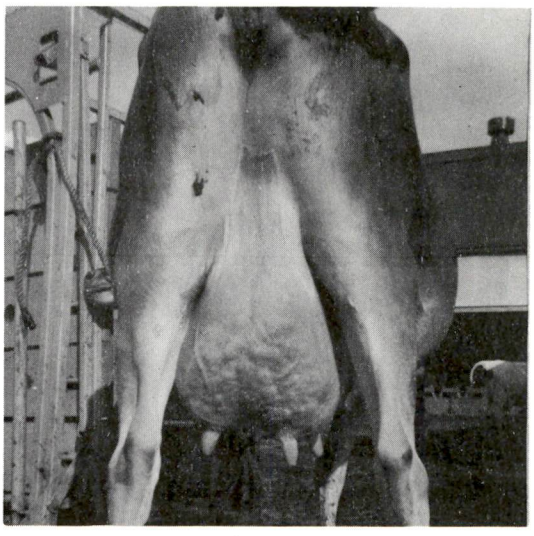
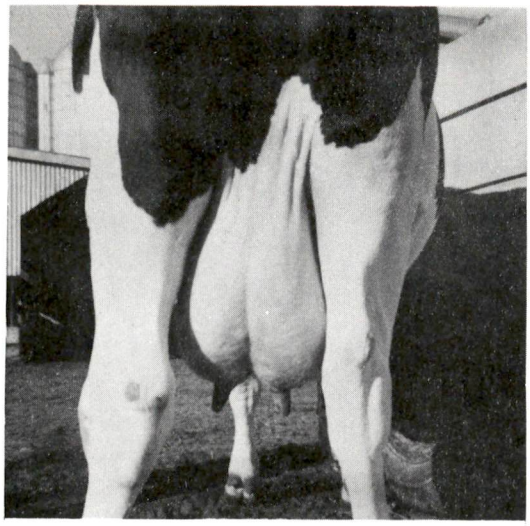


D

FIGURE 4

UDDER COMPARISONS DURING DRY PERIOD
PAIR THREE

A Control Cow T196, Day 14
B Treated Cow T192, Day 14
C Control Cow T196, Day 35
D Treated Cow T192, Day 35



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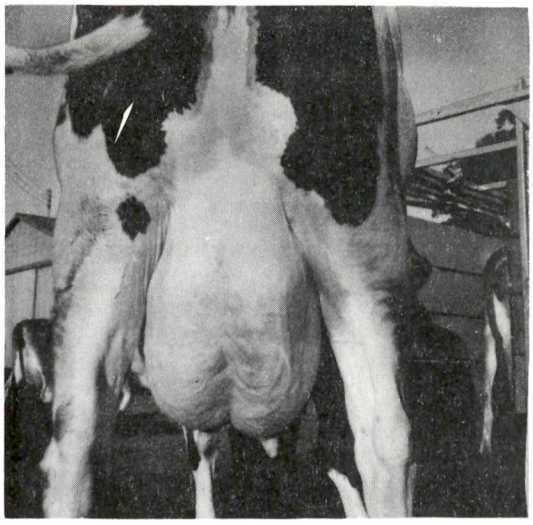

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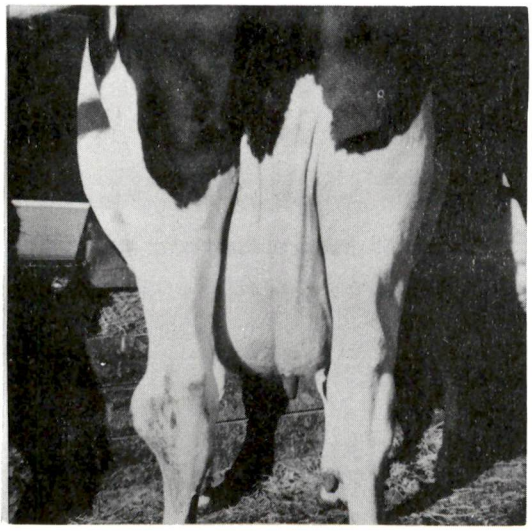

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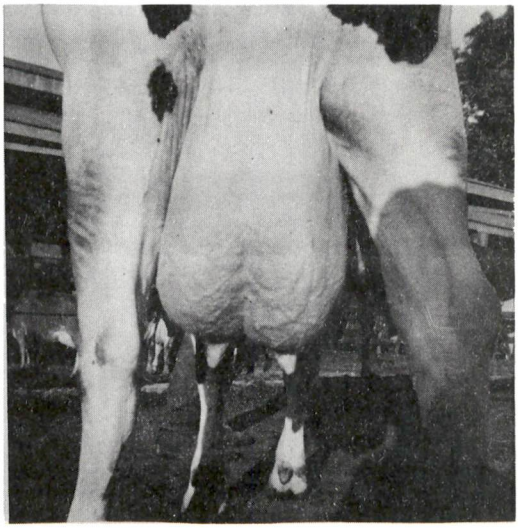

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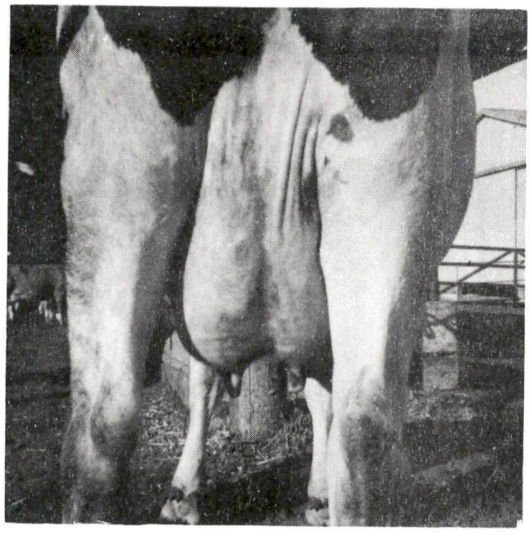
FIGURE 5

UDDER COMPARISONS DURING DRY PERIOD
PAIR SIX

A Control Cow 143, Day 1
B Treated Cow 125, Day 1
C Control Cow 143, Day 14
D Treated Cow 125, Day 14



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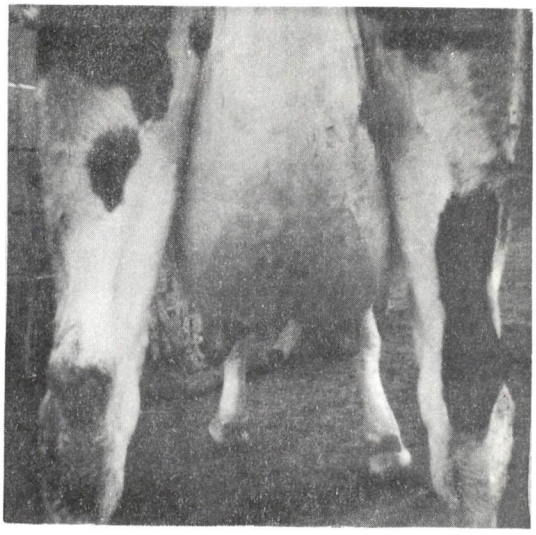

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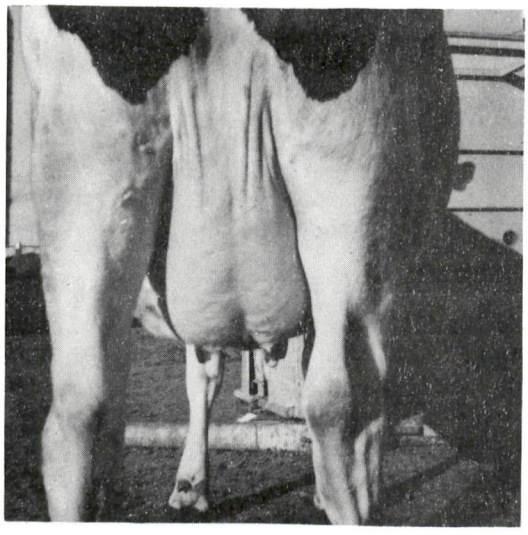

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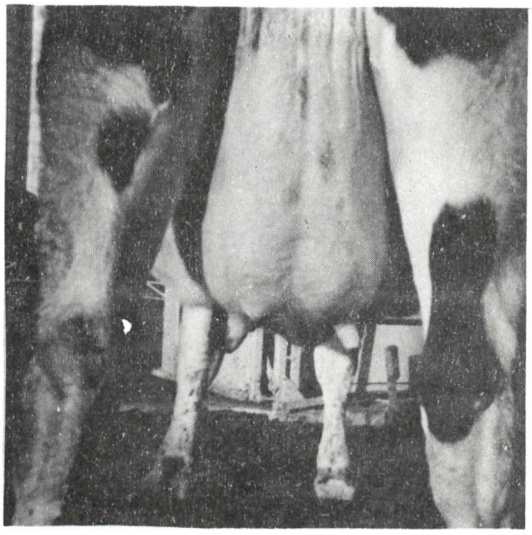

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FIGURE 6

UDDER COMPARISONS DURING DRY PERIOD
PAIR EIGHT

A Control Cow 195, Day 7
C Control Cow 195, Day 28

B Treated Cow 198, Day 7
D Treated Cow 198, Day 28

Figure 5A is of control Cow 143 the day after last milking and Figure 5B of her treated mate, Cow 125, at the same period. At 14 days post-milking the distended udder is noted in Figure 5D while the control animal (Figure 5C) has regressed normally. In this pair the treated animal produced 68.4 per cent as much milk as her control. In Pair Eight the udder of the treated animal remained distended for a relatively short period. Figures 6A and 6B of the control and treated animals, respectively, show the situation on the seventh day of the dry period. At four weeks, however, the udder of the treated cow, 198 has regressed further (Figure 6D) while the udder of control Cow 195 has not changed as much. The treated animal in this pair produced 61.7 per cent more than her control mate, a reversal of the effect noted in all other pairs.

Effect on colostrum. The results of the colostrum analysis are presented in Table III. No colostrum sample was collected from Cow 192, the control animal in Pair Two. An analysis of variance revealed a significant breed-treatment interaction ($P < .05$) for the percentage protein and total solids. The high percentage of protein characteristic of colostrum was found only in the two Jerseys, (Pairs Three and Seven) in the treated group. There was no significant breed-treatment interaction for milk fat or SNF. Likewise, there was no significant difference between treatments or breeds in percentage protein, milk fat, total solids, or SNF.

Effect on milk production. Milk production for the control and oxytocin treated groups for the first 22 weeks of lactation is shown in

TABLE III

PROTEIN, MILK FAT, TOTAL SOLIDS, AND SOLIDS-NOT-FAT IN COLOSTRUM OF CONTROL AND TREATED COWS

Pair	Protein		Milk Fat		Total Solids		Solids-not-Fat	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	(%)							
2	-----[a]	9.57	---[a]	2.9	-----[a]	16.47	-----[a]	13.57
3	10.65	12.75	1.8	6.5	17.25	22.30	15.45	15.80
5	14.40	6.43	5.9	4.5	24.37	15.63	18.47	11.13
6	12.55	5.30	2.3	2.2	19.53	12.14	17.23	9.94
7	9.55	12.57	4.0	4.4	17.49	20.41	13.49	16.01
8	8.89	6.43	3.5	5.3	16.80	15.33	13.30	10.03
Average	11.21[b]	8.84[b]	3.5	4.3	19.09[b]	17.05[b]	15.59	12.75

[a] No sample collected.

[b] Significant breed-treatment interaction ($P < .05$).

Table IV. In four of the six pairs a definitely lower milk production was observed from the treated cows. In Pair Two there was essentially no difference. In Pair Eight the treated animal had a markedly higher milk production than her control. Considerable difficulty was encountered locating the jugular vein in the treated animal during the injection period, but there is little reason to believe the injections were totally ineffective. Therefore, the atypical effect exhibited by this animal cannot be explained. It is noteworthy that not only was the treated animal's production above her control and her previous year's production, but also the control animal produced considerably less than in her previous lactation (Table I, page 16). The lack of significance between milk yields of the two groups is primarily attributed to Pair Eight. Excluding this pair would reveal a definite treatment effect.

Examination of the lactation curves during the experimental period (Figure 7) revealed that the average milk production of the control group was higher than in the previous year while the average production of the treated group was lower (Figure 1, page 19). A marked similarity was also noted between the treated group and the animals not given a dry period in Swanson's study (73), especially in the lack of a peak at the beginning of lactation.

Effect on milk constituents. The results of weekly milk analysis for milk fat, total solids, and SNF are summarized in Table V. Differences between groups for these constituents were not statistically significant.

TABLE IV

TWENTY-TWO-WEEK MILK PRODUCTION OF THE COWS TREATED PREPARTUM WITH OXYTOCIN COMPARED TO PAIRED CONTROL COWS

Pair	Group[a]	Cow Number	Milk Production
			(kg)
2	C	192	3114
	T	177	3149
3	C	T196	2489
	T	T192	789
5	C	220	4429
	T	175	3030
6	C	143	4337
	T	125	3632
7	C	L39	2383
	T	L34	1549
8	C	195	2630
	T	198	4266
Averages			
C			3230
T			2736

[a]C, control; T, treated with oxytocin twice daily in dry period.

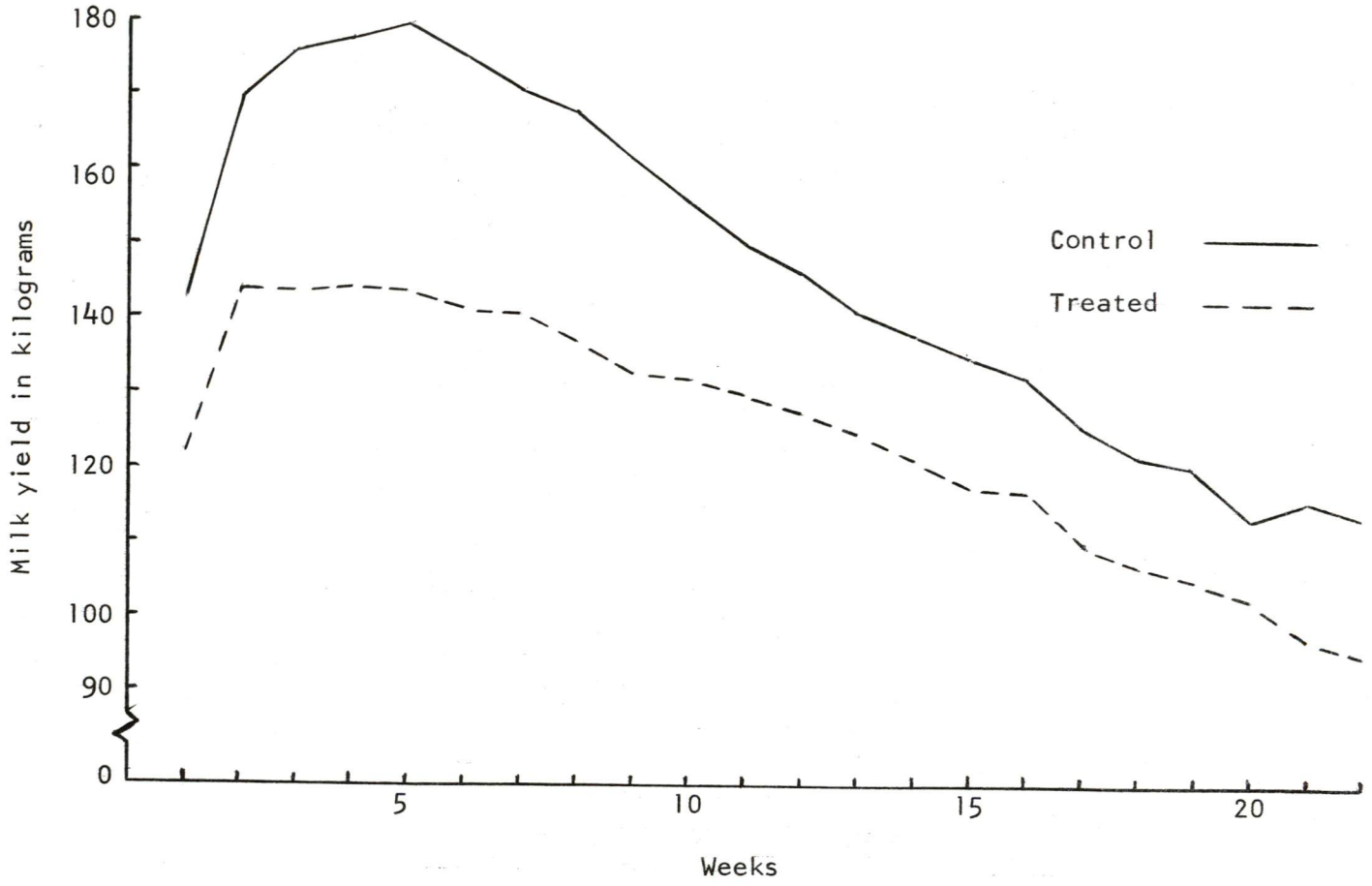

FIGURE 7

AVERAGE MILK PRODUCTION OF COWS USED
EXPERIMENTAL PERIOD 1965-1966

TABLE V

AVERAGE MILK FAT, TOTAL SOLIDS, AND SOLIDS-NOT-FAT COMPOSITION OF MILK FROM CONTROL AND OXYTOCIN TREATED COWS FOR TWENTY-TWO WEEKS OF LACTATION

Pair	Milk Fat		Total Solids		Solids-not-Fat	
	Control	Treated	Control	Treated	Control	Treated
	(%)					
2	3.9	3.7	12.74	12.62	8.85	8.89
3	5.7	5.2	15.34	14.72	9.60	9.48
5	3.6	3.6	12.24	12.48	8.60	8.90
6	3.3	3.1	11.65	11.25	8.38	8.18
7	4.9	6.1	14.20	15.84	9.26	9.73
8	3.5	3.5	11.90	12.07	8.40	8.57
Average	4.2	4.2	13.01	13.16	8.85	8.95

CHAPTER V

DISCUSSION

Results of these experiments indicate that oxytocin injected prepartum caused an inhibition of milk secretion after parturition. In Experiment I there was a significantly lower rate of gain in the pups of suckled rats which had been injected with oxytocin for a period of about seven days prior to littering than in litters of rats not injected. In Experiment II, except for one cow, the 22-week lactation records of six cows treated with oxytocin during the previoud dry period showed an average depression of milk production compared to their control mates. The reason for the reversed production records in one pair is not known but might have been due to adrenalin, caused by the difficulty of locating the jugular vein in the treated animal, dampening the effect of oxytocin. The unexpected low milk production of her control mate is also unexplainable since no disease or environmental disturbance was noted. The depressed milk production in the treated animals was similar to the results described previously in studies by Swanson (73) and Smith et al. (71) in cows not given a dry period.

The results from Experiment II would also indicate that the oxytocin treatment caused the udders to remain distended with milk for an extended period. Photographs in Figures 4, 5, and 6 (pages 27, 28, and 29) vividly illustrate this point. This effect is similar to that reported by Benson and Folley (6) and other workers (5, 7, 36, 48, 49,

51, 57) who found that injected oxytocin retarded mammary involution in rats after removal of their pups (cessation of milking) in early lactation.

The theory of Benson and Folley (6) that oxytocin stimulates the release of prolactin would appear to be validated by Experiment II. Prolactin is instrumental in the initiation of and essential to the maintenance of normal lactation. Twice daily injections of oxytocin for a period of two months would tend to deplete the prolactin stores in the pituitary thus creating a deficiency during the period of galactogenesis. The lack of a peak at the beginning of the lactation curve (Figure 7, page 34) and the depressed milk production in the treated animals could be interpreted as a lack of sufficient prolactin. However, Smith, Wheelock, and Dodd (71) found depressed milk production from the two milked quarters in a cow compared to the two quarters which were dried off normally, when all quarters could have had access to the same prolactin sources.

The results of the present experiments verify that oxytocin produced an effect on the subsequent lactation even without milk removal. Since, in the study by Smith, Wheelock, and Dodd (71), the hormonal supply for the whole udder was the same and a production depression was noted only in one-half, a hormonal action was discounted. They suggested an action as theorized by Meites and Hopkins (50) that oxytocin produced its effect by acting directly on the gland. The results of Experiment II presented in this report could substantiate this theory also. The effect could have been primarily an effect on the gland

epithelium. Alveolar constriction caused by oxytocin in itself could prevent normal preparation of the entire udder for the next lactation. This could be mediated by the failure of the epithelium to absorb or respond to the hormones involved in peak lactation. This would also explain the individual variation exhibited by the treated animals. The higher the degree of stimulation the more seriously inhibited would be the epithelium and, therefore, the lower would be the milk yield.

There are, however, other possible explanations for this phenomenon. Donker, Koshi, and Petersen (22), after using oxytocin to obtain residual milk in an experiment, found that after the oxytocin treatment was discontinued response to the usual milk ejection stimulus became essentially nonfunctional. It is possible then that the injected oxytocin made the animals refractory to normal endogenous release when injections were discontinued. This effect does not appear likely though since oxytocin is eliminated from the blood a short time after it is released, and release does not seem dependent on the level of oxytocin in the blood but rather on neurohumoral stimulation. Since the rate of gain in the treated rats was parallel to but consistently below the control group (Figure 3, page 26) and the milk production in the treated group of cows never reached the level of the controls (Figure 7, page 34), a mechanism based primarily on failure of milk ejection does not appear likely.

The possible action of oxytocin, either directly or indirectly, on storage or release of other hormones from the pituitary necessary for optimum lactation has not been disproved. The work of Smith, Wheelock, and Dodd (71) would cast doubt on this hypothesis. However,

there may be reason to believe that the control quarters could have produced more than they did had the milked ones been dried off also or that the results from the two animals used gave an atypical response to the treatment much as Pair Eight did in Experiment II.

It is recognized that this study was with a relatively small number of animals. The variation in effect observed indicates that animals may respond differently to oxytocin. A quantitative measure of the blood levels of key hormones in the animal and histological examinations of the mammary glands at intervals during the treatment would facilitate a more conclusive analysis.

CHAPTER VI

SUMMARY

Two experiments of the effects of oxytocin administered prepartum on the succeeding lactation were conducted. The first was with nine pairs of rats divided into a control group and a treated group which received oxytocin injections for about one week prepartum. Growth of the litters was used as an index of the milk production of the dam. The second experiment entailed oxytocin injections to a group of six cows throughout the dry period and a comparison of their milk production in the succeeding lactation with that of their control pair mates.

Results of these experiments indicated that oxytocin caused a depressed milk production similar to that found in cows not given a dry period. The growth rate of rat litters from dams receiving oxytocin was significantly lower than that of their controls. Five of the six treated cows showed a depressed milk yield compared to the controls. The treatment also inhibited normal regression of the udders during the dry period. It is believed that oxytocin caused a continued stimulation of the epithelium in the mammary glands of the treated groups and prevented them from becoming fully functional for the following lactation. An alternate explanation is that oxytocin affected the supply or effectiveness of galactopoietic and lactogenic hormones which are normally involved in establishing peak lactation.

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