EFFECTS ON DAIRY COWS OF FEEDING DIETHYLSTILBESTROL — AS MEASURED BY REPRODUCTIVE PHYSIOLOGY, AND THE YIELD, COMPOSITION, AND ESTROGEN CONTENT OF MILK

by

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INTRODUCTION

It has been a long recognized fact that dairy cows turned on to spring grass show an increase in yield of milk. No response was obtained in attempts by English workers (Bartlett, et al.,1948) to stimulate this galactopoietic effect of spring grass on milk yield by liberal supplements of nutrients to ordinary winter rations. These workers concluded that the stimulating effect which spring pasture had on milk production was due to some factor such as a hormone or vitamin. These same workers also demonstrated the presence of estrogenic substances in pasture crops. There is a lack of experimental work showing the definite effect of estrogenic materials on production and reproduction in dairy cattle.

In recent years, the feeding of diethylstilbestrol (a synthetic estrogen) supplemented feeds has become economically important to the beef industry (Burroughs, et al., 1955, Perry, et al., 1955). It is fairly well established that the feeding of stilbestrol in fattening rations to beef cattle results in more rapid and efficient gains (Burroughs, et al., 1955). Two things that have not been established concerning stilbestrol feeding to beef animals are - 1. the mode of action of this additive, 2. and its effect on the reproduction of the animals. These factors are of little practical importance in fattening animals, but the latter is of great concern in breeding animals.

This recent work with stilbestrol has renewed interest in the possibility that the administration of estrogenic materials to dairy cows might be profitable from the standpoint of increasing milk production.

This study was undertaken to obtain fundamental information on the physiological effects of feeding low levels of stilbestrol to dairy cattle.

REVIEW OF LITERATURE

An estrogenic compound is defined as "- any pure chemical substance which has the ability, when injected into adult ovariectomized mice or rats, to produce cornification of the vaginal epithelium similar to that occurring during estrus in the normal animal" (Allen and Doisy, 1924). According to Pope (1954), there are from 200 to 300 substances known which will satisfy this definition. He further states that these apparently behave quite similarly in exciting physiological reactions which are characteristic of estrogens, but that the various estrogens found in nature do differ greatly in potency. Dodds, et al. (1938) in 1936 synthesized a series of estrogenic agents from 4:4' dehydroxystilbene (stilbestrol), the most potent of which was 4:4' dihydroxy-alpha-beta-diethylstilbene. These workers named this synthetic estrogen diethylstilbestrol.

The similarity in responses by the animal body to all of the estrogenic substances known, whether synthetic or natural, has led to the use in literature of the term estrogen when referring to any chemical compound of estrogenic nature (Pope, 1954). The term stilbestrol is used interchangeably with the term diethylstilbestrol in modern usage (U.S. Pharmamacopeia 1950) and will be used in this thesis when referring to diethylstilbestrol.

Estrogens may be conveniently grouped into two classes, 1. endogenous, those produced normally in the physiological functions of the body and, 2. exogenous, those produced outside the body, either synthetically or naturally (Pope, 1954).

The estrogens which are normally found in the animal body are those secreted by the ovary (e.g. estradiol, estrone, estriol) (Dukes, 1947).

Pope (1954) has listed the main physiological functions of estrogen as the control of the estrus cycle, growth and function of the female accessory reproductive organs, and development of the mammary gland.

Effects of Estrogen Administration on Initiation of Lactation

Implantation. A great deal of the research on the effects of synthetic estrogens in domestic animals has been on the initiation of lactation in virgin or barren animals. Folley and Malpress (1944a) compared the effects of stilbestrol and mesohexestrol in promoting lactation when implanted subcutaneously in the neck of 19 heifers, nine cows, and two free martins. Implants of either 2.5 or 5.0 grams were used, and attempts to obtain secretions were begun on the tenth day after implantation. The animals were milked once a day until production reached five pounds, and then twice a day milking was started. These workers found much variation in response, both in degree and rate of udder development, and in degree, time of onset, and duration of lactation. Maximum daily yields varied from 0.5 to 30 pounds. In their studies stilbestrol and hexestrol were equally effective in initiating lactation, and implants of 2.5 grams were as effective as those of 5.0 grams for the initiation of production. The mean daily absorption of the synthetic estrogens ranged from 8.0 to 23.9 mg.; there was no advantage to implants that gave more than 12 mg. of absorption per day. These workers also found that there was no advantage in leaving the estrogen implant in situ after milk production had reached its peak; on the contrary, removal of the tablet at this point frequently caused a sudden rise in production. Day and Hammon (1945) also found a great deal of variation in the response of heifers to stilbestrol implants. Three groups in this experiment came into production one to six weeks post implantation. Folley et al. (1945) found that injections of anterior pituitary extract into heifers following the removal of estrogen implants caused a sharp rise in production to a peak higher than obtained with the implant alone. The heifers brought into artificial lactation received 200 ml. of anterior pituitary extract during a 40 day period at a time when the lactation curves began to flatten out. The authors point out that the rise was possibly due primarily to removal of the tablets. Lewis and Turner (1942) reported that anterior pituitary extract was effective in augmenting production artificially induced by stilbestrol implants.

Injections. In work with virgin dairy goats, Eaton and Simmons (1953) found that production was highly variable among individuals brought into lactation by subcutaneous injections of 0.5 mg. of stilbestrol in one ml. of oil three times per week. Goats 13 to 16 months of age were used, and were bred as soon as they came into heat. In general, the animals that were the best producers during the induced lactation were also the highest producers during the subsequent lactation.

Inunction of Udder. Virgin animals, both dairy cattle and goats, have been brought into lactation by inunction of the udder with an ointment containing stilbestrol. Folley et al. (1941a) initiated lactation in two dairy Shorthorn heifers 18.5 and 20 months of age respectively, by inunction of the udder with 5.0 grams of a one percent stilbestrol ointment applied three times a week. Treatment was quickly followed by secretion of an opaque fluid to a peak of 80 ml. per day. The yield gradually dropped to 20 ml. per day and remained at this level during inunction with an inactive ointment. The yield rose again when treatment at one-half the original quantity was used. When inunction with stilbestrol ointment was then discontinued, the yield rose further to a peak of 170 ml. per day and slowly declined as in a normal lactation.

In an experiment with virgin female goats, Folley, et al. (1940) induced secretion to a maximum yield of 500 ml. per day. One gram of 1 percent stilbestrol dipropionate ointment was applied three times a week to initiate secretion. After 30 days of a few ml. secretion per day, there was a sudden increase to the maximum and then a slow decline. The yield curve in these goats resembled a normal curve. When stilbestrol was omitted from the ointment, there was no effect on the rate of decline for approximately 20 days; then there was a sharp drop in production that slowly came back to the previous level when stilbestrol treatment was reinitiated.

Oral Administration. Lewis and Turner (1942) reported that estrogens orally administered are less effective in the initiation of lactation than were injections or implantations. Results of experiments with seven virgin heifers and one heifer that had aborted, when various levels and methods of oral administration were used has been reported by Folley and Malpress (1944b). Levels ranging from 20 to 100 mg. per day of stilbestrol, hexestrol and dienestrol were administered orally by incorporation into the feed and by addition to the drinking water. Treatment lasted for an average of 15 weeks with maximum daily yields ranging from 4.25 to 18 pounds per day. The type of oral administration seemed to make little difference in response by these groups of animals. Furthermore, there was no strict relationship between dosage and response. They concluded this was possibly due to the inherent capacity of animals to respond to treatment of estrogenic compounds. In a group of 32 virgin Ayrshire heifers receiving stilbestrol orally, those receiving 200 mg. per day began secretion sooner than those receiving 50 mg. per day. Increasing the daily dosage from 50 to 200 mg. per day in heifers which did not respond to ten weeks of the lower level treatment did not help; but substitution of 300 mg. for 50 at 10 weeks did produce positive results (Folley and Malpress, 1944b).

Levels Necessary for Initiation of Lactation

There seem to be definite levels of estrogen administration necessary to induce lactation in animals. It has been determined by Mixner, et al. (1944) that 0.25 mg. per day of stilbestrol injected subcutaneously into goats would initiate lactation, while 1.0 mg. per day was slightly inhibitory, and 2.0 and 4.0 mg. per day were definitely inhibitory. Inhibition seemed to be temporary since recovery in rate of production was effected when treatment ceased.

Composition of Milk from Animals in which Lactation was Artificially Induced

Folley and co-workers (1941a) found that the secretion from two heifers in which lactation was artificially induced, was never the same as normal milk even though the daily secretion was as high as 170 ml. daily. This "milk" contained less casein and more globulin than normal milk. At times, fat approached normal but solids-not-fat were usually lower than normal. Folley and Malpress (1944c) later found that a daily yield of five pounds, sometimes much less, could be regarded as assurance that the secretion was of normal composition. These results were obtained from seven heifers receiving synthetic estrogen implants and 16 heifers receiving estrogens orally. The initial secretions of these heifers were colostral in nature, with a gradual change in composition to that of normal milk, depending more upon the rate of increase of milk yield than upon the duration of estrogen treatment. Unlike the major milk constituents, phosphatase activity was unrelated to the amount of secretion. The phosphatase content rapidly decreased after the first withdrawal of milk, regardless of yield.

Effects of Stilbestrol on Normal Lactation

In the earlier work of Folley (1936a), four Guernsey cows just past the peak of lactation were used to determine the effect of intramuscular injections of estrogenic hormones on milk production. One cow received 500 mg. of estrone during a three day period plus 50 mg. of estradiol benzoate; another cow got five consecutive daily injections of 15 mg. of estradiol benzoate. The other two cows were used as controls and received injections of oil without apparent effect to milk production. Treatment of these two cows caused a slight and temporary decrease in daily milk production, accompanied by a striking increase in phosphatase of the milk, a ten percent increase in solids-not-fat, and a slight increase in total nitrogen, calcium, and lactose.

In other early work (Folley 1936b), temporary inhibition of milk secretion was shown to be the result of high level administration of estrogens. In this experiment three Guernsey cows were used, and were given injections ranging from 87 to 550 mg. of estrogenic hormones over a short period of time. The author concluded that the drop in production seemed to depend on the attainment of a high level of estrogens in the blood. There was an increase in the percentage of fat and solids-not-fat in the milk. This lasted for two to three months in the cows receiving high dosages. The treatment also caused a temporary fall in the blood serum calcium, quickly followed by a temporary considerable increase in blood serum phosphatase.

The effects of synthetic estrogens on rate of milk production and on the composition of milk of cows in normal lactation have been studied to a limited extent. Results of research by Folley and co-workers (1941b) are given in the following tabulation.

Method of administration	Experimental animals used	Stage of lactation (month)	Total dosage	Response
Oral	1 Shorthorn	3	1 gm. of stilbestrol in 100 ml. H_2O	No marked effect on production or composition of milk.
Subcutaneous injection	1 Shorthorn	4	5 daily injections of 50 mg. per day in oil of stilbestrol dipropionate	Slight increase in solids-not-fat with a slight temporary decrease in yield.
"	Shorthorn	11	400 mg. injection in oil of stilbestrol dipropionate	Increase in butterfat and S.N.F., production dropped rapidly and cow dried up.
"	Shorthorn	1	498 mg. of stilbestrol dipropionate in oil.	Increase in butterfat and S.N.F., lasted approximately 20 days. Yield declined from 27 lb. at start to 10 lb. 35 days later.
"	Shorthorn	2	1 gm. of stilbestrol in 80 ml. of H_2O.	Increase in S.N.F. which lasted 180 days, yield not affected.
"	3 Ayrshires	2	1 gm. of stilbestrol in 80 ml. of H_2O.	Increase in S.N.F. with progressive decrease in yield.
"	Shorthorn	2	375 mg. of stilbestrol in 22 ml. of H_2O	Slight increase in S.N.F. which lasted about 20 days, slight decrease in yield.

Method of administration	Experimental animals used	Stage of lactation (month)	Total dosage	Response
Subcutaneous injection	Shorthorn	2	225 mg. of stilbestrol in H_2O	No change in butterfat S.N.F., or yield.
Percutaneous inunction of udder	Shorthorn	9	8 applications at 4 day intervals, total of 400 mg. stilbestrol dipropionate.	Sharp increase in S.N. slight increase in but fat, no change in yiel effect lasted only as as treatment and retur to pretreatment level cessation of treatment
"	4 Holsteins	Two cows in mid pregnancy; and two in advanced pregnancy	Total of 25 mg. of stilbestrol in 15 days.	No change.
"	Same animals as above		Total of 60 mg. of stilbestrol in 14 days.	No change.
"	Same animals		Total of 40 mg. of stilbestrol in 2 days	The two animals in advanced pregnancy abort no change in butterfat S.N.F., or yield.
"	Guernsey	10	200 mg. of stilbestrol in 21 days	No change
			650 mg. of stilbestrol in 41 days	Increase in S.N.F. fro mean level of 9.0 perc to 9.3 percent. Production dropped from 1 lb. to 15 lb. per day during first days of h treatment, soon return to mean level of 18 lb per day.
Implantation subcutaneous in neck	Shorthorn	8	900 mg. of powdered stilbestrol	Striking increase in S percentage all during servation (96 days), n effect on yield.

In the three cases shown in the preceding tabulation where there was an increase in the butterfat and solids-not-fat and no appreciable decrease in yield, the total amount of fat and solids-not-fat secreted during a 14 day period after the beginning of treatment was greater than that secreted during a corresponding pretreatment period. In these three cases, the increases were 13.2, 28.6, and 14.3 percent, respectively, in the amount of fat produced during the treatment period as compared to the 14 day pretreatment period. Also, there were increases of 4.5, 9.4, and 8.5 percent respectively, in solids-not-fat produced during the period of treatment. The average differences in yield of fat-free milk, produced during the period of treatment, expressed as percent of the mean level before treatment were + 0.7, -6.2, and -6.4, respectively. The authors pointed out that the enrichment of the milk in these three animals represented a true increase in the amount of solids secreted, and not merely a concentration due to a reduced secretion of water.

Spielman, et al. (1941) gave intramuscular injections of stilbestrol dissolved in cottonseed oil and obtained a marked increase in the butterfat and lactose percent of 1.0 to 1.5 with no apparent effect on level of milk production. These results were obtained in an experiment with four cows receiving from 10 to 100 mg. of stilbestrol at irregular intervals for 30 days. Total protein and casein protein in the milk of these cows were not affected by the treatment. There was a marked rise of 100 mg. percent in the level of blood fat, and a significant increase in blood sugar during the period of treatment. The increase in butterfat percentage began to decline about 48 hours after termination of injections, and returned to normal within five days. The increase in lactose secretion persisted for a longer time as did the increase in blood sugar and fat. The authors concluded from this experiment that between 50 and 150 mg. of stilbestrol was necessary to give the above results.

Mode of Action of Estrogens

According to Folley (1941), the level of administration of estrogens is the most important factor in their effect on lactation. Folley also postulated that small dosages appeared to activate the anterior pituitary to produce the hormones that govern lactation, while larger dosages seemed to suppress the production of these hormones. It has also been shown by Mixner et al. (1944) that the degree of inhibition of lactation is proportional to dosage of estrogens. Folley and co-workers (1941) in their experiment on the chemical enrichment of milk by administration of stilbestrol demonstrated, in all cases that when milk yields dropped the solids-percent increased, but the reverse of this did not hold true. They postulate from this that the threshold dosage for inhibition apparently is higher than for enrichment. In some of his earliest work, Folley (1936b) suggested the possibility that estrogen hormone treatment in cattle resulted in an actual increase in the efficiency of the mammary gland cells to synthesize milk constituents. Basset and White (1955) in explaining the stimulating effect of spring grass on milk secretion postulated that the stimulation may be due to increased endogenous estrogen caused by the sudden change in feeding. Several authors (Mixner, et al., 1944, and Folley and Malpress, 1944a) suggested that the initiation and maintenance of lactation and the lactation-stimulating effects of small dosages of estrogenic compounds are due to stimulated secretion of lactogenic hormone by the anterior pituitary. Work by Meites and Turner (1942), and Lewis and Turner (1941) showed that the increase of the lactogenic hormone content of the pituitary resulting from stilbestrol treatment might also be taken as evidence that this theory is true. Folley and Malpress (1944a) suggested that inhibition of lactation, and partial or complete failure of lactional response in stilbestrol treated animals might be explained on the basis of a lack of pituitary lactogenic hormone due, perhaps to a general hypoactivity of the antêrior pituitary caused by the excessive or too-prolonged action of estrogens. This hypothesis is supported by the experiments of Folley and Young (1941), and Folley, <u>et al.</u> (1945), who found that injections of anterior pituitary extracts would further increase the peak yield of goats and cows in which lactation had been artificially induced by stilbestrol administration. That there is an inactivation of the anterior pituitary also might be shown by the fact that in most instances of stilbestrol administration, there is an inactivation of the ovaries, indicating a decrease in the secretion of the follicle-stimulating hormone and the luteinizing hormone. (Folley and Malpress, 1944a, Folley, <u>et al.</u>1944, and Marshall, <u>et al.</u> 1948). This theory of inhibition is more or less contradicted by the work of Meites and Turner (1942) who found that large dosages of stilbestrol increased the lactogenic hormone content of the pituitary in the laboratory rat, but Folley and Malpress (1944a) suggested this is true only if the observed increases indicated increased secretion and not merely an inhibition of its release. Mixner, <u>et al.</u> (1944) explain inhibition of lactation in stilbestrol treated animals on the basis of increased adrenal-cortical activity resulting in an increased rate of deaminization of the nitrogenous precursors of milk protein. However, Whitehair, et al. (1953) and Clegg and Cole (1954) have reported an increase in nitrogen retention effected through a marked decrease in the amount of nitrogen excreted in the urine in both sheep and beef cattle receiving stilbestrol.

Tepperman, et al.(1943), working with laboratory rats, have shown that stilbestrol administration causes striking adrenal hyperactivity mediated through the adrenotropic hormone of the anterior pituitary which in turn causes an increased nitrogen excretion and an increase in the liver nitrogen, glycogen, and weight. These authors state that all of these factors are evidence of increased adrenal-cortical activity, and in protein catabolism resulting in suppression of growth and milk secretion.

Since estrogen administration seems to exert an influence on yield and composition of milk, a cow in estrus might be expected to be affected by the increase in endogenous estrogens during this period, but this does not appear to be the case. Copeland (1929), through a comparison of ROM records of the American Jersey Cattle Club, found that estrus had no noticeable effect on total yield and butterfat percentage of milk. However, Folley and co-workers (1941a) have reported a regular fluctuation of milk yield in heifers brought into lactation with stilbestrol, which was correlated with the estrous cycle. The minimum secretion occurred at or near estrus and the maximum about seven days later.

Effectiveness of Orally Administered Estrogen to Ruminants

Mixner, et al. (1944) stated that the oral administration of stilbestrol in small experimental animals and in man has been quite effective, compared to subcutaneous administration. Hannan (1928) found that the estrus producing hormone was effective in rats when administered orally. Folley and Malpress (1944b) concluded that of the estrogens administered orally to bovines only about five to ten percent are utilized. These workers believed that large amounts of synthetic estrogens fail to enter the systemic circulation, due either to destruction in the rumen or failure to be absorbed, or absorption into the portal circulation and inactivation by the liver. Mixner, et al. (1944) surmised that stilbestrol administered orally to ruminants is only about one percent as effective as that administered by subcutaneous injection. Using cows receiving 10 mg. of stilbestrol per day, Turner (1956) showed the presence of estrogens in the feces, while none was found in feces from cows getting no stilbestrol.

Reproductive Performance of Stilbestrol Treated Animals

Data on virgin goats brought into lactation by injections of 1.5 mg. of stilbestrol per week failed to show any harmful effects upon their subsequent breeding performance (Eaton and Simmons, 1953). Lewis and Turner (1942) reported that in goats receiving 0.25 mg. of stilbestrol per day for long periods of treatment, there was no affect on the normal behavior of the ovaries. They also found that treatment during the last half of pregnancy seemed to cause no harmful effects. Several workers (Folley and Malpress, 1944a , Folley, et al., 1944, and Marshall, et al., 1948), found that the ovaries of cows became inactive shortly after initiation of treatment with stilbestrol, either by injection of 170 to 530 mg. or by implantation of 0.5 to 5 grams. Most of the animals included showed irregular sexual excitation unaccompanied by ovarian function. Also, there were symptoms of nymphomania such as raised tail head, relaxation of the pelvic ligaments, swelling of the vulva, and vaginal discharge exhibited by all the treated animals to varying degrees. Folley and Malpress (1944a), and Folley, et al. (1944) found that multiple large follicles or cysts occurred in one or both ovaries upon removal of the implanted stilbestrol tablet. This, in the majority of cases, was followed by a return to normal cyclic functioning of the ovaries. Parks and Bellerly (1926) found that injections of estrin during early pregnancy invariably caused the rapid reappearance of estrus and termination of the pregnancy of laboratory animals. The amount of estrin required for these results was variable, but approximately twice as much was required if the animals were in late pregnancy. Relatively large dosages of orally administered estrogenic compounds seem to be required to cause abortion in cattle. Folley, et al. (1941b) gave a subcutaneous injection of 1 gram of stilbestrol dipropionate in 22 ml. of water to each of three pregnant Ayrshires, causing two to abort within five weeks and the other to give birth to an eight month fetus. These workers also found some cows difficult to get with calf after various stilbestrol treatments.

Absorption and Metabolism of Estrogens

One of the major questions involved in the administration of estrogenic substances to animals is the fate of the substances after they are in the body. Cantarow and co-workers (1942) found that natural estrogens disappeared rapidly from the blood and urine after injection into normal animals. These workers postulated that this rapid disappearance of estrogens was caused by their inactivation or destruction in the liver with subsequent excretion principally through the bile.

Zondek and Sulman (1939) found that in contrast to the natural estrogens, stilbestrol was rendered inactive only to a small extent in laboratory animals. Large amounts of the active substance were found in the excreta, particularly the urine, after administration. Cantarow et al. (1942) found that, after administration of stilbestrol, only about one-half as much estrogen could be recovered from the bile as was recovered after administration of natural estrogens.

Yaida, as quoted by Muench (1954), postulated that estrogens are excreted into the milk, and that the content increases during gestation. Muench also stated that Letard, Szumowski and Langlois believed the mammary gland to be an outlet for estrogens administered for therapeutic purposes. Muench was able to extract estrogens from the milk of pregnant and non-pregnant cows. He stated that the estrogen level of milk fluctuates with the stage of the estrous cycle and increases during gestation.

If administered estrogens are excreted by way of the mammary gland, then it seems probable that they would appear to the greatest extent during the later stages of gestation since at this time there is an increase in the excretion of natural estrogen, according to Levin (1945), Smith et al. (1956), and Turner et al. (1930). Bovine colostrum from untreated animals has been shown by Pope and Roy (1953) to contain estrogenic activity. However, these same workers failed to show the presence of estrogens in normal milk.

Lawson, et al. (1944) extracted very large amounts of estrogen from the urine of cows receiving synthetic estrogens, and very small amounts from the blood and milk. They suggested that the high concentration of estrogens found in the urine and the low concentration found in the blood indicated that little estrogen is excreted in the milk.

Estrogens from Vegetative Sources

The occurrance of widespread breeding disorders in ewes, apparently caused by estrogens in the clover pasture which they grazed, has been reported in Australia (Pope 1954). The disorders had three main manifestations; female infertility, dystocia or uterine inertia and prolapse of the uterus. Lambing in some flocks dropped to 8 percent of the mated ewes. The problem occurred almost exclusively in sheep grazing the Dwalganup strain of subterranean clover.

Biggers and Curnow (1954) were able to prepare estrogenic extracts of a phenolic nature from subterranean clover. Curnow, according to Pope (1954) later showed that the amount of genistein (plant estrogen) found in subterranean clover of the Dwalganup strain could be responsible for the estrogen activity of the plant and, therefore, for the infertility in sheep grazing on subterranean clover pastures.

In a recent article, Pieterse and Andrews (1956a) reported that approximately 50 species of plants have been shown to possess varying degrees of estrogenic activity. These workers found alfalfa cut in various stages of growth to contain from less than 0.5 micrograms of estrogen per pound to 4.3 micrograms per pound. Pieterse and Andrews (1956b) have also demonstrated that the estrogenic content of alfalfa silage is greater than that of the original plant or of hay from the same source and concluded that estrogenic activity of alfalfa increases during fermentation.

Effects of Stilbestrol on Beef Cattle, Sheep, and Swine

Recent work has shown that both natural and synthetic estrogens when administered to beef cattle and sheep are growth stimulatory (Bell, et al., 1954; Clegg and Cole, 1954; Perry et al., 1955; and Burroughs et al., 1955). In addition to increased gains such physiological and anatomical effects as increased feed efficiency, increased nitrogen retention, mammary growth stimulation, increased sexual excitation, changes in the pelvic region of the body and lowered carcass quality have been reported (Clegg and Cole, 1954; Andrews et al., 1954; Hale et al., 1955; and Beeson et al.,1955). Andrews et al.(1954) reported that along with increased gains over controls by implantation of either 60 or 108 mg. pellets of stilbestrol, steers and heifers exhibited elevated tail heads, increased sexual behavior and slight to marked mammary stimulation. Bell et al. (1954), using 15 mg. implants of stilbestrol, increased significantly the rate of gain in feed-lot lambs. They also reported that 12 of 517 lambs were lost from prolapse of the rectum and from excessive swelling and inflammation in the rectal or perineal region.

It seems that the detrimental side-effects associated with stilbestrol administration are lessened or eliminated, by oral administration of the synthetic estrogen. Burroughs et al.(1955) observed no undesirable side-effects in heifers or steers receiving either 5 or 10 mg. of stilbestrol per day orally. However, these animals showed an average increase in weight gain of 20 percent over the control animals. Perry et al. (1955) reported slight, but recognisable pelvic and mammary changes in steers receiving 10 mg. of stilbestrol orally per day. Hale et al. (1955) reported that wethers fed 2 mg. per day of stilbestrol showed an increase in gain of 22 percent over controls, with no observable detrimental side-affects; however, wethers fed 3.6 mg. per day had a lowered carcass quality and exhibited to a slight extent some of the characteristic side effects.

Both male and female swine, receiving 2 mg. of stilbestrol orally per day showed clear cut mammary stimulation, and the females showed also an enlargement of the vulva (Beeson et al. 1955). Growth rate and feed efficiency was not improved in this group of animals. Sleeth and co-workers (1953) reported that pigs receiving 1.0 mg. of estradiol per kilogram of body weight by intramuscular injection semi-weekly did not show an increase in rate of gain over controls.

Brooks et al. (1954) found that several steroid compounds, including estrogens, stimulated cellulose digestion in vitro. They also reported that the coefficients of digestion of cellulose and protein were increased 16 and 18 percent, respectively, in yearling wethers that received 10 to 20 mg. of stilbestrol per day. Other workers, (Whitehair et al. 1953) have found no effect on digestibility of the major nutrients in a lamb ration, other than a slight increase in digestibility of the crude fiber, when the lambs were administered 24 mg. of stilbestrol by subcutaneous implant. There was however a marked increase in retention of calcium, phosphorus, and nitrogen. Clegg and Cole (1954) found an increase in efficiency of feed utilization, and nitrogen retention by steers receiving stilbestrol implants. Burroughs et al.(1955) found steers receiving stilbestrol orally had an increased feed consumption of approximately 5 percent, whereas their feed requirement for producing gain was reduced 11 percent.

Mode of Action of the Growth Stimulating Effect of Stilbestrol

In search of the mode of action of stilbestrol in increasing gains in livestock, Clegg and Cole (1954) made gross and histological studies of some of the endocrine glands of animals that received either 60 or 120 mg.

implants. These workers found an increase in size of the pituitary, adrenal, and thyroid glands taken from treated steers, and an increase in size of the pituitaries and adrenals in treated heifers. The growth of the thyroid glands of treated heifers, however, was depressed. These workers stated that there seemed to be a greater number of acidophils in the hypophysis of both heifers and steers among the treated groups. Histologically, the adrenals were not grossly different from the controls; however, there was an increase in the width of the cortex. This change in the adrenals is substantiated to a certain extent by work of Allen and Bern (1942) and Tepperman _et al._, (1943) who reported that administration of estrogens to laboratory animals caused a striking hypertrophy of the adrenals with an enlargement of the adrenal cortex. Clegg and Cole (1954) also reported a marked eosinopenic response following stilbestrol treatment in both wethers and ewes that had been implanted with 12 to 24 mg. pellets. They theorized that since a depression in blood eosinophils is related to an increase in adrenal cortical activity, stimulated by an increased secretion of adrenocorticotrophic hormone, it appeared that stilbestrol caused an increased release of this hormone from the pituitary of ruminants, thus bringing about adrenal cortical stimulation. Tepperman, _et al._ (1943) surmised that this same chain of events happened in laboratory animals following estrogen administration, leading, however, to increased protein catabolism and suppression of growth.

Estrogenic Activity of Animal Tissue After Treatment of Animal with Stilbestrol

Several investigations have been made to determine whether or not stilbestrol, or its metabolites, were deposited in tissues of treated animals.

Beeson et al. (1955), Perry et al. (1955), and Preston et al. (1956) have been unable to detect estrogenic activity in the meat of treated animals. However, Stob and co-workers (1954) were able to measure slight estrogen retention in various meat samples from beef steers, lambs and chickens that had received stilbestrol treatment. Turner (1956) reported no detectable amounts of estrogen in the liver, heart, spleen and brain of steers receiving 10 mg. of stilbestrol orally per day for 148 days. He did, however, find the kidneys, and lungs of these animals to contain measurable amounts of estrogens.

EXPERIMENTAL PROCEDURE

The experiment was divided into two phases, phase I using cows in the dairy herd at Kansas State College and phase II, using identical twin cows.

The cows used in phase I were scheduled to be sold because of poor type, low production, or breeding troubles. The objectives of phase I were to determine the following:

1. The effects of stilbestrol on reproductive functions.
2. Effective levels of stilbestrol administration.
3. The optimum time of treatment during lactation.
4. Whether stilbestrol would be excreted into the milk.
5. The effects of stilbestrol administration on the yield and composition of milk.
6. The effects of stilbestrol on anatomical structures of treated animals.

Phase II of the experiment with identical twin cows was designed primarily to give positive controlled information on total yield and composition of milk although the other factors listed above were also considered.

Phase I - Herd Cows

Animals Used. Seventeen cows, some nonpregnant, and others in various stages of gestation and lactation were used in the Phase I. These cows were selected to fit into one of the following categories:

1. Non-pregnant cows - producing 30 pounds or more of milk daily.
2. Pregnant cows - " " " " "
3. Non-pregnant cows - producing less than 30 pounds of milk daily.
4. Pregnant cows - " " " " "

These cows were divided into four groups as shown in the following table:

Table 1. Description of the cows used in phase I during the first experimental lactation.

Group number	Level of stilbestrol fed per day (mg./1000 lb. body wt.)	Identification number of cows	Breed	Production at start of experiment (lb. 4 percent F.C.M. per day)	Days in lactation at start of experiment	Days pregnant at start of experiment
I	5	125B	Holstein	52.6	35	0
	5	279A	Ayrshire	22.8	188	0
	5	123B	Holstein	28.9	357	150
	5	126B	Holstein	23.3	296	150
II	10	112B	Holstein	31.2	205	97
	10	482A	Guernsey	26.2	121	0
	10	277A	Ayrshire	23.0	290	96
	10	388B	Jersey	29.5	121	0
III	15	419B	Guernsey	40.5	28	0
	15	354B	Jersey	27.9	30	0
	15	197A	Holstein	27.1	200	90
IV	0	127B	Holstein	27.0	187	100
	0	180A	Holstein	46.7	70	7
	0	268A	Ayrshire	22.2	222	104
	0	290A	Ayrshire	40.0	65	0
	0	423B	Guernsey	20.5	98	35
	0	437B	Guernsey	23.9	253	0

Eight of the cows used in phase I were slaughtered after the start of the experiment, to provide anatomical, histological and reproductive data. Five of these animals received stilbestrol, and the other three were control cows. One treated animal was slaughtered 85 days after the start of the experiment, and the other four treated animals and two controls were slaughtered 113 days after the initiation of the study. The other control cow was slaughtered 8.5 months after the start of the experiment. The nine remaining cows of the original seventeen have all calved; the treatment they received during their second lactation on the experiment is shown in the following table.

Table 2. Description of the cows used in phase I during the second experimental lactation.

Identification number of cows	Breed	Level of stilbestrol during 1st experimental lactation (Mg/1000 lb. wt./day)	Treatment level during dry period (Mg./1000 lb. wt./ day	Level of stilbestrol during second experimental lactation (Mg. / 1000 lb. wt./ day)	Days in lactation at start of treatment	Days pregnant at start of 2nd treatment
125B	Holstein	5	0	0	-	-
123B	Holstein	5	0	20	82	0
126B	Holstein	5	5	0		
112B	Holstein	10	10	15	100	0
277A	Ayrshire	10	0	10 for 105 days then increased to 20	20	0
197A	Holstein	15	15	10 for 105 days and then none	95	9
127B	Holstein	0	0	0	-	-
180A	Holstein	0	0	0	-	-
268A	Ayrshire	0	0	10 for 105 days then increased to 15	30	0

Management of Experimental Animals. The cows in phase I received regular herd treatment, except that they were kept in a separate lot to insure full feeding of alfalfa hay, and they received no pasture. An examination of the reproductive tract by rectal palpation was made on each cow at the start of the experiment. The cows were artificially bred as soon as they came into heat after the start of the experiment, provided they had calved at least 60 days previously.

All indicated veterinary treatment for both groups of cows was administered by the college veterinarian assigned to the College herd.

Data Collected. Production. Daily milk weights were recorded, and each 14 days the milk produced during a 48 hour period was sampled for analyses. The following determinations were made on the composite samples:

Butterfat:	Babcock method
Solids-not-fat:	Mojonnier method

Milk from cows receiving 15 mg. of stilbestrol per 1000 pounds of body weight was sampled at irregular intervals for biological assay of estrogens. The tests for butterfat and solids-not-fat were made on fresh samples, and the milk extracted for estrogen assay was frozen until used. The method used in preparing the estrogen extracts was that described by Pope and Roy (1953), except that 100 ml. of milk was used instead of 50 ml. The biological assay of the extracts was made by the Department of Zoology at Kansas State College, employing the method of Evans, et al. (1941) with modifications including the use of ovariectomized mice injected once daily for four days with 0.1 cc. of an oil solution containing the extracted material. Seven mice were used for each assay. They were killed and the uteri weighed forty-eight hours after the last injection. Several methods of biological assay were tried before it was decided to use the extraction method of Pope and Roy (1953), and the assay method of Evans, _et al_.(1941). These methods are as follows:

1. Milk sample condensed and dried. The powdered milk was force-fed with a pipette to chicks and the oviduct weight used as a basis of measurement of stimulation.
2. Milk sample condensed, and the condensed milk (not dried) fed to chicks.
3. Milk sample condensed, powdered, and fed to ovariectomized mice. An increase in the uterine weight was used as a criterion of measurement of stimulation.
4. Milk sample condensed and 2000 ml. extracted by the method of Lawson, _et al_. (1944). The extract was dissolved in olive oil and injected into ovariectomized mice and the uterine weights used to measure stimulation.

Feed Weights. Except for concentrate mixture, feed weights were not recorded for the cows in phase I.

Weight. Each cow was weighed on three consecutive days at the beginning of the experiment, and thereafter on two consecutive days at the end of each 14 days. If weights on two successive days varied more than 20 pounds, the cow was reweighed on the next day, and the average of the two closest weights was recorded.

Reproductive Physiology. Reproductive tracts of the cows were examined per rectum at weekly intervals, for approximately 60 days, following the initial palpation prior to the beginning of stilbestrol feeding, and then at monthly intervals since there was no apparent change in regular cycling.

The data recorded were as follows:

1. Apparent size of ovaries
2. Presence, absence, apparent size and position of follicles and corpora lutea
3. Approximate size and tone of uterine horns.
4. Apparent size and tone of cervix
5. Apparent development of fetus

Cows were observed daily for indications of estrus. Any abnormal behavior such as nervousness, bellowing, attempts at mounting other cows, or willingness to stand to be mounted were recorded.

The vulva was examined daily for appearance of mucous discharge, hypervascularization and swelling of the labia majora.

Photographs of all animals were made at the beginning of the experiment as an aid in determining any change in physical appearance of the animals that could be attributed to stilbestrol administration.

The reproductive tracts, adrenal glands and pituitary glands were removed from all the animals which were slaughtered. Gross and histological studies of the tracts and glands were made to determine any apparent abnormalities.

Feeding Methods. All of the cows in phase I were fed average quality alfalfa hay ad libitum and sorgo silage at the rate of approximately three pounds per 100 pounds of body weight. An 18 percent crude protein concentrate mixture of the following formula was fed at a rate of one pound for each 3.5 pounds of 4 percent fat-corrected-milk produced daily.

350 lb. ground milo

200 lb. ground oats

300 lb. bran

150 lb. soybean meal

20 lb. salt

10 lb. bonemeal

Four percent fat-corrected milk was calculated according to the method of Gaines and Davidson (1927).

Stilbestrol was furnished in "Stilbosol" [1], a concentrate containing 1 gm. of diethylstilbestrol per pound.

Sufficient "Stilbosol" to furnish the desired amount of stilbestrol was used as a replacement for part of the concentrate mixture. The formula of the stilbestrol-containing feed is listed below.

400 lb. ground corn

200 lb. ground oats

96.43 lb. soybean oil meal

3.57 lb. "Stilbosol"

7 lb. salt

7 lb. steamed bonemeal

This mixture was formulated to furnish 5 mg. of stilbestrol per pound of concentrate. Samples of the grain mixture containing "Stilbosol" were taken at regular intervals and sent to Lilly and Company for chemical assay of the stilbestrol content. These samples were found to contain the following amounts per pound:

Sample 1. - 5.08 mg.
" 2. - 4.7 mg.
" 3. - 5.26 mg.

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[1] The "Stilbosol" was supplied by Eli Lilly and Company, Indianapolis, Indiana.

The amount of concentrate fed was adjusted at 14-day intervals according to the production of the previous 14-day period.

The treated cows received stilbestrol once a day as part of the concentrate mixture at the afternoon feeding. The amount of "Stilbosol"-containing concentrate fed was also adjusted at 14-day intervals, according to body weight. The cows in groups I, II, and III were fed 5, 10, and 15 mg., respectively, of stilbestrol per 1000 pounds of body weight per day. The calculated amount of experimental concentrate for each cow replaced an equal amount of the control concentrate in the daily ration.

Phase II - Identical Twins

Animals Used. Five pairs of identical twin cows were used to give further and more conclusive information on the effects of stilbestrol on milk production. Two pairs of the twins were producing in their first lactation, one in their second, and two in their third lactation. One cow of each twin pair, selected at random, was fed the experimental ration containing stilbestrol, and her twin was fed the control ration. Since results in phase I indicated that the 10 mg. level was probably safe and effective, this level was selected for all cows in phase II. Ten milligrams per 1000 pounds of body weight per day of stilbestrol was fed to the treated animal of each twin pair, beginning 60 days post partum. The animals used are listed in Table 3. Seven of the cows were bred artificially with frozen semen during the first estrus period following a 60 day postpartal rest period. The frozen semen proved to be of very low quality. Therefore, after a short time, regular day-of-collection or day-after-collection semen was used for the artificial insemination of the twins. Two of the animals in the group of twins failed to conceive to repeated artificial breedings, and were bred naturally in an attempt to effect conception.

An accident, not due to experimental treatment, caused the death of the treated animal in one set of twins after four months of treatment. The control twin of this pair was then started on stilbestrol at the 20 mg. level.

Table 3. Description of identical twin cows used in phase II of the experiment.

Twin pair number	Cow number	Breed	Level of stilbestrol administration (mg./1000 lb. body weight per day)	Lactation number
1	81A		0	1
1	82A	Jersey	10	1
2	84A	1/4 Guernsey	0-20[1]	1
2	83A	3/4 Jersey	10	1
3	86A	1/8 Angus	0	3
3	85A	7/8 Jersey	10	3
4	194B	Holstein	0	3
4	195B		10	3
5	40A	Brown Swiss	0	2
5	41A		10	2

[1] 84A was control until death of 83A. She was then put on 20 mg. per 1000 pounds of body weight per day.

<u>Management of Experimental Animals</u>. The cows in this phase of the experiment were milked twice a day, by the same person, throughout the entire experiment. They were housed in the experimental barn and turned out in a dry lot twice a day, morning and evening, for exercise and for observation of estrous symptoms.

Data Collected. Production. The production data obtained were the same as was outlined for the animals in phase I, except that a 48-hour one quart composite sample of milk was collected from each twin 48 hours before the treated animal of the pair began to receive the experimental ration. These samples were frozen and saved for estrogen extraction and biological assay. Similar samples for estrogen assay were collected two weeks after treatment had begun, and then at monthly intervals throughout the experiment.

The data collected on body weights and reproductive physiology were the same as outlined for the animals in phase I.

A nine months supply of average quality alfalfa hay was purchased from one source for the feeding of the identical twins in this study. Every 20th bale was set aside when the hay was stored and later one block from the center of each of these bales was taken. A composite sample of this hay was made for a proximate feedstuffs analysis. Aliquot samples of the concentrate mixture were also taken for chemical analyses.

Feed Weights. All hay, silage, and concentrate mixture fed to, and refused by, the identical twins was carefully weighed and recorded. The hay was weighed out to the twins a bale at a time and the hay fed off of this bale so that there would be approximately a ten percent weigh-back daily. Weigh backs were made once a day to the nearest tenth of a pound. If there was any silage or concentrate refused, it was also weighed back at this time.

Feeding Methods. As stated above, alfalfa hay was fed so that there was approximately a ten percent weigh back daily. Sorgo silage was fed at the rate of two pounds per 100 pounds of body weight. This amount was reduced for twin pairs if one animal of the pair continually left silage to be weighed back. Concentrate was fed, to the twin cows that were in their second and third lactations, at a rate of one pound for each 3.5 pounds of 4 percent fat-corrected-milk produced daily. The two pairs of identical twins producing in their first lactation were fed concentrate at a rate of one pound for each 3.0 pounds of F.C.M. produced daily, to allow for growth. Sufficient "Stilbosol"-containing concentrate to furnish the desired amount of stilbestrol was used as a replacement for part of the concentrate mixture of the treated animal of each pair. The formulas for the concentrate mixtures are as follows:

Control Mixture		Stilbestrol-containing mixture
(lb.)		(lb.)
Ground corn	400	400
Ground oats	200	200
Soybean oil meal	100	96.43
Salt	7	7
Steamed bonemeal	7	7
"Stilbosol"	-	3.57

These concentrate mixtures were calculated to contain approximately 14 percent total protein. The amount of concentrate fed to both control and experimental animals was adjusted as previously stated for the animals in phase I.

EXPERIMENTAL RESULTS

Although the two phases of the experiment were outlined separately in the procedure, for clarity, and to avoid repetition, the results of each will be discussed together.

The Effects of Stilbestrol on Milk Production

Herd Cows. Cows for this phase were selected primarily to obtain information on reproduction. It was not expected that they would yield comparable data on production because of the variation in stage of lactation, gestation, pregnancy, and age. The data on milk production are presented primarily as a matter of record, although an attempt was made to summarize the results (Table 4). The cows receiving stilbestrol declined 31.6 percent in production of 4 percent F.C.M., while the control cows declined 41.0 percent. Although these data indicate that the feeding of stilbestrol favorably affected milk production, no conclusions were justified because of the heterogenity and smallness of numbers of the groups.

Production data on a few cows in phase I were omitted from Table 4 because these cows were too far advanced in stage of gestation, and were on trial too few days.

Identical Twins. Identical twin cows were used to gain more conclusive information on the effects of stilbestrol on milk production, since it has been reported by Hancock Δ that one pair of identical twin cows is equal to about 22 random pairs of animals for measuring milk production.

Δ Hancock, John. Studies in Monozygotic Cattle Twins, p. 68, New Zealand Department of Agriculture, Animal Research Division, Publication No. 63 Hamilton, New Zealand.

Table 4. F.C.M. production data of cows in phase I fed varying amounts of stilbestrol continuously for 114 or 119 days.

Group No.	Number of cow	Breed	Level of stilbestrol (mg./1000 pounds of body weight per day)	Number of days in lactation at start of experiment	Number of days pregnant at start	No. of days on test	No. of days pregnant during test period	Production at start of experiment (lb. 4% F.C.M. per day)	Production at end of experiment (lb. 4% F.C.M. per day)	Percent decline
I	125A	Holstein	5	35	0	119	54	52.6	37.8	28.1
	279A	Ayrshire	5	188	0	114	0	22.8	12.9	43.4
	Average									35.8
II	112B	Holstein	10	205	97	119	119	31.2	22.3	28.8
	482A	Guernsey	10	121	0	114	42	26.2	16.0	38.9
	277A	Ayrshire	10	290	96	119	119	23.0	15.1	34.4
	Average									34.0
III	419B	Guernsey	15	28	0	114	0	40.5	31.9	21.2
	354B	Jersey	15	30	0	114	32	27.9	17.5	37.3
	Average									29.3
	Average of treated groups			128	28		52	32.0	21.9	31.6
IV	127B	Holstein	0	186	100	119	119	27.0	17.0	37.0
	180A	Holstein	0	70	7	119	119	46.0	30.7	33.3
	268A	Ayrshire	0	222	104	119	119	22.2	8.0	64.0
	290A	Ayrshire	0	65	0	114	0	40.0	17.0	57.5
	423B	Guernsey	0	98	35	114	114	20.5	15.2	25.9
	437B	Guernsey	0	253	0	119	0	23.9	17.2	28.0
	Average			149	52		79	30.0	17.5	41.0

Data on the milk production of the identical twins are presented in Tables 5, 6, and 7, and in Figs. 1 and 2. In Table 5, the production of the twins is compared at different four-week periods during their lactations. During the eight week pretreatment period (period I), the milk production of half of each of the twin pairs, those representing the control group, was remarkably similar to that of their twin sisters representing the group to be treated. During this period the cows in each twin pair were fed alike. The total treatment periods varied from 28 to 168 days, and for comparative purposes, were subdivided into 4-week, or 28-day periods. Differences in length of treatment periods were due to the fact that the twin pairs were not started on the same dates because the animals freshened at different times. Beginning approximately 60 days post partum, one animal of each pair received 10 mg. of stilbestrol per 1000 pounds of body weight daily, while the other received none, and served as a control.

The administration of stilbestrol did not result in identical production response in all twin pairs. This is ascribed to the differences in the inheritance of the twin pairs. Although there was a variation in response, the downward trend in production when stilbestrol was fed tended to be less than it was among the control cows.

In an attempt to summarize the results, the data available on five pairs were averaged for one treatment period, then the data on four pairs were averaged for each of three treatment periods. Similarly, three pairs were averaged for the four treatment periods for which data were available, and two pairs were averaged for five periods. Even within the same pairs, the average change in production from period to period was not consistant either for the control animals or for the treated animals. The weighted

Table 5. The effect of stilbestrol on the production of 4 percent F.C.M. by identical twin cows.

Twin pair	No. of cow	Level of stilbestrol (mg./1000 lb.wt/day)	Pre-treatment period I 1-8 wk. (lb.)	Period II 9-12 weeks (lb.)	Change from period I (%)	Period III 13-16 weeks (lb.)	Change from previous period (%)	Period IV 12-20 weeks (lb.)	Change from previous period (%)	Period V 21-24 weeks (lb.)	Change from previous period (%)	Period VI 25-28 weeks (lb.)	Change from previous period (%)	Period VII 29-32 weeks (lb)	Change from previous period (%)	Change from period I to end of experiment (%)
				Average daily production of 4 percent F.C.M. — Treatment periods												
1	81A	0	16.6	18.0	+8.4	18.2	+1.1	15.5	-14.8	16.2	+4.5	14.6	-9.9	13.8	-5.5	-16.9
1	82A	10	17.7	20.6	+16.4	21.0	+1.9	19.8	- 5.7	20.5	+3.5	20.3	- .09	19.6	-3.5	+10.7
2	84A	0	18.6	14.9	-19.9	16.1	+8.1	17.2	+ 6.8	16.6	-3.5	15.8 /2	-4.8	17.3	+9.5	-19.9
2	83A	10	18.5	15.4	-16.8	15.5 /1	+0.7	12.8	-17.4	13.5	+5.5					-16.8
3	86A	0	21.6	19.2	-11.2	15.7	-28.9	12.4	-21.0							-42.6
3	85A	10	21.6	16.2	-25.0	13.3	-17.9	10.8	-18.8							-50.0
4	194B	0	40.1	34.5	-14.0	32.6	- 5.5	33.9	+ 4.0	33.2	-2.1	30.4	-8.4	27.7	-8.9	-30.9
4	195B	10	39.0	39.1	+ 0.3	38.9	.5	35.8	- 8.0	40.7	+13.7	33.6	-17.4	31.4	-6.5	-19.5
5	40A	0	26.4	24.0	-9.1	22.2	- 7.5	20.9	- 5.9	20.3	-2.9					-23.1
5	41A	10	31.7	28.5	-10.1	25.5	-10.5	23.3	- 8.6	21.1	-9.4					-33.4
Av. for 5 pairs	Control		24.7	22.1	-10.5											-10.5
	Treated		25.7	24.0	- 6.6											- 6.6
Av. for 4 pairs	Control		26.2	23.9	- 8.8	22.2	-7.0	20.7	- 6.8							-21.0
	Treated		27.5	26.1	- 5.1	24.7	-5.4	22.4	- 9.3							-18.6
Av. for 3 pairs	Control		27.7	25.5	- 8.0	24.3	-4.7	23.4	- 3.7	23.2	-0.9					-16.3
	Treated		29.5	29.4	- 0.3	28.5	-3.1	26.3	- 7.7	27.4	+4.2					- 7.1
Av. for 2 pairs	Control		28.4	26.3	- 7.4	25.4	-3.4	24.7	- 2.8	24.7	0.0	22.5	-8.9	20.8	-7.6	-26.8
	Treated		28.4	29.9	+ 5.3	30.0	+0.3	27.8	- 7.3	30.6	+10.1	27.0	-11.8	25.5	-5.6	-10.2

/1 83A went "off" feed during this period and in calculating percent change, period II is taken as end of expt.

/2 84A put on 20 mg. level of stilbestrol during this period.

Table 6. A comparison of total production of identical twin cows, as influenced by the administration of stilbestrol.

Twin pair	No. of cow	Treatment (mg./1000 pounds body weight/day)	Total production (actual)	Average fat test (%)	Total production (4 % F.C.M) (lb.)	Average total solids test (%)	Total production of solids (lb)	Total production of fat (lb)	Experimental period (Base and treated) (weeks)	F.C.M. production of animal receiving stilbestrol as per cent of control
1	81A	0	3111	4.66	3625	14.94	465	145	32	121
1	82A	10	3746	4.70	4398	14.94	558	176		
2	84A	0	1573	4.25	1670	13.83	218	67	14	102
2	83A△1	10	1600	4.24	1697	13.73	220	68		
3	86A	0	2067	4.90	2533	15.50	320	101	20	92
3	85A	10	1964	4.75	2332	14.80	291	93		
4	194B	0	8590	3.55	7630	11.62	998	305	32	109
4	195B	10	9261	3.60	8330	11.65	1079	333		
5	40A	0	4241	3.97	4208	12.98	550	168	26	115
5	41A	10	4878	3.97	4845	13.16	642	194		

Weighted average daily F.C.M. for controls 22.6
" " " treated 24.9
+2.3 (10.2%)

△1 Total production of this pair of twins calculated only to the time 83A went off feed.

Table 7. Total F.C.M. produced by identical twin cows during period of stilbestrol administration.

Twin pair	No. of cow	Treatment (mg./1000 lb. body weight/day)	Total F.C.M. production during treatment (lb.)	Average daily F.C.M. production during treatment (lb.)	Length of treatment period (weeks)	F.C.M. production of animal rec. stilbest. as % of control
1	81A	0	2693	16.0	24	126
1	82A	10	3408	20.3	24	
2	84A	0	626	14.9	6	106
2	83A	10	661	15.7	6	
3	86A	0	1324	15.8	12	85
3	85A	10	1125	13.4	12	
4	194B	0	5386	32.1	24	114
4	195B	10	6145	36.6	24	
5	40A	0	2447	21.8	16	113
5	41A	10	2756	24.6	16	

Weighted average daily F.C.M. for controls 21.7
" " " treated 24.6
+2.9 (13.4%)

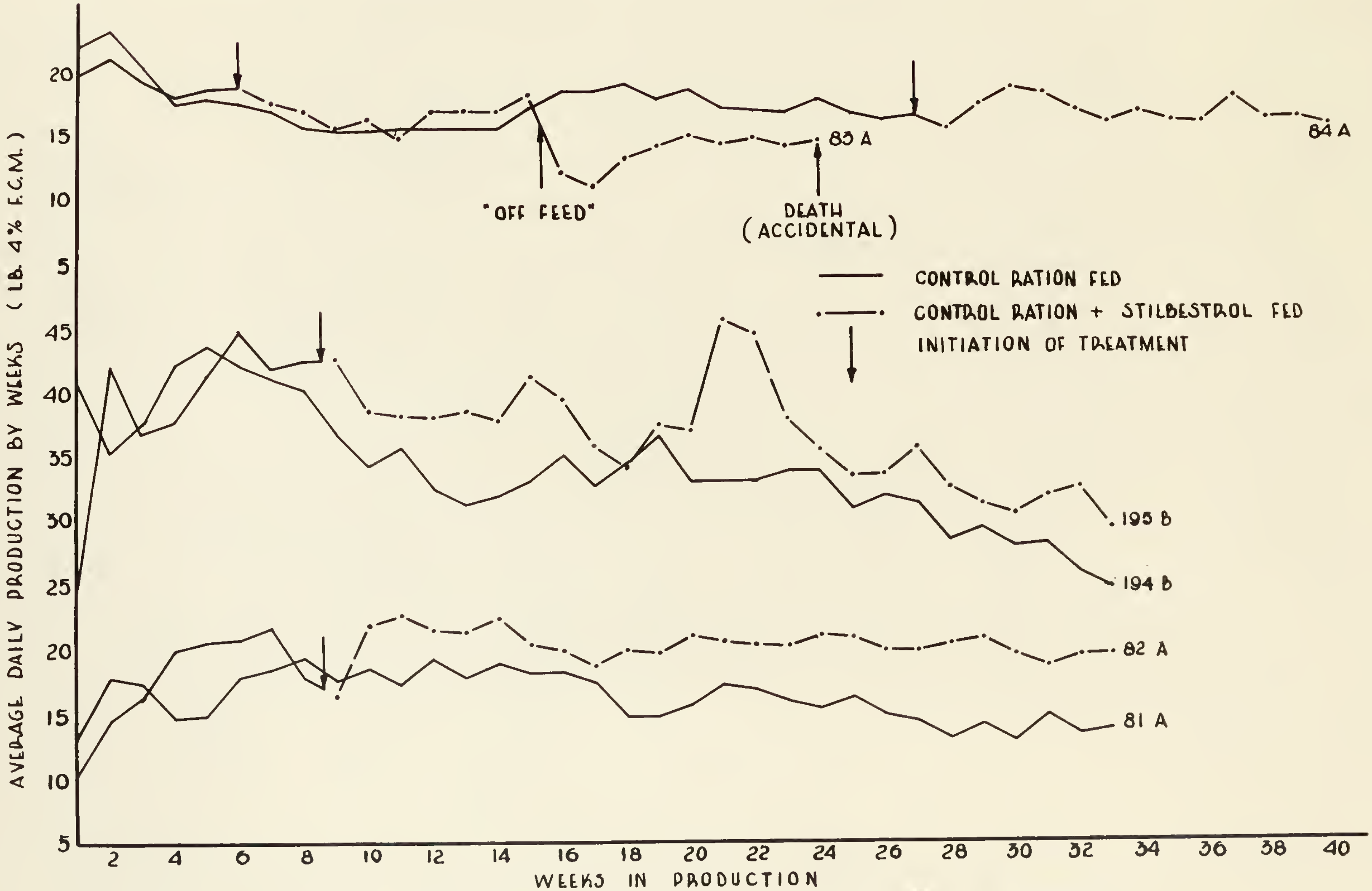

FIG.1 EFFECT OF STILBESTROL (10 mg./1000 lb. BODY WT./DAY) ON LEVEL OF 4% F.C.M. PRODUCTION OF IDENTICAL TWIN COWS.

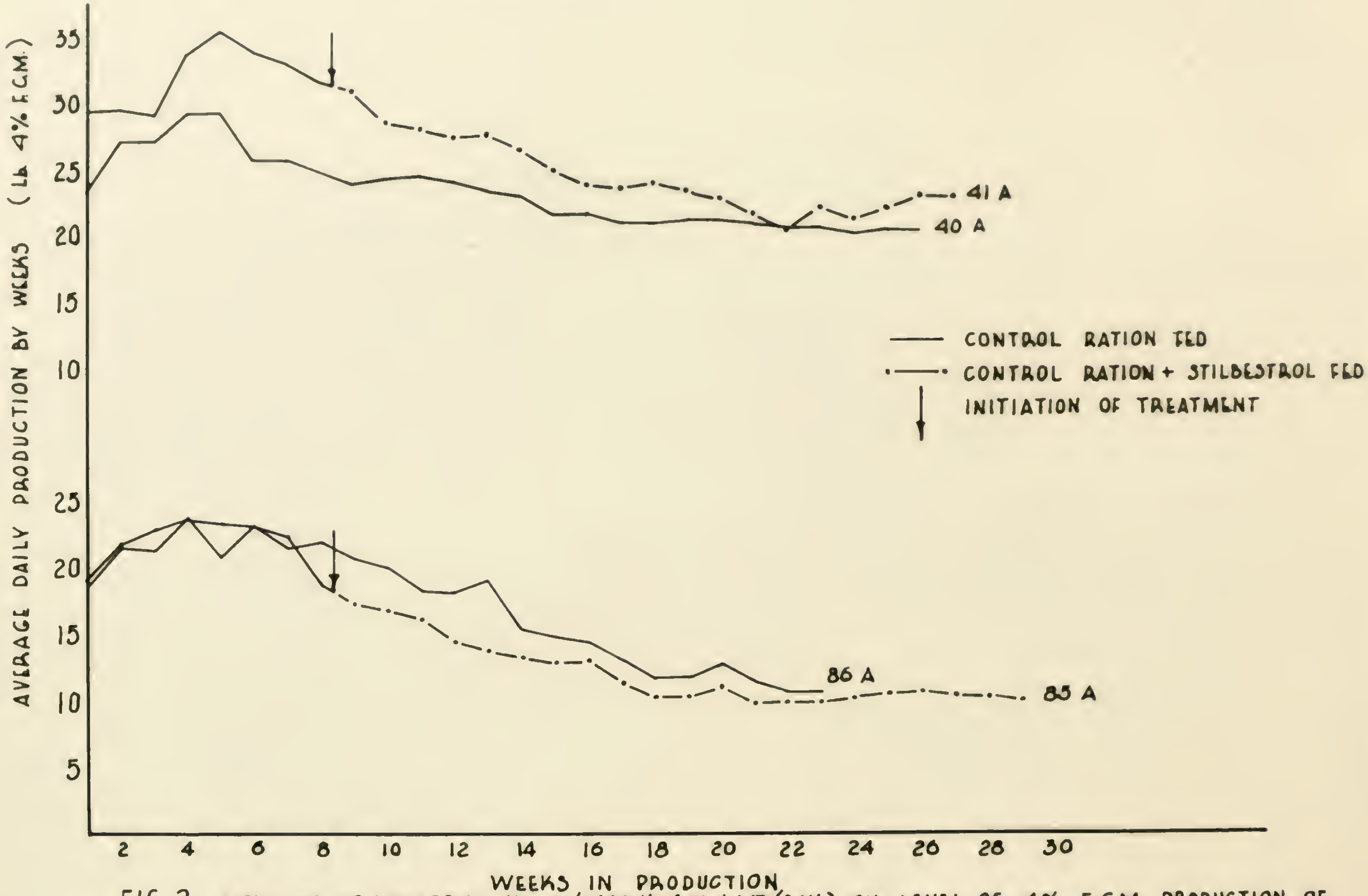

FIG. 2 EFFECT OF STILBESTROL (10 mg/1000 lb. BODY WT/DAY) ON LEVEL OF 4% F.C.M. PRODUCTION OF IDENTICAL TWIN COWS.

average decrease per 28-day period for all the periods was 9.6 percent for the control cows, and 5.2 percent for the cows fed stilbestrol. Thus, the treated cows decreased an average of 54 percent as much as the controls. In Table 7 the average daily production of all the control animals while on experiment is shown to be 21.7 pounds, while the stilbestrol fed animals is 24.6. The difference of 2.9 pounds daily is 13.4 percent more for the treated animals.

The feeding of stilbestrol seems to have no effect on the fat test of the cows of this study, as shown by the extremely close average test in Table 6. Appendix Tables 2 through 11 give additional information on production and the percent fat produced by the cows in phase II. Also shown in Table 6 is the average percent total solids produced by each twin. The closeness of these figures indicate the lack of effect by stilbestrol on the production of solids. A detailed account of solids production by the identical twins is given in Appendix Tables 12 through 16.

Efficiency of Milk Production

Presented in Table 8 is a comparison of the efficiency of production of 4 percent F.C.M. by the identical twin cows used in this study. The average daily T.D.N. requirements for maintenance [1] was subtracted from the average daily T.D.N. intake for each animal. This calculation gave the approximate amount of T.D.N. remaining after daily maintenance requirements were met. This figure, the daily T.D.N. intake above maintenance requirements, was divided into the average daily 4 percent F.C.M. production giving

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[1] Morrison, F.B. Feeds and Feeding. 21st ed. Table III, p. 1147 Morrison Publishing Co., Ithaca, N.Y. 1948.

Table 8. A comparison of efficiency of 4 percent F.C.M. production of identical twin cows when one cow of the pair received stilbestrol.

Twin pair No.	Cow No.	Level of treatment (mg./1000 lb. body wt. per day)	Pre-treatment: Average body weight (lb)	Pre-treatment: Average daily TDN intake 4 wk. (lb)	Pre-treatment: Daily T.D.N. req. for maint. (lb)	Pre-treatment: T.D.N. left for prod.	Pre-treatment: Average daily production (F.C.M.) (lb)	Pre-treatment: Lb. F.C.M./lb. T.D.N. above maint.	Treatment: Average body weight	Treatment: Average daily T.D.N. in-take	Treatment: T.D.N. req. for maintenance	Treatment: T.D.N. remaining for production	Treatment: Average daily production (FCM)	Treatment: Lb. FCM/Lb. TDN above maint.	Length of treatment period (weeks)
1	81A	0	612	12.6	9.1	3.5	17.6	5.02	666	13.1	9.5	3.6	16.0	4.44	24
1	82A	10	597	13.3	9.0	4.3	20.2	4.70	630	13.9	9.4	4.5	20.3	4.51	
				+0.7				-.38		+0.8				+.07	
2	84A	0							647	13.1	9.5	3.6	14.9	4.14	8
2	83A	10							600	12.9	9.1	3.8	15.7	4.13	
										-0.2				-.01	
3	86A	0	1126	17.4	8.8	8.6	21.8	2.53	1175	17.4	9.2	8.2	15.8	1.93	12
3	85A	10	1074	16.6	8.5	8.1	21.9	2.70	1085	16.6	8.6	8.0	13.4	1.68	
				-0.8				+.17		-0.8				-.25	
4	194B	0	998	21.4	7.9	13.5	41.5	3.07	1053	22.2	8.3	13.9	32.1	2.31	24
4	195B	10	975	19.8	7.8	12.0	42.4	3.53	1045	23.2	8.3	14.9	36.6	2.46	
				-1.6				+.46		+1.0				+.15	
5	40A	0	1088	20.0	8.6	11.4	26.2	2.30	1128	17.8	8.8	9.0	21.8	2.42	16
5	41A	10	1061	20.2	8.4	11.8	33.1	2.81	1119	21.0	8.7	12.3	24.6	2.00	
				+0.2				+.51		+3.2				-.42	
Weighted av. for controls				17.85				3.23		17.20	-3.6%			3.06	-5.26%
" " " treated				17.48				3.44		18.13	+3.7%			3.01	-12.5%
				- .37				+.21		+ .93				-.05	

the pounds of 4 percent F.C.M. produced per pound of T.D.N. intake above maintenance requirements. These calculations were made for each animal, both for the period prior to treatment, and the treatment period.

During the pretreatment period the animals scheduled for treatment consumed an average of .37 pounds less T.D.N. per day than the controls and produced .21 pounds more F.C.M. per animal per day. The animals receiving stilbestrol consumed approximately .93 pounds more T.D.N. per animal per day during the period of treatment than did the controls. The stilbestrol fed animals were approximately 12.5 percent less efficient in the production of F.C.M. during the treatment period than they were during the pretreatment period while the control animals were only 5.3 percent less efficient.

Table 9 gives a comparison of the total T.D.N. intake between the control and treated animal of each pair for the base or pretreatment period and for the treatment period.

The 28 day totals of T.D.N. consumed by each cow in phase II, in the form of alfalfa hay, grain and silage, is given in Tables 17 through 21 in the Appendix.

Stilbestrol Excretion into Milk

The results of the preliminary methods of estrogen assay, both with powdered and condensed milk using chicks and ovariectomized mice, are shown in Table 10. There was no significant difference between the uterine weights of assay animals fed milk from cows receiving stilbestrol and of those receiving the control ration.

The results of biological assays of extracts of milk from cows treated

Table 9. A comparison of T.D.N. intake by identical twins when one cow of each twin pair received stilbestrol.

Twin pair number	Periods	Total TDN from alfalfa hay(lb) Control animal	Treated animal	Total TDN from grain (lb) Control animal	Treated animal	Total TDN from silage (lb.) Control animal	Treated animal
1							
81A and	Base - 4 weeks	238.8	228.4	113.0	140.4	-	3.2
82A	Treated - 24 wks.	1163.1	1123.0	710.1	858.3	326.7	356.6
2							
83A and	Base	-	-	-	-	-	-
84A	Treated - 8 wks.	500.9	481.3	232.5	239.2	-	-
3							
85A and	Base - 4 weeks	250.7	259.1	137.5	139.4	99.9	66.6
86A	Treated - 12 wks.	825.2	784.9	334.1	305.7	302.4	301.5
4							
194B and	Base - 4 weeks	354.4	306.5	243.2	247.8	2.9	-
195B	Treated - 24 wks.	1888.5	1935.8	1233.9	1383.8	601.7	576.8
5							
40A and	Base - 4 weeks	293.3	266.3	166.5	202.8	100.8	97.2
41A	Treated - 16 wks.	1038.2	1299.7	554.2	649.6	403.2	401.4

with stilbestrol and results of injecting known amounts of stilbestrol into mice are shown in Table 11. The results of assay number V , expressed as uterine weights, indicated that there was no positive response to any of the various levels of treatment. A qualitative determination of uterine response to estrogenic stimulation can be made by noting hyperemia of the uterine blood vessels. No such response was noted in any of the assays mentioned above.

The results of assay VI, Table 11, in which known amounts of stilbestrol were injected into mice, expressed as uterine weights, indicated there was stimulation in all treated groups, however, there was no marked difference in response between the various levels of dosage. This indicated that each

Table 10. The effect on uterine weights of feeding powdered and condensed milk obtained from stilbestrol treated cows to immature chicks and ovariectomized mice.

Assay number	Assay animal used	Groups	Number in group	Average amount of test product consumed per animal	Average uterus weight (mg.)	Source of milk
I	Chicks	I	7	29.6 gm. powdered milk	22.00	Composite sample from cows in phase I on 15 mg.
		II	7	Control	20.34	
		III	7	52.50 gm. powdered milk	34.50	
		IV	7	Control	35.8	
II	Chicks	I	6	180 ml. condensed milk	73.8	Composite sample from cows in phase I on 15 mg.
		II	6	Control	66.6	
III	Ovariectomized mice	I	8	27 gm. powdered milk	22.05	Composite sample from cows in phase I on 15 mg.
		II	7	29 gm. powedered milk	21.78	Control cow
		III	5	Control	23.90	
IV	Ovariectomized mice	I	8	25 gm. powdered milk	23.38	83A prior to treatment
		II	7	31 gm. powdered milk	21.12	84A prior to treatment
		III	7	Control	27.14	

dose level caused a maximum response. Data on the cows from which milk was collected for extraction and biological assay are presented in Table 12.

The results of the assays of extracts made using the method of Pope and Roy (1953) are presented in Table 13. In assay number VII, there was no significant difference in response as measured by uterine weights of ovariectomized mice between the extract of milk from 83A (treated animal) and

Table 11. The effect on the uterine weight of ovariectomized mice of injecting extracts of milk from cows receiving stilbestrol.

Assay number	Source of material	Amount of material	Method of extraction	Group number	No. of mice	Equivalent dose (ml. milk per mouse)	Average uterine weight (mg)
V	83A 43 days on stilbestrol	475 ml. whole milk	Pope and Roy (1953)	I	4	14.25	9.10
				II	4	28.50	7.67
				III	4	47.50	8.97
	Composite sample from 3 cows in phase I on 15 mg. level	2000 ml. condensed milk	Lawson et. al (1944)	IV	4	60.00	9.82
				V	4	120.00	10.93
				VI	4	200.00	11.67
				VII	5	Control	8.48
VI	Known amounts of stilbestrol added in oil	0.025 mg./ mouse		I	7	-	78.87
		.05 mg./ mouse		II	7	-	77.14
		0.1 mg./ mouse		III	7	-	79.31
		0.2 mg./ mouse		IV	7	-	68.52
		Control		V	7	-	19.37

84A. Nor was there a difference between these two groups and the control group.

In assay VIII (Table 13) there was a significant difference ($P < .05$) between the extract of the treated cow 84A (on 20 mg. level 13 days at this collection) and the extract of 195B (on 10 mg. level 124 days at collection). There was however, no significant difference in response between this group and the group receiving the lowest level standard. This is the only case in which an estrogen extract of a milk sample (to which stilbestrol was not added) showed a response comparable to the response elicited by a standard dosage of stilbestrol.

Table 12. Information on cows presented in Table 13 from which estrogen extracts of milk were made.

Sample number	Cow number	Level of stil-bestrol (mg./1000 lb.wt./day)	Days on stilbestrol at collection of sample	Days pregnant at collection of sample	Days past last estrous at collection
3	83A	10	112	81	
4	84A	0	0	0	3
5 /1	84A	20	13	0	6
6	194B	0	0	0	10
7	195B	10	124	51	
8	123B	20	124	120	
9	40A /2	0	0	60	
10	40A	0	0	60	

/1 Sample collected 13 days after 84A put on 20 mg. at the death of 82A.

/2 This sample had 21.84 u g. of stilbestrol added to it prior to extraction.

In assay IX, sample 7, milk from 40A, a control cow, had 21.84 micrograms of stilbestrol added to it before extraction. The stilbestrol was dissolved in methyl alcohol and added to the milk immediately before the extraction procedure was started. This was done to measure recovery of stilbestrol from the milk. There was a significant ($P<.05$) difference between uterine weights of the mice receiving this extract and the uterine weights of mice receiving an extract of the same milk to which no stilbestrol had been added. Sample number 6 in assay IX was from 123B, a stilbestrol fed cow on the 20 mg. level. One mouse was treated with this extract. This mouse received a milk equivalent of 80 ml. and had a uterine weight of 10.8 mg. as compared to 10.31 mg. which was the average uterine weight of the

Table 13. Summary of results of uterine weights of mice receiving injections of extracts of milk extracted for estrogen content.

Assay number	Source of sample and number of extract	Volume of milk extracted (ml.)	Volume extract and olive oil (cc.)	Stilbestrol added ug/0.1 cc	Total dose in 4 days (cc.)	Number of mice	Milk equivalent per mouse (cc)	Average body weight (gm.)	Average uterine weight (mg.)	Ratio (mg. uterine weight / gm. body weight x 100)
VII	1-83A	100	3	—	0.4	7	13.33	21.71	13.62	64.52 [1]
	2-84A	100	3	—	0.4	7	13.33	22.11	13.90	63.16 [1]
	St. I	—	-	0.78	0.4	7	—	21.11	62.90	298.48 [2]
	St. II	—	-	0.087	0.4	7	—	21.40	45.32	212.71 [2]
	St. III	—	-	0.012	0.4	7	—	18.54	22.61	122.31 [2]
	Control	—	-	—	0.4	7	—	19.84	13.18	67.45
VIII	3-84A	100	3	—	0.4	7	13.33	21.28	19.30	92.50 [3]
	4-194B	100	3	—	0.4	7	13.33	22.60	11.42	51.89 [1]
	5-195B	100	3	—	0.4	7	13.33	24.18	10.77	45.19 [1]
	St. I	—	-	0.78	0.4	7	—	19.47	54.52	282.69 [2]
	St. II	—	-	0.097	0.4	7	—	23.95	49.84	208.80 [2]
	St. III	—	-	0.012	0.4	7	—	22.57	15.84	71.67 [3]
	Control	—	-	—	0.4	7	—	22.22	9.81	45.93
IX	6-123B	100	5	—	0.4	1	80.0	18.8	10.8	57.44 [1]
	7-40A	100 + 21.84 ug. stilbestrol	3	—	0.4	7	13.33	23.51	31.97	136.64 [3]
	8-40A	100	3	—	0.4	7	13.33	24.51	13.44	54.91 [1]
	St. I	—	-	0.78	0.4	7	—	20.72	43.50	210.49 [2]
	St. II	—	-	0.097	0.4	7	—	19.68	35.44	182.29 [2]
	St. III	—	-	0.012	0.4	7	—	24.21	17.83	74.80 [3]
	Control	—	-	—	0.4	7	—	22.18	10.31	46.39

St. = Standard (prepared by adding known amounts of Stilbestrol to oil)
[1] No significant difference from control
[2] Statistically significant ($P < .01$) from control
[3] Statistically significant ($P < .05$) from control

control group. This difference is not significant, but there was some indication of stimulation as witnessed by a slight hyperemia of the uterine blood vessels. The standards in each experiment consisted of three dosage levels; 3.12 ug., 0.39 ug., and 0.048 ug. of stilbestrol. A significant ($P < .05$) difference between the means of the standards of each experiment was shown.

The Effects of Feeding Stilbestrol to Dairy Cows on their Reproductive Physiology.

Herd Cows. The effects of feeding stilbestrol on the reproductive performance of the cows in phase I during the first experimental lactation are shown in Table 14. Of the eleven cows receiving stilbestrol, five cows (123B, 126B, 112B, 277A, 197A) were from three to five months pregnant at the beginning of stilbestrol feeding. Three of these cows (126B, 112B, 197A) were fed stilbestrol continuously from the beginning of the experiment (April 4) until parturition, while two cows (123B, 277A) were fed stilbestrol continuously from April 4 until the termination of their lactation, but were not fed stilbestrol during the dry period. Due to an error in recording the breeding date, 197A did not have a dry period, therefore she was fed stilbestrol continuously from April 4 until she freshened.

Each of these five pregnancies terminated with the birth, without difficulty, of a normal healthy calf. Plate I shows pictures of treated cows, 112B, 125B and 277A before the initiation of treatment and also after the birth of their calves. The calf born to 197A was healthy at birth, but since the cow had no dry period, there was no colostrum and the calf developed scours and died. Death was attributed to an infection which probably resulted from lack of protective antibodies normally present in colostrum.

Table 14. Summary of reproductive behavior of cows in phase I during first experimental lactation.

Number of cow	Breed	Stage of lactation at beginning of experiment	Level of stilbestrol feeding (mg./1000 lb. wt.)	Interval of feeding stilbestrol (date)	Date of estrous period and breeding	Pregnancy	Calving date	Remarks
123B	Holstein	357 days	5	4-4-55 to 6-15-55		10-20-54 5 months pregnant at start of feeding	8-5-55 normal pregnancy	Calf normal. Cow retained placenta.
125B	Holstein	35 days	5	4-4-55 to 1-17-56	5-1-55 6-8-55	Pregnant to 6-8-55 service	3-17-56	Calf normal. Cow cleaned.
126B	Holstein	296 days	5	4-4-55 to 8-1-55		5 months pregnant at start 10-26-54	8-1-55	Calf weighed 107 pounds healthy, normal.
279A	Ayrshire	188 days	5	4-4-55 to 7-26-55	12-5-54 12-26-54 1-31-55 /1 4-26-55 5-16-55 6- 5-55 6-27-55 7-19-55			Slaughtered 7-26-55. No embryo recovered. Problem cow before treatment.
112B	Holstein	205 days	10	4-4-55 to 10-7-55		3 months pregnant at start of experiment. Received stilbestrol treatment 6 months of gestation 12-28-54	10-7-55	Normal, healthy calf. Weight 104 pounds.

Table 14. (cont.)

Number of cow	Breed	Stage of lactation at beginning of experiment	Level of stilbestrol feeding (mg./1000 lb. wt.)	Interval of feeding stilbestrol (date)	Date of estrous period and breeding	Pregnancy	Calving date	Remarks
277A	Ayrshire	290 days	10	4-4-55 to 8-12-55		3 months pregnant at start of experiment. Received stilbestrol treatment 130 days of lactation, but not during dry period	10-6-55	Calf normal.
388B	Jersey	121 days	10	5-5-55 to 6-1-55	11-19-54 [1] 3-9-55 4-25-55 6-5- 55	Not pregnant at slaughter		Permanently dilated cervix, not due to stilbestrol
472A	Guernsey	121 days	10	4-4-55	12-22-54 [1] 1-4-55 Bred 3-20-55 Bred 4-8- 55 5-22-55 Bred 6-14-55 Bred	Pregnant 42 days at slaughter. Normal embryo		Bred 2 times pre-stilbestrol. Two times following treatment
197A	Holstein	200 days	15	4-4-55 to 10-7-55		5 months pregnant at start of expt. Received stilbestrol treatment 5 mo. of pregnancy. Through error in breeding dates, no dry period. 11-5-54	7-31-55	Calf normal at birth, but since she received no colostrum died 5 days after birth.

Table 14. (Cont.)

Number of cow	Breed	Stage of lactation at beginning of experiment	Level of stilbestrol feeding (mg./1000 lb. wt.)	Interval of feeding stilbestrol (date)	Date of estrous period and breeding	Pregnancy	Calving date	Remarks
354B	Jersey	Fresh 30 days at start	15	4-4-55 to 7-26-55	5-12-55 (bred) 6-1-55 (bred) 6-24-55 (bred)	Pregnant 22 days at slaughter. Normal		Normal embryo at slaughter.
419B	Guernsey	Fresh 28 days at start	15	4-4-55 to 7-26-55	3-28-55 5-5-55 (bred) 5-28-55 (bred)	No embryo recovered at slaughter. Enlarged uterine horn. Possible resorption. 12-21-54		Slaughtered 7-26-54.
127B	Holstein	187 days	Control	-	12-7-54 (bred) 12-25-54 (bred)	Pregnant to 12-25-54 breeding. 3 months at start of expt.	9-28-55	Normal 96 pound female calf.
180A	Holstein	72 days	Control	-	3-28-55 (bred)	Pregnant 3 days at start of expt.	1-5-56	Normal calf.
268	Ayrshire	189 days	Control	-	11-16-54 (bred) 12-1-54 (bred) 12-21-54 (bred)	Pregnant 103 days at start	9-29-55	Calf died at birth. Cow had milk fever.

Table 14. (concl.)

Number of cow	Breed	Stage of lactation at beginning of experiment	Level of stilbestrol feeding (mg./1000 lb. wt.)	Interval of feeding stilbestrol (date)	Date of estrous period and breeding	Pregnancy	Calving date	Remarks
290A	Ayrshire	64 days	Control	-	4-4-55 (bred) 4-26-54 (bred)	Pregnant 90 days at slaughter		Normal embry recovered
423B	Guernsey	120 days	Control	-	1-21-55 3- 1-55 (bred)	Pregnant 120 days at slaughter		Normal embryo recovered
437B	Guernsey	240 days	Control	-	Bred 9 times - then slaughtered	-		Slaughtered 12-6-55. Re-absorption of embryo taking place. Appeared to have been pregnant number of times and not maintained. Uterus hard and meaty.

1 Dates above line indicate breeding pre-treatment. Dates below line indicate breeding after treatment was started.

One cow (125B) was bred twice after the beginning of stilbestrol feeding. She conceived to a service on 6-8-55, 64 days after the initiation of stilbestrol feeding. She calved normally on March 17, 1956, after having received stilbestrol for 352 days.

Two cows (482A, 354B) were bred and conceived after the beginning of the experiment. Apparently normal embryos were recovered from these cows at slaughter.

Three cows (279A, 388B, 419B) were bred several times following the initiation of stilbestrol feeding. No embryos were recovered from these cows at slaughter, however two (279A, 388B) were classified as problem cows before treatment and would not be expected to conceive. The other cow (419B) appeared to be pregnant approximately 60 days, and there was a slight distention of the uterus but no embryo was recovered. More detailed data on the reproductive behavior of the cows in phase I is given in Table 1 of the Appendix.

The effects on conception of feeding stilbestrol in subsequent lactations are shown in Table 15. Of the five treated cows (123B, 126B, 112B, 277B, 197A) which have been bred in their second experimental lactation, three (123B, 126B, 197A) conceived to the first service. Of the other two, 112B is not pregnant after two services and 277A has not been diagnosed pregnant. Another cow, (268A) that served as a control during the first experimental lactation conceived to her second service, 90 days after initiation of stilbestrol feeding, during the second experimental lactation.

As shown in Table 14, 123B, conceived to her first service which was nine days post initiation of treatment. This cow was on the 20 mg. level and received 26 mg. of stilbestrol in her feed per day. She aborted on the

EXPLANATION OF PLATE I

A. (125B) Picture on left was taken prior to stilbestrol feeding.
Picture on right shows same cow and her normal calf which was born after the cow had received 5 mg. of stilbestrol per 1000 pounds of body weight per day for 352 days. The cow did not receive stilbestrol during her dry period.

B. (277A) Picture on left was taken prior to stilbestrol feeding.
Picture on right shows same cow and her normal calf which was born after the cow received 10 mg. of stilbestrol per 1000 pounds of body weight per day for 118 days. The cow did not receive stilbestrol during her dry period.

C. (112B) Picture on left was taken prior to stilbestrol feeding.
Picture on right shows same cow and her normal calf which was born after the cow received 10 mg. of stilbestrol per 1000 pounds of body weight per day until calving (210 days).

PLATE I

A

B

C

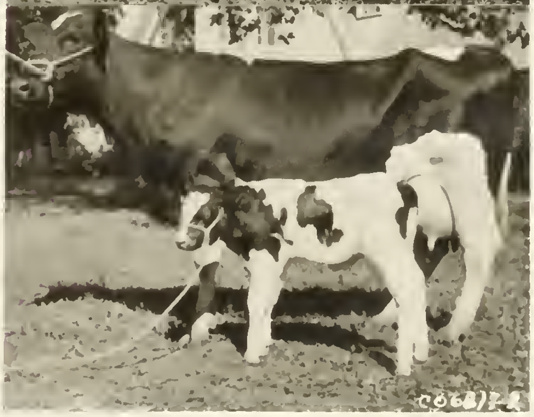


Table 15. Summary of reproductive behavior of cows in phase I during second experimental lactation.

Number of cow	Breed	Level of stilbestrol during first experimental lactation (mg./1000 lb.body wt.)	Level during dry period	Level during second experimental lactation (mg./1000 lb. body weight)	Days pregnant at start of treatment	Dates of estrous period and breeding	Pregnancy	Days on stilbestrol at conception	Remarks
125B	Holstein	5	0	0	0	-	-	-	
123B	Holstein	5	0	20	0	10-6-55 11-4-55 (bred)	Pregnant to 11-4-55 first service	9	Aborted 3-23-56 140 days.
126B	Holstein	5	5	0	0	9-24-55 10-13-55 (bred)	Pregnant to 10-13-55 first service	0	Apparently normal pregnancy
112B	Holstein	10	10	15	0	11-8- 55 12-2- 55 2-14-56 bred 3- 1-56 bred 4-12-56			Not pregnant to two breedings.
277A	Ayrshire	10	0	10 for 105 days, then increased to 20	0	11-2- 55 11-20-55 12-10-55 bred 1-24-56 bred 2-15-56 bred 3-28-56 bred			Uterus hard and meaty, poor tone.

Table 15. (concl.)

Number of cow	Breed	Level of stilbestrol during first experimental lactation (mg./1000 lb. body wt)	Level during dry period	Level during second experimental lactation (mg./1000 lb. body weight)	Days pregnant at start of treatment	Dates of estrous period and breeding	Pregnancy	Days on stilbestrol at conception	Remarks
197A	Holstein	15	15	10 for 105 days then none	9	8-29-55 9-25-55 10-18-55 bred	Pregnant to 10-18-55 first service		Apparently normal pregnancy
127B	Holstein	0	0	0	0	12-23-55 (bred)	Pregnant to first service		
180A	Holstein	0	0	0	0	3-26-56 (bred)			To early to determine pregnancy
268A	Ayrshire	0	0	10 for 105 days then increased to 15	0	11- 2-55 11-25-55 12-13-55 bred 1-26-56 bred	Pregnant to second service	90	Apparently normal pregnancy

140th day of gestation. She had never been a Brucellosis reactor and showed a negative test after aborting. The cause of her abortion is not known, but could be due to the high daily level of stilbestrol intake.

Identical Twins. Some of the identical twin cows were bred the first few times with frozen semen obtained from one collection of one bull. The use of the frozen semen, it was thought, would remove the variable factor of differences in the quality of the semen which was to be used to inseminate both treated and control animals.

One cow (83A) conceived to her first service, which was with the freshly frozen semen. It was not realized for some time that after this breeding the frozen semen inadvertently deteriorated due to faulty refrigeration; therefore, six other twins (81A, 82A, 84A, 85A, 194B, 195B) failed to conceive to repeated breedings with nonviable semen. Since the failure to conceive of both treated and control animals was due to their being inseminated with dead spermatozoa, all services using frozen semen were excluded from reproductive data presented in Table 16.

Treated cows, 82A, 195B, and 41A, conceived to their first service with fresh semen. The average number of services per conception in the treated group of twins was 2.25. This includes the five services without conception of 85A. The average number of services per conception in the control group was 2.0. This does not include the five breedings of 84A who was both a control and treated animal during the course of the experiment. The cause of failure to conceive to repeated breedings in treated animal 84A was determined at slaughter. This cow's right oviduct was collapsed and the lumen of the left oviduct was completely obstructed by cartilaginous capsule growths. Treated twin cows 82A, 83A, 195B and 41A conceived 59, 30, 75 and

Table 16. Effect of stilbestrol on reproductive behavior of identical twin cows.

Number of cow	Level of treatment (mg./1000 lb. body weight	Number of times bred	Average length of cycle (days)	Number of days pregnant during treatment period	Days on stilbestrol	Number of days received stilbestrol prior to conception
81A	0	2	23.1	57	0	
82A	10	1	22.6	104	163	59
84A	0 /1	5	19.7	0	0	
83A	10	1	19.0	25	55	30
86A	0	1	25.0	64	0	
85A	10	5	21.8	0	83	
194B	0	3	23.5		0	
195B	10	1	22.0	88	163	75
40A	0	2	24.6	90	0	
41A	10	1	21.5	103	113	10

/1 Put on 20 mg. level at the death of 83 A.

10 days, respectively, after the inception of stilbestrol feeding. An apparently normal embryo was recovered from 83A at her death.

From these data it seems apparent that the feeding of stilbestrol in amounts varying from five to 15 mg. per 1000 pounds of body weight daily has no adverse effect on pregnancy that is established, nor on the ability of cows to conceive.

Effect on Estrus. At about 20 to 30 days after the beginning of stilbestrol feeding, there was a tendency for the vulvas of both pregnant and non-pregnant cows of phases I and II to become hyperemic and enlarged. From outward appearances this condition could have been mistaken for estrus, however, this condition did not persist, nor interfere with normal cycling.

That cows in both phases of the experiment cycled normally is illustrated by the data presented in Tables 14, 15, and 16. There was no evidence of nymphomania in cows being fed stilbestrol at any time during the experiment. The ovaries, as determined by rectal palpation, remained functional throughout the experiment. Also the ovaries of all the stilbestrol-fed cows which were slaughtered during the course of the experiment showed no indications of abnormal function. Histological study of the ovaries, oviduct, and uterus is being made; however, the results of this study are not available at the writing of this thesis. While there was a variation in the length of estrous cycles of the treated cows, this variation was no greater than that normally observed in dairy cattle or that of the control animals. The regularity of estrous cycles of the identical twins in this study is shown clearly in Appendix Tables 22 to 31 inclusive.

The Effect of Feeding Stilbestrol on the Anatomy of Dairy Cattle

There was no evidence of elevated tailheads, relaxed pelvic ligaments, or sunken loins in any of the cows receiving stilbestrol. Pictures taken of cows before the initiation of stilbestrol feeding and pictures taken at the close of the experiment are shown in Plate I and II. The absence of any abnormal change in physical appearance of cows which received stilbestrol is shown in Plate II.

Effect of Stilbestrol on Body Weight in Dairy Cattle

As is shown in Figs. 3 and 4, there was no stimulation of growth that could be attributed to treatment in the stilbestrol-fed cow of each twin pair. It was coincidental that in all but twin pair 5 (40A, 41A), the

EXPLANATION OF PLATE II

Pictures in the column on the left were made at the beginning of the experiment, those in the column on the right were taken at the end of the experiment.

A. 81A - Control cow, twin pair 1.

B. 82A - Treated cow, twin pair 1, picture on right shows cow after receiving 10 mg. of stilbestrol per 1000 pounds of body weight per day for 180 days.

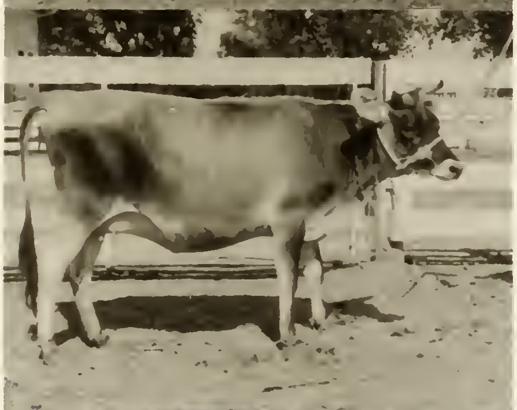
C. 194B- Control cow, twin pair 4.

D. 195B- Treated cow, twin pair 4, picture on right shows cow after receiving 10 mg. of stilbestrol per 1000 pounds of body weight per day for 180 days.

PLATE II

A

B



C

D

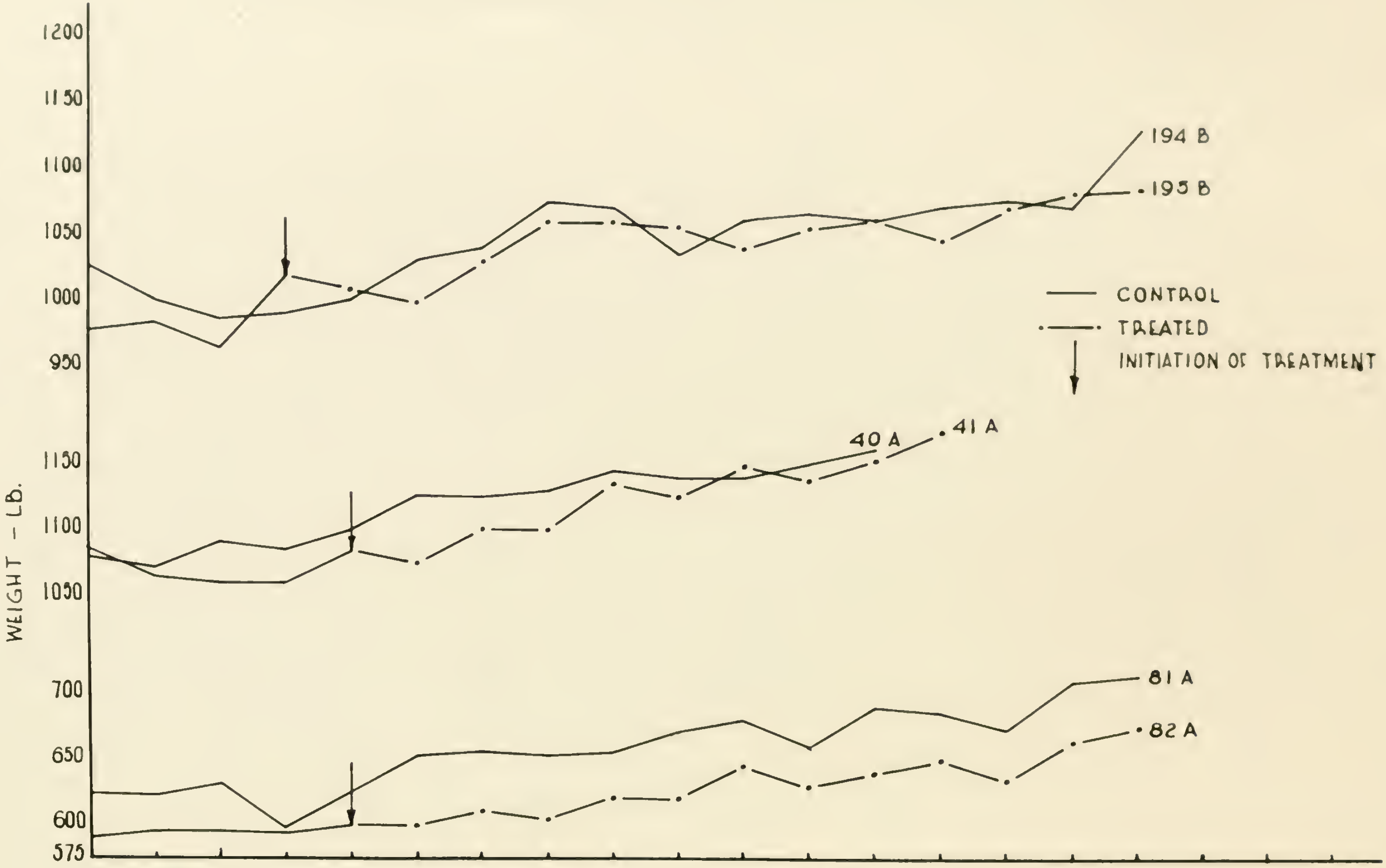


FIG. 3 EFFECT OF STILBESTROL ON BODY WEIGHT OF IDENTICAL TWIN COWS.

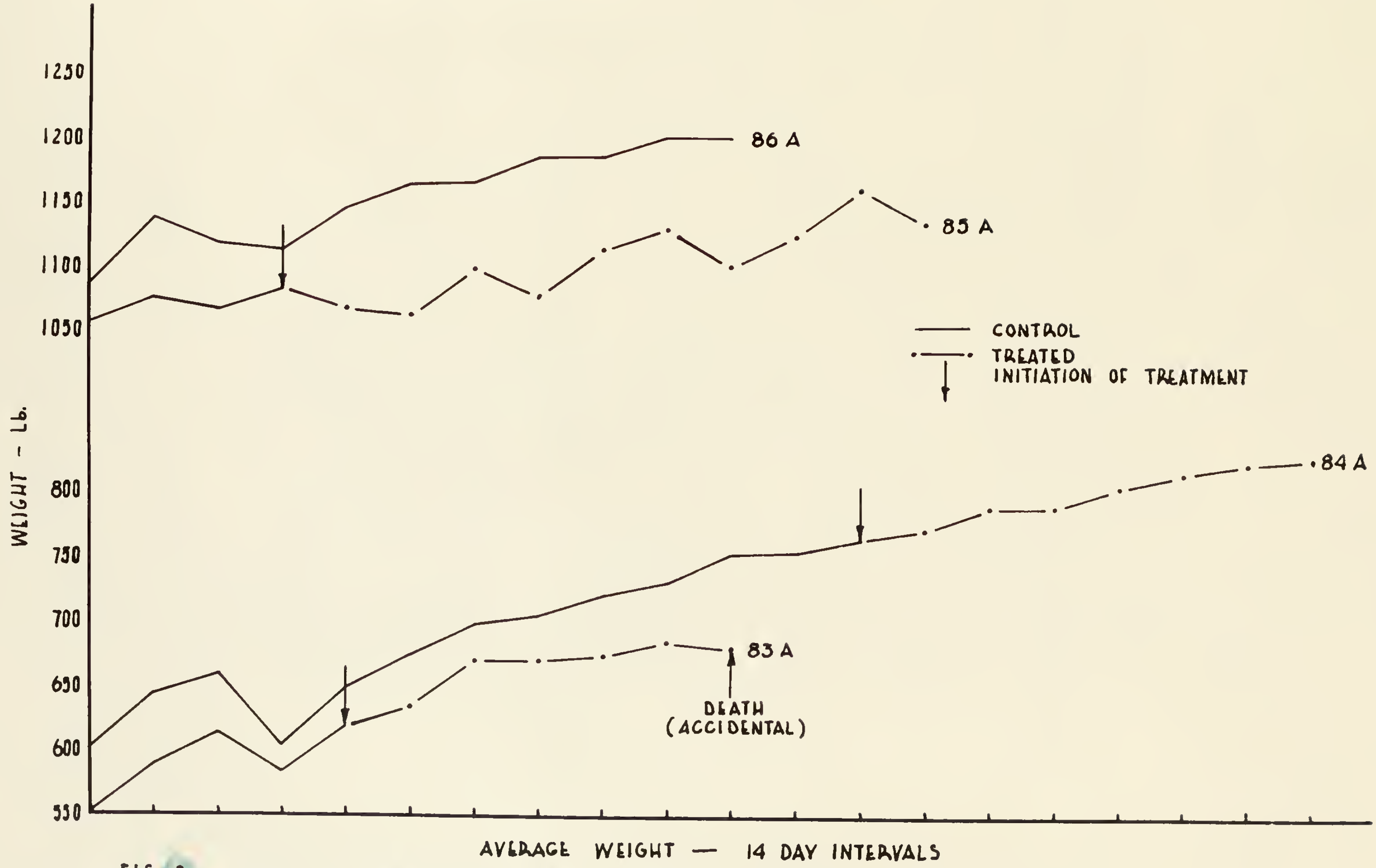


FIG. 4 EFFECT OF STILBESTROL ON BODY WEIGHT OF IDENTICAL TWIN COWS.

animal scheduled to receive stilbestrol was the lightest in weight at the first weighing post partum.

It seems apparent from these data that stilbestrol had no effect on rate of gain or growth.

The Gross and Histological Effect of Stilbestrol on the Glands of the Slaughtered Animals of this Study.

The pituitary and adrenal glands were removed from all animals slaughtered. The weights and sizes of the glands were recorded and sections taken for histological study.

Data on the gross study of these glands are presented in Table 17. In most cases there seemed to be an increase in gland weight as body weight increased.

It appears from these data that stilbestrol had no effect on pituitary and adrenal size.

Histological work was incomplete at the writing of this thesis.

DISCUSSION

Production

In early work, (Folley, et al. 1941b) the administration of estrogenic materials to lactating cows either had no effect on production or caused a drop which was in a few cases accompanied by an increase in the percentage of fat and/or total solids. The data of this study are not in agreement with those findings. When lactating cows were fed stilbestrol at levels varying from 5 to 15 mg. per 1000 pounds of body weight daily, the results indicated that there was very little, if any, initial stimulatory effect on

Table 17. Pituitary and adrenal gland weights of cows slaughtered during experiment.

Cow number	Breed	Treated or control	Body weight (lb.)	Body weight (Kg.)	Stage of pregnancy	Weight of adrenal glands (gm)	Ratio body wt. (kg.) / adrenal wt. (gm.)	Pituitary weight (gm.)	Ratio body wt. (kg.) / pituitary weight (gm.)
83A	Jersey-Guernsey	Treated	682	309	85 days	20.5	151/10	2.0	155/1
448B	Guernsey	Control	760	344	Open	17.0	202/10	1.5	229/1
84A	Jersey-Guernsey	Treated	825	374	Open	20.0	187/10	2.0	187/1
242B	Ayrshire	Control	830	376	93 days	16.5	228/10	1.7	221/1
423B	Guernsey	Control	973	442	146 days	18.5	239/10	-	
246B	Ayrshire	Control	990	449	25 days	19.5	230/10	1.7	264/1
419B	Guernsey	Treated	994	451	Embryonic abortion	26.5	170/10	1.7	265/1
354B	Jersey	Treated	1002	455	31 days	29.6	150/10	2.0	228/1
482B	Guernsey	Treated	1083	480	41 days	30.0	160/10	1.8	267/1
437B	Guernsey	Control	1134	512	Embryonic abortion	32.0	160/10	3.8	135/1
279A	Ayrshire	Treated	1143	520	Open. Possible embryonic abortion	32.7	159/10	1.7	306/1
290A	Ayrshire	Control	1262	573	59 days	34.8	165/10	2.5	229/1
183A	Holstein	Control	1670	753	72 days	39.0	193/10	2.8	269/1

production, but that there was a decrease in the rate of decline. This regulating effect of stilbestrol on persistance of production appears to have increased total yield of the identical twins receiving stilbestrol approximately 13 percent. The results also showed that in four out of five pairs the treated animal produced more milk than the control. Also, this study failed to show that the feeding of stilbestrol had any effect on the percentage of fat or total solids produced.

The outstanding differences in procedure between the early work with stilbestrol and the procedures used in this study are the level and means of administration. Subcutaneous injection, subcutaneous implantation, and percutaneous inunction of the udder with high doses were the chief means of administration of stilbestrol used by Folley and co-workers (1941b) as compared to the feeding, of comparatively small amounts in this study. It is probable that the differences in response are due to differences in experimental procedures.

In only one twin pair did the treated animal (85A) produce less than the control. This cow and her twin (86A) were Jersey-Angus crossbreds of poor dairy character. One might postulate from this that cows with an inherent ability for low production will not respond to estrogen treatment. This possibility is supported by the work of Eaton and Simmons (1953), who found that goats that responded best to initiation of lactation by stilbestrol injections were also the highest producers in their subsequent lactation.

It seems probable that the amount of stilbestrol that is absorbed and enters the systemic circulatory system of the animal is the factor governing the response brought about. It is likely that only a small portion of the active stilbestrol fed to ruminants is actually absorbed. It is thought that this is due either to the poor digestibility of the material or the inactivation of it by rumen microorganisms. Turner (1956) found that dairy cows fed 10 mg. of stilbestrol per day showed as much estrogenic activity in the dried feces as was present in the feed. Folley and Malpress (1944b) indicate that only 5 to 10 percent of estrogens orally administered to bovines are utilized. Since there appeared to be a stimulation to production and no effect on the percent of fat or solids produced by the cows on this study, and since they were on comparatively low levels of orally administered stilbestrol, it is postulated that the level necessary for stimulation of production falls below that which results in the inhibition of lactation or causes an increase in milk solids.

Feed Efficiency

No attempt was made to determine efficiency of production for the cows in phase I. Although there were insufficient data obtained from phase II (identical twin study) for reliable evaluation, the twins receiving stilbestrol produced milk with greater net efficiency than the controls in only two of the five twin pairs (Table 8). The average efficiency of production of the treated group (stilbestrol fed cow of each pair) was less than that of the control group during the treatment period; however, the difference in average efficiency was only .05 of a pound of F.C.M. per animal per day. This difference in efficiency of production in favor of the controls indicates that the greater production of the cows fed stilbestrol was due to some factor other than improved efficiency.

The control animals of the identical twin pairs consumed an average of 0.37 pounds more T.D.N. per day during the pre-treatment period than the animals scheduled for treatment, but then, during the period of stilbestrol feeding, the treated animals took in an average of 0.93 pounds more T.D.N. per day than did the controls. This 7 percent greater T.D.N. intake by the twins receiving stilbestrol is in agreement with the work of Burroughs et al. (1955) where a 5 percent increase in feed consumption by beef steers was reported, but in the present study it is thought that no more than an indirect relationship to stilbestrol feeding and an increase nutrient intake exists. This theory is based on the fact that stilbestrol in some way caused an increase in production that was compensated for by an increase in the grain ration, thereby increasing the T.D.N. intake of the animals receiving stilbestrol (Table 9). The amount of grain consumed was controlled, and increased according to production, therefore, the increase in production can not be attributed to a greater ad. libitum intake of T.D.N. In only two twin pairs (4 and 5) was the increase in grain accompanied by a greater intake of hay by the treated over the control animals. Whether or not it is of significance, these two pairs contained the highest producing cows of phase II.

It may be that the mode of action of the effect which stilbestrol has on milk production is through its action on the pituitary gland. According to Lewis and Turner (1941) stilbestrol administration causes an increase in the lactogenic hormone. If this is true, then the increase in the amount of lactogenic hormone present in the animal body could well be the factor involved in the slower rate of decline in production in the majority of cows of this study.

Excretion of Estrogens into the Milk

One of the most important considerations, if stilbestrol is to be of practical value in dairy rations, is whether the feeding of low levels causes, or increases estrogenic activity of the milk.

There are very few reports in the literature concerning the amount of estrogens normally found in milk, and even fewer concerning the increase, if any, in estrogenic compounds in milk of cows treated with stilbestrol. Pope and Roy (1953) estimate that bovine colostrum contains estrogenic activity equivalent to 5.2 ug. estradiol per liter of colostrum. These same workers failed, however, to detect any estrogenic activity in normal milk. In this study, biological assays on six different samples of milk from untreated cows showed no estrogenic activity. The data in Table 13 indicate that the assay used in this study was sensitive to amounts of estrogen equivalent to 0.048 ug. of stilbestrol per 100 cc. of milk. A positive response equivalent to approximately this amount was obtained from the extract prepared from a milk sample of 84A (Table 13). This is approximately one half the amount which Lawson, et al. (1944) estimated was present in a sample of milk from a cow receiving stilbestrol. Even though this one positive response indicates that the feeding of stilbestrol caused a slight increase in the estrogenic activity of milk, it should not be taken as conclusive in view of all of the assays in this study showing no response. It is not altogether unlikely that the stage of gestation or period of estrous cycle could have more effect on the estrogen content of milk than does the feeding of small amounts of stilbestrol if one considers the work by Muench (1954) who showed that the estrogenic activity of normal milk fluctuated with the estrous cycle and increased with gestation.

Approximately 70 percent of the stilbestrol added to a sample of normal milk was recovered in the extract and measured by biological assay. This does not necessarily indicate that 70 percent of any estrogens present in milk would be extracted by the method used, but does indicate that the method is quantitatively accurate for any estrogens that are soluble in the solvents used.

It would appear that the mammary gland is of minor importance in the excretion of estrogens from the animal body, and that the feeding of 5 to 20 mg. of stilbestrol per 1000 pounds of body weight does not add sufficient amounts so that detection in milk by the method used in this study can readily be made.

Reproduction

The results of this experiment show rather conclusively that the reproductive behavior of dairy cattle fed stilbestrol at levels varying from 10 to 15 mg. per 1000 pounds of body weight per day will not be affected in the same manner as beef or dairy cattle receiving injections or implants of stilbestrol.

Numerous reports of undesirable side effects accompanying the administration of stilbestrol to livestock are found in the literature (Bell, et al. 1954, Perry, et al. 1955, and Sleeth, et al. 1953). The most important detrimental manifestation, concerning reproduction, usually accompanying stilbestrol administration is the inactivation of the ovaries. However, inactivation of ovaries was not found to take place in beef heifers fed stilbestrol at levels of 5 to 10 mg. per day (Burroughs, et al., 1955). So far as could be determined, there was no effect whatsoever on the ovaries of any of the dairy cows fed stilbestrol during the course of this study. Probably this lack of characteristic effect on the ovaries is due to the small amount of estrogenic material that is absorbed and made available to exert a physiological response in the animal body. Even though the small amount of estrogenic material absorbed is insufficient to cause detrimental side effects, there seems to be enough present to trigger the mechanism necessary to affect milk production. Other points affirming the lack of effect to the ovaries was the normal cycling and conception of the treated animals.

Another major concern to the practical aspect of stilbestrol administration to dairy cattle is its effect on pregnancy. The number of cases studied in this experiment indicate with fair certainty that 10 to 15 mg. per day of stilbestrol orally administered to dairy cattle will have no harmful effect on pregnancy. To ascertain the effect of levels higher than this, more work must be done since there was one case of abortion in this experiment when the cow received approximately 26 mg. per day for 140 days.

The only problem routinely encountered in this experiment so far as reproductive behavior is concerned was the hypervascularization of the vulva in most treated animals. This condition did not persist but when present did simulate symptoms of estrus from the standpoint of the condition of external genitalia. The degree of hypervascularization varied with animals and apparently was not related to the level of estrogen administration.

Physical Appearance

Another factor leading to the postulation that only small amounts of stilbestrol entered the systematic circulation of the animals of this study is the lack of effect on the physical appearance, and the apparent lack of hypertrophy of the pituitary and adrenal glands that has been reported to take place in animals receiving stilbestrol implants (Clegg and Cole, 1954).

The changes that have been noted by some workers in some of the endocrine glands as a result of stilbestrol administration are thought to be evidence of systemic circulation through the animal body of active stilbestrol on its metabolites. If it is presumed that orally administered stilbestrol is absorbed by the portal circulation, it must pass through the liver, an organ known for its ability to inactivate estrogens, before it can enter the systemic circulation to cause hypertrophy of the adrenal and pituitary. In view of the work of Zondak and Sulman (1939), and Canterow, et al. (1942), who report that synthetic estrogens are detoxified to a smaller extent by the liver than or natural estrogens, it is presumed that small amounts of active stilbestrol do reach the systemic circulation following oral administration.

SUMMARY AND CONCLUSIONS

Seventeen cows from the Kansas State College purebred dairy herd (phase I) and five pairs of identical twin cows (phase II) were used to determine the physiological effects of low level feeding of stilbestrol on milk production and reproductive behavior. In phase I, five cows served as controls and the others received stilbestrol as part of their daily grain ration at levels of 5 to 20 mg. per 1000 pounds of body weight. In phase II, one animal of each twin pair received stilbestrol at the 10 mg. level while the other cow of each pair received the control ration.

Treated and control animals were fed the same ration, except for the addition of sufficient "Stilbosol" to the treated animal's ration to furnish the desired amount of stilbestrol.

The results for phase I which included the production of 13 cows for 114 or 119 days, showed that the animals receiving stilbestrol declined 31.6 percent in production during the treatment period, while the control cows declined 41 percent.

The experimental period of the cows in phase II (identical twins) was of variable length. It ranged from 14 to 32 weeks. The results of this study showed that the treated animals produced an average of 2.9 pounds (13.4 percent) per day more of 4 percent F.C.M. during the period of stilbestrol intake, than did the control animals. Graphs of production are presented, showing that this increase was due to a maintenance of greater persistency throughout the period of stilbestrol intake. Also shown in the results is the fact that the administration of stilbestrol in this experiment had no effect on the percent of fat or percent of total solids produced.

It is concluded that the oral administration of stilbestrol by the addition of "Stilbosol" to the feed in amounts to furnish 10 mg. per 1000 pounds of body weight daily increased production approximately 13 percent.

It is indicated from the data that the addition of stilbestrol to the feed of dairy cattle apparently does not effect efficiency of milk production, but that it does increase total feed consumption approximately 7 percent. This increase in consumption of feed was shown to be due to the increase in grain intake to balance the increased production and not to a stimulatory effect on hay consumption.

Twenty-two biological assays were conducted to determine the effect of stilbestrol administration on the estrogenic activity of milk. Seven assays were made using immature chicks and ovariectomized mice as the assay animals. Powdered and condensed milk from cows receiving stilbestrol was shown to contain insufficient amounts of estrogenic material to evoke a response in these animals. Fifteen biological assays were made using oil solutions of extracts of milk from both treated and control animals. Uterine weights of ovariectomized mice were used as a measurement of stimulation. There was only one case where there was a positive response to an extract of milk from a treated cow. This was from the milk of a cow receiving 20 mg. of stilbestrol per 1000 pounds of body weight per day. It was estimated that the 100 ml. of milk this extract was removed from contained less than .048 ug. of stilbestrol equivalent.

Approximately 70 percent of the stilbestrol added to a sample of milk was recovered when the sample was extracted by the method of Pope and Roy (1953). This showed that the method of extraction was effective in removing soluble estrogens from the milk.

It is concluded that if the treated cows of this study excreted estrogens in their milk, the amounts were below that capable of causing stimulation to the uteri of mice equal to the stimulation obtained by injections of .048 ug. of stilbestrol.

It was shown in the results of phase I, that the feeding of 5 to 15 mg. of stilbestrol per 1000 pounds of body weight per day throughout the gestation of five cows three to five months pregnant, had no adverse effect on gestation. Five normal calves were born to these cows. It was also shown that stilbestrol at these levels did not have any harmful effect on conception rate of cows receiving treatment.

It is shown by data from twins in phase II that the treated animals, all of which received stilbestrol before the first breeding, had an average service per conception of 2.25. This includes five services without conception in the case of one treated animal. All the treated animals of phase II were on the 10 mg. level of stilbestrol feeding. The control animals of phase II conceived to an average of 2.0 services.

It is concluded from these data that the feeding of stilbestrol to dairy cows at the rate of 5 to 15 mg. per 1000 pounds of body weight per day had no adverse effect on pregnancy or conception of treated animals.

The administration of stilbestrol to the cows of this study resulted in no detrimental effects to their physical appearance, nor did it seem to have any effect on rate of growth or gains of body weight.

From the limited data presented in the results it is concluded that the administration of stilbestrol in this experiment did not grossly affect pituitary or adrenal glands.

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APPENDIX

Table 1. Information on lifetime reproductive behavior of cows used in Phase I.

Cow	Gestation number	Times bred	Length of cycle (days)	Gestation (days)	Remarks
112B Goody	1	1		281	
Born 3-28-51	2	4	32, 19, 46	242	Calf born dead
10 mg. level stilbestrol - dry	3	1	(12-28-54)		Due 10-6-55. Pregnant at start of experiment.
123B America	1	5	49,29,49,19	274	
Born 10-11-51	2	7	52, 5,19,29,15,20		
5 mg. level, no stilbestrol dry period			11-9-54 last breeding	270	Calved 8-5-55 - normal. Pregnant at start.
125B Beloved	1	1		279	
Born 12-1-51	2	2	18	281	
5 mg. level	3	2	39 6-8-55 last bred		Not pregnant at start
126B Cinnamon	1	2	39	273	
Born 2-4-52	2	3	38,59	280	
5 mg. level, Stilbestrol dry period			10-26-54 last bred		Calved 8-1-55. Normal Pregnant at start
197A Modesty	1	2	19	274	
Born 2-17-50	2	2		288	
15 mg. level	3	3	25,42	279	
Stilbestrol	4	2	43		Conceived to first service
dry period			11-5-54 first 12-18-54 last No colostrum calf died	269	Calved 7-31-55 14 days early. Pregnant at start.
277A Eve	1	12	27,23,38,21,22,80 41,40,37,20,19	280	
Born 9-7-47					
10 mg. level	2	2	21	280	
No Stilbestrol	3	2	71	273	
Dry period	4	1		280	
	5	4	45,68,22 12-30-54 last bred		Pregnant at start Due 10-8-55.

Table 1. (cont.)

Cow	Gestation number	Times bred	Length of cycle (days)	Gestation (days)	Remarks
279A Gardenia	1	8	42,58,34,39,19,19,20	297	
Born 3-1-48	2	3	18,20	275	
5 mg. level	3	2	24	276	
	4	1		275	
		8 x to slaughter	21,36,85,20,20,22,22 last bred 7-19-55 slaughtered 7-26-55 Bred 5 times on expt.		No embryo recovered Not pregnant at start.
354B Frosting	1	8	21,21,65,44,20,40,86	283	
Born 12-21-49	2	1		285	
15 mg. level	3	1		279	
	4	3	20,23 Last bred 6-24-55 slaughtered		7-26-55 (Preg. 32 days) Not pregnant at start
388B Banter	1	6	22,64,23,79,19	275	Dead calf (Cesarean)
Born 11-30-51		2 x to slaughter	41		Slaughtered 6-28-55. No em bryo recovered Not pregnant at start.
10 mg. level					
419B Honesty	1	2	30	279	
Born 7-13-50	2	5	22,42,20,41	287	
15 mg. level	3	1		290	
	4	2	23 both on experiment		Slaughtered 7-26-55 No embryo recovered. Not pregnant at start.
482A Halo	1	1		278	
Born 4-29-47	2	1		278	
10 mg. level	3	3	23,19	288	
	4	1		278	
	5	1		287	
	6	2	43	293	
	7	4	65, (19),45,23		
		1 Heat not bred	Last two breedings on experiment		Last bred 6-14-55 Slaughtered 7-26-55 Pregnant (42 days) Not pregnant at start
127B Desirous	1	2	70	274	
Born 2-16-52	2	2	18		
Control			Last bred 12-25-54	(Preg)	Pregnant at start

Table 1. (Concl.)

Cow	Gestation number	Times bred	Length of cycle (days)	Gestation (days)	Remarks
180A Brunette	1	1		282	
Born 4-15-49	2	4	19,21,42	285	
Control	3	2	7	288	
	4	2	39	283	
	5	1	Bred 3-28-55		Pregnant at start
268A Hanna	1	1		276	
Born 7-1-46	2	1	(20), (19), (19) cycled 3 times - not bred	279	
Control	3	4	18,19,19	283	
	4	1	(22), (19)	280	
	5	1		285	
	6	3	32, 37	287	
	7	3	21, 20 Last bred 12-21-54		Pregnant at start
290B Elegant	1	8	20,27,42,16,36,20,41	289	
Born 10-24-48	2	2	18	282	
Control	3	2	26	297	
	4	1		287	
	5	2	22 Bred 4-4-55 and 4-26-55 Slaughtered 7-26-55		Not pregnant at start Pregnant 90 days
423B Caramel	1	6	19,9,31,18,60	295	
Born 4-21-51	2	1	(20)	290	
Control	3	1	3-1-55 bred Slaughtered 6-28-55		Pregnant at start Pregnant 119 days
437B Cheer	1	1	Aborted	252	
Born 12-21-43	2	3	20,4	284	
Control	3	1		285	
	4	6	19,24,45,44,20 Last bred 7-15-55		

Table 2. Weekly milk production of control cow, twin pair No. 1 (81A).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Aug. 1 - 7	92.6	4.0	3.704	92.6	13.2		
2. Aug. 8 -14	124.2	4.0	4.968	124.2	17.7	216.8	216.8
3. Aug.15 -21	120.8	4.0	4.832	120.8	17.3	337.6	337.6
4. Aug.22 -28	110.3	3.5	3.861	102.0	14.6	447.9	439.6
5. Aug.29 to Sept. 4	112.3	3.5	3.931	103.9	14.8	560.2	543.5
6. Sept. 5 -11	118.5	4.3	5.096	123.8	17.7	678.7	667.3
7. Sept.12 -18	122.6	4.3	5.272	128.1	18.3	801.3	795.4
8. Sept.19 -25	102.8	4.7	4.832	136.0	19.4	904.1	931.4
9. Sept.26 to Oct. 2	110.6	4.7	5.198	122.2	17.5	1014.7	1053.6
10. Oct. 3 - 9	110.5	5.1	5.636	128.7	18.4	1125.2	1182.3
11. Oct.10 -16	103.0	5.1	5.253	120.0	17.1	1228.2	1302.3
12. Oct.17 -23	109.0	5.5	5.995	133.5	19.1	1337.2	1435.8
13. Oct.24 -30	106.7	5.5	5.869	124.7	17.8	1443.9	1560.5
14. Oct.31 to Nov. 6	109.3	5.3	5.793	130.6	18.7	1553.2	1691.1
15. Nov. 7 -13	105.7	5.3	5.602	126.3	18.0	1658.9	1817.4
16. Nov.14 -20	98.7	5.9	5.823	126.8	18.1	1757.6	1944.2
17. Nov.21 -27	93.7	5.9	5.528	120.4	17.2	1851.3	2064.6
18. Nov.28 to Dec. 4	83.2	5.5	4.576	101.9	14.6	1934.5	2166.5
19. Dec. 5 -11	84.5	5.5	4.648	103.5	14.8	2019.0	2269.0
20. Dec.12 -18	86.6	5.7	4.936	108.7	15.5	2105.6	2377.7
21. Dec.19 -25	94.9	5.7	5.409	119.1	17.0	2200.5	2496.8
22. Dec.26 to Jan. 1	94.5	5.6	5.292	117.2	16.7	2295.0	2614.0
23. Jan. 2 - 8	89.4	5.6	5.006	110.9	15.8	2384.4	2724.9
24. Jan. 9- 15	86.1	5.6	4.822	106.8	15.3	2470.5	2831.7
25. Jan.16 -22	90.8	5.6	5.085	112.6	16.1	2561.3	2944.3
26. Jan.23 -29	83.9	5.6	4.698	104.0	14.8	2645.2	3048.3
27. Jan.30 to Feb. 5	80.8	5.6	4.525	100.2	14.3	2726.0	3148.5
28. Feb. 6 -12	73.2	5.6	4.099	90.8	13.0	2799.2	3239.3
29. Feb.13 -19	79.1	5.6	4.430	98.1	14.0	2878.3	3337.4
30. Feb.20 -26	72.0	5.7	4.104	90.4	12.9	2950.3	3427.8
31. Feb.27 to Mar. 4	82.9	5.7	4.725	104.0	14.9	3033.2	3531.8
32. Mar. 5 -11	77.7	5.3	4.118	92.9	13.3	3110.9	3624.7
33. Mar.12 -18	79.7	5.3	4.224	95.2	13.6	3190.6	3719.9

Table 3. Weekly milk production of experimental cow, twin pair No. 1 (82A).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Aug. 15-21	77.1	3.5	2.699	71.3	10.2		
2. Aug. 22-28	109.0	3.5	3.815	100.8	14.4	186.1	172.1
3. Aug. 29 to Sept. 4	121.6	3.5	4.256	112.5	16.1	307.7	284.6
4. Sept. 5 -11	127.9	4.6	5.883	139.4	19.9	435.6	424.0
5. Sept.12 -18	131.9	4.6	6.067	143.8	20.5	567.5	567.8
6. Sept.19 -25	128.5	4.9	6.297	145.9	20.8	696.0	713.7
7. Sept.26 to Oct. 2	133.0	4.9	6.517	151.0	21.6	829.0	864.7
8. Oct. 3 - 9	130.9	3.7	4.843	125.0	17.9	959.9	989.7
9. Oct.10 -16	119.9	3.7	4.436	114.5	16.4	1079.8	1104.2
10. Oct.17 -23	123.1	5.6	6.894	152.7	21.8	1202.9	1256.9
11. Oct.24 -30	128.2	5.6	7.189	159.0	22.7	1331.1	1415.9
12. Oct.31 to Nov. 6	124.1	5.4	6.701	150.2	21.5	1455.2	1566.1
13. Nov. 7 -13	122.7	5.4	6.626	148.5	21.2	1577.9	1714.6
14. Nov.14 -20	124.9	5.7	7.119	156.7	22.4	1702.8	1871.3
15. Nov.21 -27	114.3	5.7	6.515	143.4	20.5	1817.1	2014.7
16. Nov.28 to Dec. 4	113.9	5.5	6.265	139.5	19.9	1931.0	2154.2
17. Dec. 5 -11	106.3	5.5	5.847	130.2	18.6	2037.3	2284.4
18. Dec.12 -18	115.3	5.4	6.226	139.5	19.9	2152.6	2423.9
19. Dec.19 -25	114.2	5.4	6.167	138.2	19.7	2266.8	2562.1
20. Dec.26 to Jan. 1	117.0	5.7	6.669	146.8	21.0	2383.0	2708.9
21. Jan. 2 - 8	114.5	5.7	6.527	143.7	20.5	2498.3	2852.6
22. Jan. 9 -15	117.3	5.4	6.334	141.9	20.3	2615.6	2994.5
23. Jan.16 -22	115.6	5.4	6.242	139.9	20.0	2731.2	3134.4
24. Jan.23 -29	117.3	5.7	6.686	147.2	21.0	2848.5	3281.6
25. Jan.30 to Feb. 5	116.8	5.7	6.658	146.6	20.9	2965.3	3428.2
26. Feb. 6 -12	110.8	5.7	6.316	139.1	19.9	3076.1	3467.3
27. Feb.13 -19	111.1	5.7	6.333	139.4	19.9	3187.2	3706.7
28. Feb.20 -26	113.4	5.7	6.464	142.3	20.3	3300.6	3849.0
29. Feb.27 to Mar. 4	115.5	5.7	6.584	145.0	20.7	3416.1	3994.0
30. Mar. 5 -11	112.7	5.4	6.086	136.4	19.5	3528.8	4130.4
31. Mar.12- 18	108.2	5.4	5.843	130.9	18.7	3637.0	4261.3
32. Mar.19 -25	108.5	5.7	6.185	136.2	19.5	3745.5	4397.5
44. Mar.26 to April 1	108.6	5.7	6.190	136.3	19.5	3854.1	4533.8

Table 4. Weekly milk production of experimental cow, twin pair No. 2 (83A)

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. June 4 -10	117.3	5.1	5.982	136.7	19.5		
2. June 11-17	125.0	5.1	6.375	145.6	20.8	242.3	282.3
3. June 18-24	125.2	4.4	5.508	132.7	19.0	367.5	415.0
4. June 25 to July 1	118.4	4.4	5.210	125.5	17.9	485.9	540.5
5. July 2 - 8	126.6	4.1	5.191	128.5	18.4	612.5	669.0
6. July 9 -15	128.4	4.1	5.264	130.3	18.6	740.9	799.3
7. July 16-22	117.5	4.2	4.935	121.0	17.3	858.4	920.3
8. July 23-29	112.6	4.2	4.729	116.0	16.6	971.0	1036.3
9. July 30 to Aug. 5	102.6	4.2	4.309	105.7	15.1	1073.6	1142.0
10. Aug. 6 -12	106.0	4.3	4.558	110.8	15.8	1179.6	1252.8
11. Aug. 13-19	95.1	4.3	4.089	99.4	14.2	1274.7	1352.2
12. Aug. 20-26	107.8	4.4	4.743	114.3	16.3	1382.5	1466.5
13. Aug. 27 to Sept. 2	109.0	4.4	4.796	115.5	16.5	1491.5	1582.0
14. Sept. 3 - 9	108.5	4.4	4.774	115.0	16.4	1600.0	1697.0
15. Sept. 10-16	112.3	4.7	5.278	124.1	17.7	1712.3	1821.1
16. Sept. 17-23	60.7	6.2	3.763	80.7	11.5	1773.0	1901.8
17. Sept. 24-30	55.0	6.2	3.410	73.2	10.5	1828.0	1975.0
18. Oct. 1 - 7	86.6	4.2	3.637	89.2	12.7	1914.6	2064.2
19. Oct. 8 -14	92.4	4.2	3.881	95.2	13.6	2007.0	2159.4
20. Oct. 15-21	89.2	4.9	4.371	101.2	14.5	2096.2	2260.6
21. Oct. 22-28	85.8	4.9	4.204	97.4	13.9	2182.0	2358.0
22. Oct. 29 to Nov. 4	90.2	4.8	4.330	101.0	14.4	2272.2	2459.0
23. Nov. 5 -11	85.1	4.8	4.085	95.3	13.6	2357.3	2554.3
24. Nov. 12-Dec. 15	44.0	4.8	2.112	49.3	14.1	2401.3	2603.6

Table 5. Weekly milk production of control cow, twin pair No. 2 (84A). [1]

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. June 4 - 10	124.4	5.5	6.842	152.4	21.8		
2. June 11-17	131.5	5.5	7.233	161.1	23.0	255.9	313.5
3. June 18-24	133.9	4.4	5.892	141.9	20.3	389.8	455.4
4. June 25 to July 1	113.9	4.4	4.012	120.7	17.2	503.7	576.1
5. July 2 - 8	129.1	3.7	4.777	123.3	17.6	632.8	699.4
6. July 9 -15	126.3	3.7	4.673	120.6	17.2	759.1	820.0
7. July 16-22	112.8	4.2	4.783	116.2	16.6	871.9	936.2
8. July 23-29	104.5	4.2	4.389	107.6	15.4	976.4	1043.8
9. July 30 to Aug. 5	101.8	4.2	4.276	104.9	15.0	1078.2	1148.7
10. Aug. 6 -12	101.0	4.3	4.343	105.5	15.1	1179.2	1254.2
11. Aug. 13-19	94.5	4.3	4.064	98.8	14.1	1273.7	1353.0
12. Aug. 20-26	100.6	4.4	4.426	106.6	15.2	1374.3	1459.6
13. Aug. 27 to Sept. 2	99.6	4.4	4.382	105.6	15.1	1473.9	1565.2
14. Sept. 3 - 9	98.9	4.4	4.352	104.8	15.0	1572.8	1670.0
15. Sept. 10-16	104.9	4.7	4.931	115.9	16.6	1677.7	1785.9
16. Sept. 17-23	104.5	5.3	5.539	124.9	17.8	1782.2	1910.8
17. Sept. 24-30	104.3	5.3	5.528	125.6	17.8	1886.5	2035.4
18. Oct. 1 - 7	108.9	5.2	5.663	128.5	18.4	1995.4	2163.9
19. Oct. 8 -14	102.6	5.2	5.335	121.1	17.3	2098.0	2285.0
20. Oct. 15-21	90.5	5.2	4.706	106.8	15.3	2188.5	2391.8
21. Oct. 22-28	98.0	5.2	5.096	115.6	16.5	2286.5	2507.4
22. Oct. 29 to Nov. 4	97.4	5.2	5.065	114.9	16.4	2383.9	2622.3
23. Nov. 5 -11	96.6	5.2	5.023	114.0	16.3	2480.5	2736.3
24. Nov. 12-18	94.3	5.8	5.469	119.8	17.1	2574.8	2856.1
25. Nov. 19-25	89.0	5.8	5.162	113.0	16.1	2663.8	2969.1
26. Nov. 26 to Dec. 2	88.4	5.7	5.039	110.9	15.8	2752.2	3080.0
27. Dec. 3 - 9	89.4	5.7	5.096	112.2	16.0	2841.6	3192.2
28. Dec. 10-16	83.8	5.7	4.777	105.2	15.0	2925.4	3297.4
29. Dec. 17-23	93.6	5.7	5.335	117.5	16.8	3019.0	3414.9
30. Dec. 24-30	98.2	6.0	5.892	127.7	18.2	3117.2	3542.6
31. Dec. 31 to Jan. 6	94.7	6.0	5.682	123.1	17.6	3211.9	3665.7
32. Jan. 7 -13	95.2	5.4	5.141	115.2	16.5	3307.1	3780.9
33. Jan. 14-20	89.5	5.4	4.833	108.3	15.5	3396.6	3889.2
34. Jan. 21-27	91.7	5.7	4.227	115.1	16.4	3488.3	4004.3
35. Jan. 28 to Feb. 3	87.3	5.7	4.976	109.6	15.7	3575.6	4113.9
36. Feb. 4 -10	82.3	6.1	5.020	108.2	15.5	3657.4	4222.1

Table 5. (Concl.)

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
37. Feb. 11-17	93.3	6.1	5.691	122.7	17.5	3751.2	4344.8
38. Feb. 18-24	86.7	5.9	5.115	111.4	15.9	3837.9	4456.2
39. Feb. 25 to Mar. 2	87.1	5.9	5.139	111.9	16.0	3925.0	4568.1
40. Mar. 3- 9	88.6	5.5	4.873	108.5	15.5	4013.6	4676.6
41. Mar. 10-16	80.5	5.5	4.428	98.6	14.1	4044.1	4775.2
42. Mar. 17 to April 21 (4½ days)	49.4	5.5	2.717	60.5	13.4	4143.5	4835.7

$\underline{/1}$ This animal was started on 20 mg. of stilbestrol per 1000 pound of body weight per day on December 7, 1955.

Table 6. Weekly milk production of experimental cow, twin pair No. 3 (85A).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Sept. 14-20	117.5	4.6	5.410	128.2	18.3		
2. Sept. 21-27	138.2	4.6	6.357	150.6	21.5	255.8	278.8
3. Sept. 28 to Oct. 4	137.4	4.6	6.320	149.8	21.4	393.2	428.6
4. Oct. 5 -11	143.7	5.0	7.185	165.3	23.6	536.9	593.9
5. Oct. 12-18	142.6	5.0	7.130	164.0	23.4	679.5	757.9
6. Oct. 19-25	138.9	5.1	7.084	161.8	23.1	818.4	919.7
7. Oct. 26 to Nov. 1	135.0	5.0	6.750	155.3	22.2	953.4	1075.0
8. Nov. 2 - 8	114.6	5.0	5.730	131.8	18.8	1068.0	1206.8
9. Nov. 9 -15	96.5	5.7	5.501	121.1	17.3	1164.5	1327.9
10. Nov. 16-22	94.0	5.7	5.358	118.0	16.9	1258.5	1445.9
11. Nov. 23-29	92.4	5.5	5.082	113.2	16.2	1350.9	1559.1
12. Nov. 30 to Dec. 6	82.1	5.5	4.516	100.6	14.4	1433.0	1659.7
13. Dec. 7 -13	77.5	5.7	4.418	97.3	13.9	1510.5	1757.0
14. Dec. 14-20	74.6	5.7	4.252	93.6	13.4	1585.1	1850.6
15. Dec. 21-27	69.7	5.9	4.112	89.6	12.8	1654.8	1940.2
16. Dec. 28 to Jan. 3	70.6	5.9	4.165	90.7	13.0	1725.4	2030.9
17. Jan. 4 -10	64.3	5.7	3.665	80.7	11.5	1789.7	2111.6
18. Jan. 11-17	57.1	5.7	3.255	71.7	10.2	1846.8	2183.3
19. Jan. 18-24	57.0	5.8	3.306	72.4	10.3	1903.8	2255.7
20. Jan. 25-31	60.4	5.8	3.503	76.7	11.0	1964.2	2331.9
21. Feb. 1 to 7	59.0	5.2	3.068	69.6	9.9	2023.2	2401.5
22. Feb. 8 -14	58.6	5.2	3.047	69.1	9.9	2081.8	2470.6
23. Feb. 15-21	58.4	5.4	3.154	70.7	10.1	2140.2	2541.3
24. Feb. 22-28	58.8	5.4	3.175	71.1	10.2	2199.0	2612.4
25. Feb. 29 to Mar. 6	59.9	5.6	3.354	74.3	10.6	2258.9	2686.7
26. Mar. 7 -13	60.7	5.6	3.399	75.3	10.8	2319.6	2762.0
27. Mar. 14-20	59.8	5.5	3.289	73.3	10.5	2374.4	2835.3
28. Mar. 21-27	59.5	5.5	3.273	72.9	10.4	2438.9	2908.2
29. Mar. 28 to Apr. 3	58.5	5.5	3.218	71.7	10.2	2497.4	2979.9

Table 7. Weekly milk production of control cow, twin pair No. 3 (86A).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Nov. 4 -10	117.3	4.7	5.513	129.6	18.5		
2. Nov. 11-17	128.2	5.1	6.538	149.4	21.3	245.5	279.0
3. Nov. 18-24	136.2	5.1	6.946	158.7	22.7	381.7	437.7
4. Nov. 25 to Dec. 1	134.8	5.4	7.279	163.1	23.3	516.5	600.8
5. Dec. 2 - 8	119.7	5.4	6.464	144.8	20.7	636.2	745.6
6. Dec. 9 -15	122.6	6.1	7.479	161.2	23.0	758.8	906.8
7. Dec. 16-22	114.0	6.1	6.954	149.9	21.4	372.8	1056.7
8. Dec. 23-29	118.8	5.9	7.009	152.7	21.8	991.6	1209.4
9. Dec. 30 to Jan. 5	112.0	5.9	6.608	143.9	20.6	1103.6	1353.3
10. Jan. 6 -12	111.5	5.7	6.356	139.9	20.0	1215.1	1493.2
11. Jan. 13-19	101.0	5.7	5.757	126.8	18.1	1316.1	1620.0
12. Jan. 20-26	97.5	6.0	5.850	126.8	18.1	1413.6	1746.8
13. Jan. 27 to Feb. 2	102.4	6.0	6.144	133.1	19.0	1516.0	1874.9
14. Feb. 3 - 9	91.3	5.2	4.748	107.7	15.4	1607.3	1982.6
15. Feb. 10-16	88.2	5.2	4.586	104.1	14.9	1695.5	2086.7
16. Feb. 17-23	81.5	5.5	4.483	99.8	14.3	1777.0	2186.5
17. Feb. 24 to Mar. 1	75.9	5.5	4.175	93.0	13.3	1852.9	2279.5
18. Mar. 2 - 8	72.2	5.0	3.610	83.0	11.9	1925.1	2362.5
19. Mar. 9 -15	71.3	5.0	3.565	82.0	11.7	1996.4	2444.5
20. Mar. 16-22	70.5	5.7	4.019	88.5	12.6	2066.9	2533.0
21. Mar. 23-29	64.2	5.7	3.659	80.6	11.5	2131.1	2613.6
22. Mar. 30 to Apr. 5	59.7	5.6	3.343	74.0	10.6	2190.8	2687.6
23. Apr. 6 -12	60.6	5.6	3.394	75.1	10.7	2251.4	2762.7

Table 8. Weekly milk production of control cow, twin pair No. 4 (1948).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Aug. 14-20	256.4	4.7	12.051	283.3	40.5		
2. Aug. 21-27	289.2	3.0	8.676	245.8	35.1	545.6	529.1
3. Aug. 28 to Sept. 3	305.1	3.0	9.153	259.3	37.1	850.7	788.4
4. Sept. 4 -10	357.7	2.8	10.016	293.3	41.9	1208.4	1081.7
5. Sept. 11-17	369.3	2.8	10.340	302.8	43.3	1577.7	1384.5
6. Sept. 18-24	351.6	2.9	10.196	293.6	41.9	1929.3	1678.1
7. Sept. 25 to Oct. 1	343.1	2.9	9.950	286.5	40.9	2272.4	1964.6
8. Oct. 2 - 8	335.2	2.9	9.721	279.9	40.0	2607.6	2244.5
9. Oct. 9 -15	306.0	2.9	8.874	255.5	36.5	2913.6	2500.0
10. Oct. 16-22	290.6	2.8	8.137	238.3	34.0	3204.2	2738.3
11. Oct. 23-29	302.7	2.8	8.476	248.2	35.5	3506.9	2986.5
12. Oct. 30 to Nov. 5	274.5	2.8	7.686	225.1	32.2	3781.4	3211.6
13. Nov. 6- 12	265.9	2.8	7.445	218.0	31.1	4047.3	3429.6
14. Nov. 13-19	252.0	3.2	8.064	221.8	31.7	4299.3	3651.4
15. Nov. 20-26	262.0	3.2	8.384	230.6	32.9	4561.3	3882.0
16. Nov. 27 to Dec. 3	266.8	3.4	9.071	242.8	34.7	4828.1	4124.8
17. Dec. 4 -10	249.2	3.4	8.473	226.8	32.4	5077.3	4351.6
18. Dec. 11-17	245.4	3.8	9.325	238.0	34.0	5322.7	4589.6
19. Dec. 18-24	262.8	3.8	9.986	254.9	36.4	5585.5	4844.5
20. Dec. 25-31	259.7	3.2	8.310	228.5	32.6	5845.2	5073.0
21. Jan. 1 - 7	261.3	3.2	8.362	229.9	32.8	6106.5	5302.9
22. Jan. 8 -14	244.5	3.6	8.802	229.8	32.8	6351.0	5537.7
23. Jan. 15-21	250.1	3.6	9.004	235.1	33.6	6601.1	5767.8
24. Jan. 22-28	253.4	3.5	8.869	234.4	33.5	6854.5	6002.2
25. Jan. 29 to Feb. 4	232.1	3.5	8.124	214.7	30.7	7086.6	6216.9
26. Feb. 5 -11	228.3	3.8	8.675	221.5	31.6	7314.9	6438.4
27. Feb. 12-18	224.3	3.8	8.523	217.6	31.1	7539.2	6656.0
28. Feb. 19-25	217.2	3.4	7.385	197.7	28.2	7756.4	6853.7
29. Feb. 26 to Mar. 3	224.1	3.4	7.619	203.9	29.1	7980.5	7057.8
30. Mar. 4 -10	204.6	3.7	7.570	194.9	27.8	8185.1	7252.5
31. Mar. 11-17	206.2	3.7	7.629	196.9	28.1	8391.3	7449.4
32. Mar. 18-24	198.6	3.4	6.752	180.7	25.8	8589.9	7630.1
33. Mar. 25-31	189.1	3.4	6.429	172.1	24.6	8779.0	7802.2

Table 9. Weekly milk production of experimental cow, twin pair No. 4 (195B).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Aug. 4 -10	190.4	3.8	7.235	184.7	24.6		
2. Aug. 11-17	300.0	3.8	11.400	291.0	41.6	490.4	475.7
3. Aug. 18-24	320.4	2.7	8.651	257.9	36.8	810.8	733.6
4. Aug. 25-31	326.3	2.7	8.810	262.7	37.5	1137.1	996.3
5. Sept. 1 -7	357.7	3.2	9.658	288.0	41.1	1494.8	1284.3
6. Sept. 8-14	355.5	3.2	11.376	312.8	44.7	1850.3	1597.1
7. Sept. 15-21	362.1	2.7	9.777	291.5	41.6	2212.4	1888.6
8. Sept. 22-28	367.7	2.7	9.928	296.0	42.3	2580.1	2184.6
9. Sept. 29 to Oct. 5	355.5	2.9	10.310	296.9	42.4	2935.6	2481.5
10. Oct. 6 -12	320.1	2.9	9.283	267.3	38.2	3255.7	2748.8
11. Oct. 13-19	312.8	3.0	9.384	265.9	38.0	3568.5	3014.7
12. Oct. 20-26	311.6	3.0	9.348	264.9	37.8	3880.1	3279.6
13. Oct. 27 to Nov. 2	319.9	2.9	9.277	267.1	38.2	4199.2	3546.7
14. Nov. 3 - 9	314.0	2.9	9.106	262.2	37.5	4513.2	3808.9
15. Nov. 10-16	315.3	3.4	10.720	286.9	41.0	4828.5	4095.8
16. Nov. 17-23	300.7	3.4	10.224	273.6	39.1	5129.2	4369.4
17. Nov. 24-30	272.8	3.4	9.275	248.2	35.5	5402.0	4617.6
18. Dec. 1 - 7	260.5	3.4	8.857	237.1	33.9	5662.5	4854.7
19. Dec. 8 -14	268.9	3.8	10.218	260.8	37.3	5931.4	5115.5
20. Dec. 15-21	265.3	3.8	10.081	257.3	36.8	6196.7	5372.8
21. Dec. 22-28	287.9	4.7	13.531	318.1	45.4	6484.6	5690.9
22. Dec. 29 to Jan. 4	281.0	4.7	13.207	310.5	44.4	6765.6	6001.4
23. Jan. 5 -11	279.8	3.6	10.073	263.0	37.6	7045.4	6264.4
24. Jan. 12-18	262.7	3.6	9.457	246.9	35.3	7308.1	6511.3
25. Jan. 19-25	250.9	3.5	8.782	232.1	33.2	7559.0	6743.4
26. Jan. 26 to Feb. 1	252.1	3.5	8.824	233.2	33.3	7811.1	6976.6
27. Feb. 2 - 8	255.2	3.8	9.698	247.5	35.4	8066.3	7224.1
28. Feb. 9 -15	234.1	3.8	8.896	227.1	32.4	8300.4	7451.2
29. Feb. 16-22	239.2	3.4	8.133	217.7	31.1	8539.6	7668.9
30. Feb. 23-29	252.0	3.4	8.568	212.1	30.3	8791.6	7881.0
31. Mar. 1 to 7	233.1	3.7	8.625	222.6	31.8	9024.7	8103.6
32. Mar. 8 -14	236.6	3.7	8.754	226.0	32.3	9261.3	8329.6
33. Mar. 15-21	222.5	3.5	7.788	205.8	29.4	9483.8	8535.4
34. Mar. 22-28	227.8	3.5	7.973	210.7	30.1	9711.6	8746.1

Table 10. Weekly milk production of control cow, twin pair No. 5 (40A).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Oct. 9 -15	171.5	3.6	6.174	161.2	23.0		
2. Oct. 16-22	200.0	3.6	7.200	188.0	26.9	371.5	349.2
3. Oct. 23-29	200.1	3.6	7.204	188.1	26.9	571.6	537.3
4. Oct. 30 to Nov. 5	203.2	4.0	8.128	203.2	29.0	774.8	740.5
5. Nov. 6 -12	203.6	4.0	8.144	203.6	29.1	978.0	944.1
6. Nov. 13-19	188.5	3.7	6.975	180.0	25.7	1166.5	1124.1
7. Nov. 20-26	188.5	3.7	6.975	180.0	25.7	1355.0	1304.1
8. Nov. 27 to Dec. 3	174.9	3.9	6.821	172.3	24.6	1529.9	1476.4
9. Dec. 4 -10	168.6	3.9	6.575	166.1	23.7	1698.5	1642.5
10. Dec. 11-17	166.4	4.1	6.822	168.9	24.1	1864.9	1811.4
11. Dec. 18-24	166.7	4.1	6.835	169.2	24.2	2031.6	1980.6
12. Dec. 25-31	164.0	4.1	6.724	166.5	23.8	2195.6	2147.1
13. Jan. 1 -7	160.0	4.1	6.560	162.4	23.2	2355.6	2309.5
14. Jan. 8-14	160.1	4.0	6.404	160.1	22.9	2515.7	2469.6
15. Jan. 15-21	149.0	4.0	5.960	149.0	21.3	2664.7	2618.6
16. Jan. 22-28	146.0	4.2	6.132	150.4	21.5	2810.7	2769.0
17. Jan. 29 to Feb. 4	141.1	4.2	5.926	145.3	20.8	2951.8	2914.3
18. Feb. 5 -11	145.6	4.0	5.824	145.6	20.8	3097.4	3059.9
19. Feb. 12-18	147.0	4.0	5.880	147.0	21.0	3244.4	3206.9
20. Feb. 19-25	144.3	4.1	5.916	146.5	20.9	3388.7	3353.4
21. Feb. 26 to Mar. 3	143.3	4.1	5.875	145.4	20.8	3532.0	3498.8
22. Mar. 4 -10	141.4	4.0	5.656	141.4	20.2	3673.4	3640.2
23. Mar. 11-17	142.7	4.0	5.708	142.7	20.4	3816.1	3782.9
24. Mar. 18-24	140.2	4.0	5.608	140.2	20.0	3956.3	3923.1
25. Mar. 25-31	142.9	4.0	5.716	142.9	20.4	4099.2	4066.0
26. April 1 to May 7	142.1	4.0	5.684	142.1	20.3	4241.3	4208.1

Table 11. Weekly milk production of experimental cow, twin pair No. 5 (41A).

Week of lactation and date	Total production	Percent fat	Pounds fat	Total F.C.M.	Average daily F.C.M.	Total actual production	Total F.C.M.
1. Sept. 27 to Oct. 3	235.8	3.1	7.310	204.0	29.1		
2. Oct. 4 -10	237.9	3.1	7.375	205.8	29.4	473.7	409.8
3. Oct. 11-17	234.5	3.1	7.270	202.9	29.0	708.2	612.7
4. Oct. 18-24	223.8	4.3	9.623	233.9	33.4	932.0	846.6
5. Oct. 25-31	234.6	4.3	10.088	245.2	35.0	1166.6	1091.8
6. Nov. 1 - 7	228.1	4.2	9.580	234.9	33.6	1394.7	1326.7
7. Nov. 8 -14	223.3	4.2	9.379	230.0	32.9	1618.0	1556.7
8. Nov. 15-21	220.4	4.0	8.816	220.4	31.5	1838.4	1777.1
9. Nov. 22-28	214.7	4.0	8.588	214.7	30.7	2053.1	1991.8
10. Nov. 29 to Dec. 5	202.0	3.9	7.878	199.0	28.4	2255.1	2190.8
11. Dec. 6 -12	198.2	3.9	7.730	195.2	27.9	2453.3	2386.0
12. Dec. 13-19	186.9	4.1	7.663	189.7	27.1	2640.2	2575.7
13. Dec. 20-26	189.2	4.1	7.757	192.0	27.4	2829.4	2767.7
14. Dec. 27 to Jan. 2	182.3	4.1	7.474	185.0	26.4	3011.7	2952.7
15. Jan. 3 - 9	169.6	4.1	6.954	172.1	24.6	3181.3	3124.8
16. Jan. 10-16	165.1	4.0	6.604	165.1	23.6	3346.4	3289.9
17. Jan. 17-23	163.1	4.0	6.524	163.1	23.3	3509.5	3453.0
18. Jan. 24-30	161.5	4.2	6.783	166.3	23.8	3671.0	3619.3
19. Jan. 31 to Feb. 6	158.6	4.2	6.661	163.4	23.3	3829.6	3782.7
20. Feb. 7 -13	156.6	4.1	6.421	158.9	22.7	3986.2	3941.6
21. Feb. 14-20	148.6	4.1	6.093	150.8	21.5	4134.8	4092.4
22. Feb. 21-27	138.4	4.1	5.674	140.5	20.1	4273.2	4232.9
23. Feb. 28 to Mar. 15	150.8	4.1	6.183	153.1	21.9	4424.0	4386.0
24. Mar. 6 -12	147.0	4.0	5.880	147.0	21.0	4571.0	4533.0
25. Mar. 13-19	153.6	4.0	6.144	153.6	21.9	4724.6	4686.6
26. Mar. 20-26	153.6	4.2	6.451	158.2	22.6	4878.2	4844.8
27. Mar. 27 to Apr. 2	153.7	4.2	6.455	158.3	22.6	5031.9	5003.1

Table 12. Total solids production of twin pair 1 during experiment.

	81A (Control)			82A (Treated)		
	Total milk produced 14 days	Percent total solids	Total solids produced 14 days	Total milk produced 14 days	Percent total solids	Total solids produced 14 days
	216.8	13.4	29.1	186.1	12.9	24.0
	231.1	12.6	29.1	249.5	14.5	36.2
	230.8	13.7	31.6	260.4	14.5	37.8
	225.4	14.2	32.0	263.9	13.3	35.1
	221.1	14.9	32.9	243.0	14.8	36.0
	212.0	15.4	32.6	252.3	14.7	37.1
	216.0	15.2	32.8	247.6	15.7	38.9
	204.4	15.9	32.5	228.2	15.5	35.4
	176.9	15.5	27.4	221.6	15.2	33.7
	171.1	15.9	27.2	231.2	15.6	36.1
	189.4	15.8	29.9	231.8	15.1	35.0
	175.5	15.8	27.7	232.9	15.6	26.3
	174.7	15.7	27.4	227.6	15.6	35.5
	154.0	15.8	24.3	224.5	15.6	35.0
	151.1	16.1	24.3	228.2	14.8	33.8
	160.6	14.8	23.8	216.7	15.7	34.0
Total	3110.9	14.94	464.8	3745.5	14.94	559.7

Table 13. Total solids production of twin pair 2 during experiment.

	83A (Treated)			84A (Control)		
	Total milk produced 14 days	Percent total solids	Total solids produced 14 days	Total milk produced 14 days	Percent total solids	Total solids produced 14 days
	242.3	14.1	34.2	255.9	14.5	37.1
	243.6	13.6	33.1	247.8	13.9	34.4
	255.0	13.7	34.9	255.4	13.5	34.5
	230.1	13.4	30.8	217.3	13.8	30.0
	208.6	13.5	28.2	202.8	13.5	27.4
	202.9	13.7	27.8	195.1	13.6	26.5
	217.5 /1	14.1	30.7	198.5	13.9	27.6
Total	1600.0	13.73	219.7	1572.8	13.83	217.5
	173.0	15.0	26.0	209.4	14.7	30.8
	141.6	13.3	18.8	213.2	15.1	32.2
	181.6	13.9	25.2	193.1	14.5	28.0
	176.0 /2	14.1	24.8	195.4	14.7	28.7
				190.9	15.1	28.8
				177.4	15.3	27.1
				173.2 /3	15.4	26.7
				191.8	16.0	30.7
				189.9	15.0	28.5
				181.2	15.5	28.1
				169.6	16.0	27.2
				180.0	15.8	28.4
				175.7	15.2	26.7
Total				1629.7	15.47	252.2

/1 Went off feed at the end of 14 weeks.

/2 Death (accidental).

/3 Put on 20 mg. level.

Table 14. Total solids production of twin pair 3 during experiment.

	85A (Treated)			86A (Control)		
	Total milk produced 14 days	Percent total solids	Total solids produced 14 days	Total milk produced 14 days	Percent total solids	Total solids produced 14 days
	255.8	14.1	36.1	245.5	14.1	34.6
	281.1	13.4	37.7	271.0	15.6	42.3
	281.5	14.8	41.7	242.3	15.8	38.3
	249.6	15.6	36.4	232.8	15.8	36.8
	190.5	15.6	29.7	223.5	16.1	36.0
	174.5	14.8	25.8	198.5	15.6	31.0
	152.1	15.4	23.4	193.7	16.1	31.2
	140.3	16.0	22.4	169.7	15.1	25.6
	121.3	15.5	18.8	148.1	16.4	24.3
	117.4	15.8	18.5	141.8	14.4	20.4
Total	1964.2	14.80	290.6	2066.9	15.50	320.4
	117.6	15.8	17.9			
	117.2	15.5	18.2			
	120.6	14.7	17.7			
	119.3	14.5	17.3			

Table 15. Total solids production of twin pair 4 during experiment.

	194B (Control)			195B (Treated)		
	Total milk produced 14 days	Percent total solids	Total solids produced 14 days	Total milk produced 14 days	Percent total solids	Total solids produced 14 days
	545.6	13.5	74.7	490.4	11.9	58.4
	662.8	10.7	71.0	646.7	10.4	67.3
	720.9	11.0	79.3	713.2	11.0	78.5
	678.3	11.0	74.6	729.8	10.7	78.1
	596.6	11.0	65.6	675.6	11.0	74.3
	577.2	11.1	64.1	624.4	11.6	72.4
	517.9	11.0	57.0	633.9	10.9	69.1
	528.8	11.9	62.9	616.0	11.7	72.1
	494.6	12.0	59.4	533.3	11.9	63.5
	522.5	11.9	62.2	534.2	11.9	63.6
	505.8	11.8	59.7	568.9	14.0	79.6
	503.5	11.6	58.4	542.5	12.1	65.6
	460.4	12.2	56.2	503.0	11.8	59.4
	441.5	12.3	54.3	489.3	12.2	59.7
	428.7	12.1	51.9	491.2	12.0	58.9
	404.8	11.8	47.8	469.7	12.4	58.2
Total	8589.9	11.62	997.8	9261.3	11.65	1078.6

Table 16. Total solids production of twin pair 5 during experiment.

	40A (Control)			41A (Treated)		
	Total milk produced 14 days	Percent total solids	Total solids produced 14 days	Total milk produced 14 days	Percent total solids	Total solids produced 14 days
	371.5	12.7	47.7	473.7	12.4	58.7
	403.3	13.2	53.2	458.3	13.6	62.3
	392.1	12.4	48.6	462.7	13.6	62.9
	363.4	13.1	47.6	443.7	12.4	55.0
	335.0	13.1	43.9	416.7	12.5	52.1
	330.7	13.2	43.7	385.1	13.4	51.6
	320.1	12.8	41.0	371.5	13.6	50.5
	295.0	13.6	40.1	334.7	12.9	43.2
	286.7	13.0	37.3	324.6	13.6	44.1
	291.3	13.2	38.5	315.2	13.5	42.6
	284.7	12.9	36.7	287.0	13.5	38.7
	282.9	12.8	36.2	297.8	12.8	38.1
	285.0	12.8	36.5	307.2	13.6	41.8
Total	4241.3	12.98	550.4	4878.2	13.16	641.7

Table 17. Total digestible nutrient and total protein intake of twin pair 1.

	28 day periods	D.P. Intake (lb.) Source Alfalfa hay (10%)	Grain (10%)	Silage (1.5%)	Total	T.D.N. Intake (lb.) Source Alfalfa hay (50%)	Grain (75%)	Silage (18%)	Total	4% F.C.M. T.D.N. (lb.)
81A										
Control	1	38.4	15.8	-	54.2	192.2	118.6	-	310.8	1.41
	2	47.8	15.1	-	62.9	238.8	113.0	-	351.8	1.40
	3	49.5	16.6	2.1	68.2	247.3	124.7	25.6	397.6	1.45
	4	46.8	17.3	5.0	69.1	203.8	130.0	59.8	393.6	1.29
	5	34.2	17.4	5.0	56.6	170.8	130.4	59.8	361.0	1.20
	6	44.6	14.9	5.0	64.5	222.9	112.1	60.5	395.5	1.15
	7	33.9	15.0	5.0	53.9	169.5	112.2	60.5	342.2	1.19
	8	29.8	13.4	5.0	48.2	148.8	100.7	60.5	310.0	1.24
82A										
Treated	1 /1	31.0	7.4	-	38.4	154.8	55.2	-	210.0	1.20
	2	45.7	18.7	2.7	67.1	228.4	140.4	3.2	372.0	1.52
	3	43.9	19.1	4.3	67.3	219.5	142.7	51.8	414.0	1.39
	4	40.0	20.4	5.0	65.4	199.9	153.0	60.5	413.4	1.42
	5	36.0	17.8	5.0	58.8	180.2	133.7	59.8	373.1	1.48
	6	42.7	18.9	5.0	66.6	213.7	142.1	60.5	416.3	1.38
	7	28.3	19.4	5.0	52.7	141.6	145.5	60.5	347.6	1.63
	8	33.6	18.8	5.0	57.4	168.1	141.3	50.6	369.9	1.48

/1 14 days

Table 18. Total digestible nutrient and total protein intake of twin pair 2.

	28 day periods	D.P. Intake (lb.) Source: Alfalfa hay (10%)	D.P. Intake (lb.) Source: Grain (10%)	D.P. Intake (lb.) Source: Silage (1.5%)	D.P. Intake (lb.) Total	T.D.N. Intake (lb.) Source: Alfalfa hay (50%)	T.D.N. Intake (lb.) Source: Grain (75%)	T.D.N. Intake (lb.) Source: Silage (18%)	T.D.N. Intake (lb.) Total	4% F.C.M. / T.D.N. (lb.)
83A										
Treated	1	40.3	17.1	-	57.4	201.3	128.0	-	329.3	1.38
	2	56.0	14.8	-	70.8	279.9	111.2	-	391.1	1.14
	3	33.3	13.9	-	47.2	166.3	104.2	-	270.5	1.36
	4	53.7	12.8	4.6	71.1	268.5	95.7	54.7	418.9	.94
	5 [1]	13.6	5.0	1.8	20.4	68.0	37.3	21.6	126.9	1.14
84A										
Control	1	43.0	16.9	-	59.9	215.0	126.8	-	341.8	1.27
	2	57.2	14.1	-	71.3	285.9	105.7	-	391.0	1.06
	3	66.2	15.4	-	81.6	331.1	115.4	-	446.5	1.11
	4	51.1	16.5	4.6	72.2	255.5	124.0	54.7	434.2	1.06
	5	43.2	15.7	5.0	63.9	215.8	118.1	60.5	394.4	1.16
	6	49.8	15.3	5.6	70.7	249.2	114.5	67.7	431.4	1.07
	7	48.2	16.1	5.9	70.2	241.0	120.6	70.6	432.2	1.07
	8	45.0	15.4	5.9	66.3	225.2	115.7	70.6	411.5	1.10
	9 [2]	33.8	15.0	5.3	54.1	168.8	112.5	63.0	344.3	1.10

[1] 11.5 days

[2] 25.5 days

Table 19. Total digestible nutrient and total protein intake of twin pair 3.

	28 day periods	D.P. Intake (lb.) Source: Alfalfa hay (10%)	D.P. Source: Grain (10%)	D.P. Source: Silage (1.5%)	D.P. Total	T.D.N. Intake (lb.) Source: Alfalfa hay (50%)	T.D.N. Source: Grain (75%)	T.D.N. Source: Silage (18%)	T.D.N. Total	4 % F.C.M. / T.D.N. (lb.)
85A										
Treated	1 /1	21.8	16.5	.8	39.1	109.2	123.5	9.0	241.7	1.30
	2	51.8	18.6	5.5	75.9	259.1	139.4	66.6	465.1	1.32
	3	51.0	17.2	8.4	76.6	254.8	129.2	100.8	484.8	.93
	4	55.6	13.3	8.3	77.2	277.9	94.8	99.9	477.6	.78
	5	50.4	10.2	8.4	69.0	252.2	76.7	100.8	429.7	.70
	6	43.8	8 .5	8.3	60.6	219.2	63.7	99.0	381.8	.73
	7	47.4	8.3	8.4	64.1	237.2	62.0	100.8	400.0	.74
86A										
Control	1 /2	29.5	12.4	6.9	48.8	147.3	93.2	82.8	323.3	1.50
	2	50.1	18.3	8.3	76.7	250.7	137.5	99.9	488.1	1.25
	3	49.3	17.3	8.4	75.0	246.4	130.1	100.8	477.3	1.13
	4	55.0	15.1	8.4	78.5	275.1	113.5	100.8	489.4	.90
	5	60.7	12.1	8.4	81.2	303.7	90.5	100.8	495.0	.70

/1 14 days

/2 21 days

Table 20. Total digestible nutrient and total protein intake of twin pair 4.

	28 day periods	D.P. Intake (lb.) Source: Alfalfa hay (10%)	D.P. Intake (lb.) Source: Grain (10%)	D.P. Intake (lb.) Source: Silage (1.5%)	D.P. Intake (lb.) Total	T.D.N. Intake (lb.) Source: Alfalfa hay (50%)	T.D.N. Intake (lb.) Source: Grain (75%)	T.D.N. Intake (lb.) Source: Silage (18%)	T.D.N. Intake (lb.) Total	4% F.C.M. T.D.N. (lb.)
194B										
Control	1 /1	29.0	14.9	-	43.9	145.1	111.5	-	256.6	2.15
	2	70.9	32.4	.2	103.5	354.4	243.2	2.9	600.5	1.94
	3	64.2	31.9	8.1	105.2	321.1	239.0	97.7	657.8	1.47
	4	53.9	27.1	8.4	89.4	269.4	203.6	100.8	573.8	1.59
	5	64.7	27.4	8.4	100.5	321.1	205.7	100.8	630.1	1.50
	6	66.5	27.3	8.4	102.2	332.3	204.5	100.8	637.6	1.46
	7	66.9	26.7	8.4	102.0	334.3	200.3	100.8	635.4	1.36
	8	61.6	24.1	8.4	94.1	307.8	180.8	100.8	589.4	1.32
195B										
Treated	1	37.1	24.6		61.7	185.5	184.5	-	370.0	2.69
	2	61.3	33.0		94.3	306.5	247.8	-	554.3	2.14
	3	71.8	32.9	5.2	109.9	359.1	246.5	62.8	668.4	1.64
	4	70.7	32.4	8.8	111.9	353.4	242.9	105.8	702.1	1.55
	5	62.4	30.3	8.8	101.5	311.9	227.2	105.8	644.9	1.56
	6	66.8	31.7	8.4	106.9	334.0	237.4	100.8	672.2	1.69
	7	55.5	30.5	8.4	94.4	277.6	228.9	100.8	607.3	1.55
	8	60.0	26.8	8.4	95.2	299.8	200.9	100.8	601.5	1.46

/1 14 days

Table 21. Total digestible nutrient and total protein intake of twin pair 5.

	28 day periods	D.P. Intake (lb.) Source: Alfalfa hay (10%)	Grain (10%)	Silage (1.5%)	Total	T.D.N. Intake (lb.) Source: Alfalfa hay (50%)	Grain (75%)	Silage (18%)	Total	4 % F.C.M. / T.D.N (lb.)
40A										
Control	1 /1	44.8	15.0	5.4	65.2	223.9	112.5	65.2	401.6	1.42
	2	58.7	22.2	8.4	89.3	293.3	166.5	100.8	560.6	1.31
	3	57.8	20.6	8.4	86.8	289.1	154.8	100.8	544.7	1.23
	4	48.5	19.2	8.4	76.1	242.4	143.7	100.8	486.9	1.28
	5	44.6	17.5	8.4	70.5	222.9	131.3	100.8	455.0	1.28
	6	56.8	16.6	8.4	81.8	283.8	124.4	100.8	509.0	1.12
41A										
Treated	1 /2	26.9	10.9	3.1	40.9	134.7	81.9	37.1	253.7	1.72
	2	53.3	27.0	8.1	88.4	266.3	202.8	97.2	566.3	1.64
	3	69.0	25.6	8.4	103.4	345.0	191.9	100.8	637.7	1.25
	4	63.9	22.6	8.4	94.9	319.4	169.3	100.8	589.5	1.21
	5	70.2	20.6	8.4	99.2	351.1	150.5	100.8	602.4	1.08
	6	56.8	18.4	8.3	83.5	284.2	137.9	99.0	521.1	1.13

/1 21 days

/2 14 days

Table 22. Reproductive history for control cow 81A (Twin pair 1)

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
8-10-55				8-31-55	Signs of heat
8-28-55	p.m.				
8-29-55	a.m.		20	9-19-55	
8-31-55					Post estrous bleeding
9-19-55	p.m.		21	10-10-55	
9-20-55					Post estrous bleeding
10-13-55	a.m.	5:00 p.m.	24	11- 2-55	
10-16-55					Post estrous bleeding
11- 6-55	a.m.	5:00 p.m.	25	11-26-55	
11- 9-55					Post estrous bleeding
11-28-55	a.m.	5:00 p.m.	23	12-18-55	
12-22-55 /1	p.m.	a.m. 23rd	25	1-11-55	
1- 4-56	a.m.	p.m.	24	2- 3-56	
1-16-56					Post estrous bleeding
2-20-56					Pregnant to J-12

/1 All identical twins bred with frozen semen up to December 2, 1955, after this, regular semen used, except were natural service is indicated.

Table 23. Reproductive history for experimental cow 82A (Twin pair 1)

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
8-22-55	a.m.			9-12-55	
8-23-55	a.m.				Post estrous bleeding p.m.
8-24-55					Riding other cattle
9- 9-55	p.m.		18	9-30-55	
10-3-55	p.m.		23	10-22-55	
10-26-55	a.m.	3:00 p.m.	25	11-17-55	
10-28-55					Post estrous bleeding
11-19-55	a.m.	p.m.	23	12- 9-55	
11-21-55					Post estrous bleeding
12-12-55	a.m.	p.m.	24	1- 1-56	
12-14-55					Post estrous bleeding
2-20-56					Pregnant to J-13

Table 24. Reproductive history for experimental cow 83A (Twin pair 2)

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
7-23-55					Vulva slightly swollen
7-28-55				8-18-55	Riding other cows
8- 9-55					Coming in heat
8-15-55	Noon	5:00 p.m.	19 days		Bred to J-12 - frozen semen
8-16-55		11:00 a.m.		9- 5-55	Bred to J-12 - frozen semen
8-17-55					Post estrous bleeding
9-30-55					Pregnant

Table 25. Reproductive history for control cow 84A (Twin pair 2)

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 days cycle	Remarks
8 - 7 -55	2:00 p.m.			8-28-55	
8 - 8 -55		9:00 a.m.			
8 - 9 -55					Post estrous bleeding
8 -23 -55	a.m.	p.m.	16	9-13-55	
8 -24 -55		a.m.			
9 - 6 -55					Mucous discharge
9 -13 -55	9-12-55	9-12			
	12:00 a.m.	9-13	21	10- 1-55	
9 -29- 55	Noon	9-29			
		9-30	19	10-19-55	
10-18- 55	a.m.	p.m.	20	11- 7-55	
11- 5- 55	p.m.	9-6 a.m.	19	11-25-55	
11-25-55	a.m.	p.m.	21	12-15-55	
11-26- 55					Post estrous bleeding
12-14- 55	a.m.	p.m.	20	1- 3-56	
12-17- 55	a.m.				Post estrous bleeding
1- 2- 56	?		20	1-23-56	Did not notice positively
1- 4- 56					Post Estrous bleeding
1-21-56	p.m.	p.m.	19	2-10-56	Bred natural to Sparkle
1-24- 56					Post estrous bleeding
2- 9- 56	p.m.	p.m.	20	2-29-56	Bred to Sparkle
2-11- 56					Post estrous bleeding
2-29- 56	a.m.	p.m.	21	3-20-56	Bred natural to single toe
3- 3- 56					Post estrous bleeding
3-20- 56	p.m.	3-21 a.m.	21		Bred natural to Sparkle

Table 26. Reproductive history for experimental cow 85A (Twin pair 3).

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
9-18-55	p.m.				
9-24-55	Noon				In heat every 4 to 6 days.
11-17-55	a.m.	11-17 a.m.			
		11-18 a.m.		12-7-55	
12- 8-55	p.m.	p.m.	22	12-28-55	
12-24-55	p.m.	p.m.	22	1-18-56	
1-20-56	p.m.	p.m.	23	2- 9-56	
2-11-56	p.m.	p.m.			
		a.m.	19	3- 2-56	Bred to Sparkle
3- 4-56	a.m.	p.m.	23	3-24-56	Bred to Sparkle and Guernsey
3- 7-56					Post estrous bleeding
3-25-56	p.m.	-	22	4-14-56	

Table 27. Reproductive history for control cow 86A (Twin pair 3).

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
11-12-55	a.m.			12-2- 55	
12- 3-55	p.m.		22	12-23-55	
12-29-55	p.m.		27	1-18-56	
1-18-56	p.m.	p.m.	21	2- 7-56	
3- 5-56					Pregnant to J-12

Table 28. Reproductive history for control cow 194B (Twin pair 4).

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
9-24-55	p.m.			10-13-55	
10- 1-55	p.m.		8	10-21-55	Probably from injection of 50 mg. stilbestrol
10-26-55	a.m.	6:00 p.m.	26	11-17-55	Could not ascertain definite heat-bred on chance.
11- 2-55	p.m.	11-3-a.m.	7	11-22-55	
11- 6-55					Post estrous bleeding.
11-24-55	a.m.	p.m.	23	12-16-55	
11-26-55					Post estrous bleeding
12-17-55	a.m.	a.m. p.m.	22	1- 6-56	
12-19-55					Post estrous bleeding
1- 8-56	a.m.	p.m.	23	1-28-56	
1-11-56					Post estrous bleeding
1-29-56	a.m.		22	2-18-56	
1-31-56					Post estrous bleeding
2-22-56	a.m.	p.m.	25	3-13-56	Bred natural to single toe and Guernsey
2-24-56					Post estrous bleeding
4- 6-56					Pregnant.

Table 29. Reproductive history for experimental cow 195B (Twin pair 4).

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
9 -5-55	a.m.			9-27-55	Suspect heat
9-24-55	p.m.		20	10-13-55	
10- 2-55	p.m.				From stilbestrol injection given
10-14-55	a.m.	5:00 p.m.	22	11- 2-55	
11- 2-55	a.m.	p.m.	21	11-22-55	
11- 7-55	a.m.	8:00 a.m.			Still showing signs of heat
11-24-55	a.m.	p.m.	23	12-14-55	
11-26-55					Post estrous bleeding
12-17-55	a.m.	a.m. p.m.	24	1- 5-56	
12-18-55					Post estrous bleeding
1-25-56					Pregnant to H-22

Table 30. Reproductive history for control cow 40 A (Twin pair 5).

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
10-30-55	a.m.			11-19-55	
11-21-55	p.m.		23	12-11-55	
11-23-55					Post estrous bleeding
12-15-55	p.m.	p.m.	25	1- 3-55	
1- 8-56	p.m.	p.m.	26	1-28-56	
1-11-56					Post estrous bleeding
3-15-56					Pregnant to H-7

Table 31. Reproductive history for experimental cow 41A (Twin pair 5).

Date	Heat	Bred	Length of cycle (days)	Next heat with 21 day cycle	Remarks
10-20-55	p.m.			11-11-55	
11-11-55	p.m.		21	12- 1-55	
12- 2-55	a.m.	p.m.	22	12-21-55	
2- 1-56					Pregnant to B-8

Table 32. Medical treatment and observations of control cow 81A (Twin pair 1).

July 27, 55	Calved; 35 lb. female, healthy. Cow did not clean.
Aug. 2-3, 55	Grain not eaten - after birth discharge.
Aug. 4, 55	Veterinarian's examination found clean. Gave one pint of glucose.
Aug. 7, 55	Grain eaten.
Sept. 3, 55	Holding milk up in rear quarters.
Sept. 4, 55	2 cc. Pitocin intramuscular. Still held up.
Sept. 14, 55	End of teat scabbed. Machine causing - difficulty to milk with machine or by hand.
Sept. 19, 55	Using teat tube on right rear.
Sept. 22, 55	Right rear teat swollen and constricted. A.M. - Ruptured tissue in canal by use of teat tube - using medicated wax dialators to hold open between milkings, treated with antibiotics (udder infusion) - teat tube to milk.
Oct. 10, 55	Production from right rear quarter continued to drop with use of teat tube - 1 tube penicillin given and quarter dried off.
Nov. 11, 55	Swelling right front quarter - "off feed". Penicillin given right front quarter. OK - Nov. 15.
Jan. 7, 56	Switch to portable DeLaval milker.
Jan. 14, 56	All cows vaccinated (Blackleg) caused swelling at injection site, off feed and drop in production in some.
March 6, 56	Blood taken from all cows for bang's test.

Table 33. Medical treatment and observations of experimental cow 82A (Twin pair 1).

Aug. 14, 55	Calved; 37 lb. female. Calf died on 8-15-55. Cow did not clean.
Aug. 16, 55	After birth expelled - cow difficult to milk. Kicks - holds up milk.
Aug. 20, 55	After birth expelled.
Aug. 23, 55	Still holding up milk - becoming adjusted to milking. 2 cc. Pitocin given intramuscular to cause complete let-down - apparently helped.
Aug. 25, 55	2 cc. Pitocin A.M. - Complete let-down.
Jan. 9, 56	Swollen right leg at knee (H_2O accumulation from fall).

Table 34. Medical treatment and observations of experimental cow 83A (Twin pair 2).

May 10, 55	Calved. (approximate date).
Sept. 19,55	"Off feed" PM - Refused hay and grain.
Sept. 20,55	Vet's treated - arsenic - strychnine salt to stimulate rumen - milk production dropped rapidly.
Sept. 21,55	Repeat treatment - diarrhea.
Sept. 22,55	Pint glucose and Mg. SO_4 drench; vulva swollen abnormally, Possibly from switching tail and diarrhea.
Sept. 23,24 1955	Began to eat again. All during sickness temperature normal. Rumen motility slow to normal - diarrhea.
Nov. 15, 55	Fell in stanchion 10:00 a.m. and strangled to death. Embryo and pituitary and adrenals removed - embryo normal. 85 days. Weight of pituitary - 2.0 g. Weight of adrenals - 20.5 g.

Table 35. Medical treatment and observations of control cow 84A (Twin pair 2).

May 10, 55	Calved. (approximate date).
Oct. 19, 55	1 cc. streptomycin - penicillin mixture given intrauterine.
Feb. 1, 56	Tom says this cow became dull and sleepy after starting on stilbestrol.
Feb. 29, 56	Cycles regularly and tract appears normal, as determined by palpation per rectum.
March 22,56	Reproductive tract removed at slaughter, March 22. One oviduct collapsed, the other plugged with growths on inner wall. Weight of pituitary - 2.0 g. Weight of adrenals - 20.0 g.

Table 36. Medical treatment and observations of experimental cow 85A (Twin pair 3).

Sept. 9, 55	Calved. 54 lb. female. Cleaned - no trouble.
Sept. 30, 55	Cow in heat every 4 - 6 days since calving. Cystic graffian follicle r.o.
Oct. 7, 55	10 rat units pituitary gonadotrophin intravenously - slight regression 10-14-55.
Oct. 25, 55	10,000 I.u. in 10 cc. physio. NaCl sol. intramuscularly of "Antuitrin S", chorionic gonadotropin.
Nov. 12, 55	10 rat units gonadotropin - Ovary still cystic; seems to be some lutein tissue present. Regressing in size.
Dec. 1, 55	Ovaries seem normal, cycling regularly now.
Jan. 22, 56	Hand milking started because of low production.

Table 37. Medical treatment and observations of control cow 86A (Twin pair 3).

Oct. 31, 55	Calved. 60 lb. female, normal - cow cleaned.
Nov. 7, 55	Nervous at milking - kicks.
Dec. 5, 55	O.K. at milkings now - no kicking.
Jan. 15, 56	Very aggressive to other cows in heat. Often develops an edemateous condition on belly from riding other cows.
Feb. 8, 56	Started hand milking to be uniform with twin cow 85A.

Table 38. Medical treatment and observations of control cow 194B (Twin pair 4).

Aug. 8-9, 55	Cow suffering badly from hot weather. Ca Gluconate given for strength.
Aug. 10, 55	Calf pulled - very large calf (131 lb. 34.5" at withers) Hard on cow.
Aug. 15, 55	Uterus and horns in poor shape. 3 Million units penicillin and 25 mg. stilbestrol (intramuscular) given to clear up and out infection in uterus. Putrifying tissue. From ruptured condition of uterus. Udder infusion of penicillin for mastitis.
Aug. 16, 55	3 million units penicillin (intramuscular) and uterine capsules.
Aug. 17, 55	3 million units (I.M.) penicillin, also penicillin for mastitis in left rear quarter. Cow leaving grain.
Aug. 18, 55	Uterine capsules - cervix still open.
Aug. 19, 55	1 tube H.P. penicillin left rear quarter. Grain mixed with H_2O.
Sept. 11, 55	Mastitis continues to flare up every 6 - 10 days.
Sept. 30, 55	50 mg. stilbestrol (I.M.) given to help clear out small amount of fluid and extraneous matter in uterus.
Oct. 1, 55	False heat - due to stilbestrol and injection? Uterus and horns in comparatively good shape.
Dec. 1, 55	Mastitis continuing.
Jan. 26, 56	Pus in vagina.
Jan. 29, 56	In heat - not bred. Infection in tract. Seems to be caused by fecal matter drawn into vagina. Vagina and cervix badly prolapsed when cow is down
Feb. 27, 56	Bred naturally because of difficulty encountered in attempting to pass pipette through cervix.
April 15, 56	Uterus still protrudes when cow is down. Pregnant. Possibly twins.

Table 39. Medical treatment and observations of experimental cow 195B (Twin pair 4).

June 30, 55	Cow suffering from hot weather.
Aug. 2, 55	Calved. 110 lb. male. Cow did not clean.
Aug. 3, 55	Poor condition after calving. Did not clean, eating poorly - mastitis.
Aug. 4, 55	1 pint dextrose.
Aug. 5- 6	Penicillin, all four quarters.
Aug. 7, 55	Vaginal odor - putrid.
Aug. 8,55	40 mg. intramuscular stilbestrol and uterine capsule.
Aug. 10, 55	Leaving grain. Flies bad around pelciv region and vulva (odor).
Aug. 11, 55	3 million units penicillin (intramuscular) and uterine capsule.
Aug. 12, 55	Black vaginal discharge.
Aug. 17, 55	Mastitis left rear quarter - leaving grain.
Aug. 20, 55	Cleaning up grain in H_2O mixture.
Aug. 24, 55	Mastitis left rear - 1 tube penicillin.
Sept. 8, 55	Mastitis recurring every 6 - 8 days.
Sept.29, 55	50 mg. intramuscular stilbestrol to help clear out extraneous matter from uterus.
Oct. 6, 55	Signs of heat; from stilbestrol injection?
Oct. 6, 55	Off grain - eats OK when wetted.
Dec. 1, 55	Mastitis continues.
Jan. 56	Mastitis continues.
Feb. 28, 56	Slight prolapse of uterus when cow is down.

Table 40. Medical treatment and observations of control cow 41A (Twin pair 5).

Oct. 4, 55	Calved. 87 lb. female. Cleaned - no trouble.
Oct. 14,55	Right rear quarter swollen.
Oct. 27,55	Quarter OK - no treatment. No sign of mastitis.
Jan. 7, 56	Injection of penicillin and sulfa, for hoof rot - also local treatment.

Table 41. Medical treatment and observations of experimental cow 41A (Twin pair 5).

Sept. 22, 55	Calved - 92 lb. female. Normal birth.
Oct. 7, 55	Did not finish grain.
Oct. 11, 55	Leaves small amount of grain.

EFFECTS ON DAIRY COWS OF FEEDING DIETHYLSTILBESTROL — AS MEASURED BY REPRODUCTIVE PHYSIOLOGY, AND THE YIELD, COMPOSITION, AND ESTROGEN CONTENT OF MILK

by

CHARLES BENTON BROWNING

B. S., Texas Technological College, 1955

AN ABSTRACT OF A THESIS

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Diethylstilbestrol, commonly called stilbestrol, is a synthetic estrogen-like compound that has been used in the past few years to increase body weight gains and to lower feed requirements per unit of gain in fattening animals. Since stilbestrol was effective in stimulating growth in fattening animals, the question was raised as to its possible effect on milk secretion in dairy cows, and reproductive physiology of breeding animals in general.

The purpose of this study was to obtain information on the various physiological effects of feeding low levels of stilbestrol to dairy cows.

The experiment was divided into two phases, phase I, in which herd cows were used and phase II, in which identical twin cows were used.

In phase I, 12 dairy cows of four different breeds, some non-pregnant and others in various stages of gestation, were fed stilbestrol at levels ranging from 5 to 20 mg. per 1000 pounds of body weight per day. The objectives of phase I were - 1. to determine the effect of stilbestrol on reproduction, 2. to determine whether stilbestrol administration would cause or increase the estrogenic activity of milk from treated cows, and 3. to determine whether feeding stilbestrol to dairy cows would result in unfavorable anatomical changes of the treated animals.

The major problem encountered in setting up an experiment to compare milk production between groups of animals is the difference in inheritance of the cows. Identical twin cows were used in phase II of this experiment to eliminate this variable factor. Theoretically, one pair of identical twin cows will replace about 22 random pairs of cows without loss of statistical efficiency in measuring milk production. Five pairs of identical twin cows were used. Since results in phase I indicated that the 10 mg. level was probably safe and effective, this level was selected for phase II. One animal of each twin pair was fed stilbestrol at the level of 10 mg. per 1000 pounds of body weight per day, beginning 60 days post partum. The other animal of each pair was used as a control.

It was concluded from this experiment that the feeding of stilbestrol at the 10 mg. level increased the average amount of 4 percent fat-corrected-milk produced by approximately 13 percent. This greater production was the result of a 54 percent lower rate of decline in the treated animals rather then by an immediate increase in production. There was no measurable effect on the percent of fat or solids produced by the treated animals.

It was determined by assaying known amounts of stilbestrol that estrogens in extracts of milk in amounts equivalent to .048 micrograms of stilbestrol could be determined by the biological assay methods used in this study.

Thirteen biological assays were made on extracts of milk from cows on the experiment. Ten of these were from cows receiving stilbestrol, and three were from control cows. Only one of the thirteen extracts contained a measurable amount of estrogen. This was an extract from the milk of a cow receiving 20 mg. of stilbestrol per 1000 pounds of body weight per day. This extract contained an amount of estrogen capable of producing approximately the same response in ovariectomized mice as .048 micrograms of stilbestrol.

So far as could be determined, no detrimental effects on reproductive physiology of the cows were brought about by the continued feeding of 5 to

15 mg. of stilbestrol per 1000 pounds of body weight per day. There seemed to be no effect on conception, early pregnancy, or normal calving in animals receiving stilbestrol.

There were no signs of nyphomania in any of the treated cows, nor were there any undesirable effects to physical appearance.

Preliminary studies of the pituitary and adrenal glands of cows that received stilbestrol revealed no gross change in weight or structure that could be attributed to the feeding of stilbestrol.

Date Due

Jul 7 '58 P		
Jul 21 '58 C		
Jul 29 '58 Y		
Aug 5 '58 C		