INFLUENCE OF THE ESTROUS CYCLE ON LEVEL OF BLOOD PLASMA LIPASE ACTIVITY

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CHAPTER I

INTRODUCTION

Impaired fertility is one of the larger problems causing losses from our dairy cow populations. Rollinson (39) stated that the annual wastage from sterility in dairy herds was about 5% of the dairy cow population in Great Britain with 61% of these disposals due to infertility. According to Rickey (35), from 1963 through 1965, 35% of the cattle leaving the Oklahoma State University herd were culled because of fertility problems. Muller (30) reported that in Austria 30-40% of culled cows had fertility problems. These losses are very real in terms of genetic progress and production of pounds of milk and fat, as well as the expense of repeat breeding and veterinary treatment.

Over the years, research has been done on this problem and has resulted in a rather wide acceptance of hormonal treatment of problem cows; however, only limited progress has been made. It has become obvious that we know very little about the basic chemical and biological mechanisms involved in mammalian reproduction. A brief review of present concepts of reproduction in the cow will be presented as a foundation for this study.

The Estrous Cycle

The normal estrous cycle of the cow consists of four different phases known as proestrus, estrus, metestrus, and diestrus. The

physiological events occurring during the estrous cycle may be summarized within these four phases as follows. During proestrus (2-3 days in length), follicular growth is stimulated by FSH (follicle stimulating hormone) which is secreted by the anterior lobe of the pituitary gland. The follicle as it develops produces follicular fluid containing estradiol. The presence of estradiol causes an increase in the blood supply to and growth of the tubular genitalia. The vulva swells slightly and the vestibule becomes congested and bright red in color. The mucous cells begin to swell causing the vaginal part of the cervix to enlarge. The vascularity of the uterine mucosa is increased and the cervical cells begin to secrete mucus.

In the following phase of the cycle known as estrus (15-17 hours in length), the increased levels of estrogenic hormones emanating from the developing follicle cause the animal to display characteristic signs of estrus, ie, restlessness, bellowing, or allowing mounting by other animals. The vulva swells more and the color of the vestibule becomes a darker red. Considerable swelling and protrusion of the folds of the mucosa membranes of the cervix occurs with concurrent secretion of mucus being characteristic. As the follicle continues to grow rapidly, the nervous system of the cow becomes refractory to the high estradiol concentrations and acceptance of mounting ceases. Toward the last of this estrus period, the pituitary hormone balance shifts from FSH to LH (luteinizing hormone), which is also secreted by the anterior lobe of the pituitary.

Metestrus (3 1/2 to 4 days in length) is characterized by the reorganization of the follicular nest of cells into the corpus luteum. During this phase estrus ceases, ovulation occurs (approximately 11

hours after the end of estrus), and the tract gradually loses its congestion with the vulva becoming wrinkled and reduced with the cervix subsiding in mucus flow.

The final phase, diestrus (14-15 days in length), is characterized by the presence of the fully developed corpus luteum and its secreted hormone, progesterone. Progesterone secretion results in a thickening of the uterine wall endometrium, development of the glands and muscles of the uterus, and a general preparation of the uterus for nourishment of the embryo and formation of the placenta. With conception, there is a prolongation and intensification of the diestrus reactions under the influence of the embryo. If no pregnancy occurs, the corpus luteum will remain functional to about the 19th day of the cycle with degeneration starting on about the 17th day. The corpus luteum regression and the proestrus phase of the next cycle occurs simultaneously, thus setting the stage for another cycle.

Hormonal Control of the Estrous Cycle

The hormones which control the estrous cycle are largely supplied by the pituitary gland and the cycling ovary with complex interrelationships apparent in controlling the events which occur during the estrous cycle.

Follicle stimulating hormone (FSH) is responsible for the growth of the Graafian follicle and the maturation of the ovum. Luteinizing hormone (LH) initiates the preovulatory enlargement of the follicles, causing ovulation and the subsequent formation of the corpus luteum. FSH in conjunction with LH causes the theca interna cells of the follicle to produce estrogens. The maturing follicle secretes estrogens in

increasing amounts until its action on the nervous system produces behavioral estrus. Increased amounts of estrogens also inhibit the production of FSH by the pituitary gland. When ovulation is completed, the corpus luteum is formed from the ruptured follicle under the influence of LH and, within a period of 3 to 5 days, begins to secrete progesterone. As long as the corpus luteum remains active in the production of progesterone, the secretion of FSH remains low and the ovarian follicles show little growth and development. If pregnancy does not occur, progesterone secretion by the corpus luteum wanes, FSH production increases, and a new cycle of follicular activity commences. It is apparent that progesterone suppresses the secretion of FSH by the pituitary gland.

Although much is known about the reproductive cycle of the dairy cow, it is obvious from the large percentage of infertility problems that much has yet to be learned about the characteristic causes and possible treatments of functional reproductive problems. This is especially true in those areas which result in reduced fertility. Salisbury and VanDemark (45) listed several possible problems which may result from a hormone imbalance. These included: failure of ovulation to occur, silent estrus, pregnant animals showing estrus, and failure of zygote formation. While much is known about these problems, no clear cause and effect relationships have been established. Additional research is therefore necessary to understand and eliminate the difficulties.

Current research efforts are directed toward measuring hormonal levels or their influences in body tissue and fluids. This study was initiated to determine if the level of activity of blood plasma lipase can be used as an indicator of reproductive function in the individual cow.

CHAPTER II

LITERATURE REVIEW

There have been many studies dealing with the effect of various hormones on the lipid level of the blood stream and various tissues. Davis et al. (5) found that in the maturing rat testes there was a decrease in triglyceride concentration which occurred as puberty commenced and hormone level increased. If the lipid level of the blood stream or body tissues is changed, there must necessarily be a change in the blood serum activity level of lipase, an enzyme which hydrolyzes fats into fatty acids and glycerol.

Previous work has established a definite relationship among lipase and various hormones. Hirsch et al. (21) injected pituitary extracts high in hormone content into rabbits which caused an increase in the hydrolysis of fat deposits into free fatty acids. If the hormone level is related to lipase activity, then specific periods of the cycle, pregnancy, and various hormonal reproductive problems should be reflected in the lipase level of activity in the blood stream.

The Relationship of the Reproductive Cycle to Body Lipids

In order to relate hormone levels to lipase levels, a cycle of lipids in the blood must be established. Dempsey and Wislocki (6), as well as Guarna (18), found that in general the situation seemed to be that at those times in the estrous cycle when the activity of the

tissues is of diminishing intensity and the rate of oxygen utilization and anaerobic metabolism is decreasing fat is deposited in uterine tissues. In pregnancy, too, the fatty deposits were increased when the rate of uterine growth diminished. This coincided with the time when the uterine circulation became progressively less efficient and the transfer rate of substances across the placental barrier increased. During involution, the growth stimulus of distention was likewise lacking and fatty degeneration took place along with reduction of the tissue mass. McBride and Korn (28) found that lipase activity in the guinea pig mammary gland is constant during pregnancy with a large increase occurring at parturition. Popjak (32) found that when an animal miscarried there was an immediate rise in plasma lipids.

The relationship between lipase and lipid levels of the body apparently is as follows. As blood lipase rises, there is a corresponding increase in blood lipids resulting from the breakdown of fatty storage tissue. As lipase levels decrease, there is a decrease in the blood lipid levels. Robinson (38) found that higher levels of lipase in tissue are related to periods of stress when energy is needed. Similarly, McBride and Korn (29) working with guinea pigs stated that the lactating mammary gland takes up free fatty acids 20 times more rapidly than that of animals in the middle of the pregnancy period.

Okey et al. (31), working with the sow, found that the phospholipid content of uterine mucosa changed significantly during the cycle. The most significant increase coincided with the phase of maximum glandular proliferation under the influence of the corpus luteum. It was concluded that fatty degeneration takes place in certain parts of the uterus as the estrous cycle advances. Also, it was found that the

uterine mucosa contained more lipase at this time than at other times in the cycle. Saito (44) found that fatty degeneration occurs in the uterus mucosa during estrus and that the level of lipase in the tissue is high at this time. Erpf-Lefkovics and Rosenbloom (10) found identical relationships in sheep.

Rudman (42) stated that ovulation in the pigeon and dove is associated with a 4 to 7-fold increase in the concentration of total lipids in the plasma as compared to the concentration before or after ovulation. Similarly, Taurog et al. (50) found that in laying birds, when contrasted with nonlaying birds, there was a marked increase in the levels of triglycerides, phospholipids, and free cholesterol of the blood. Entenman et al. (8) found that a relationship existed between the rise in blood lipids and oviduct size. Under the conditions studied, a rise in blood lipids occurred when an oviduct growth of at least 10 gm. had been attained.

Popjak (32), working with rabbits, found that pregnant rabbits, when fed cholesterol, developed a heavy deposition of fat in the placenta. The deposition was so heavy that it interfered with the nutrition of the fetuses which were 1/3 lighter than the controls. This suggested a decreased level of lipase especially during the latter part of the gestation period when maximum fetal growth occurs.

Takeda et al. (49) obtained results which would tend to support this in that it was found that an increase in fat content of the endometrium in pregnancy was associated with a decrease in serum lipase.

Several studies have been conducted concerning the alteration of epithelial fat in the uterus during the reproductive cycle. Reynolds (34) stated that in the rat the uterine mucosa was free of osmophilic

granules at estrus when the uterus was distended with fluid. In metestrus, at the time when vacuolar degeneration of the endometrium was maximum, granules appeared and increased in size and number. They decreased as diestrus proceeded.

Alden (2) obtained somewhat differing results with rats in that fat in uterine epithelial tissue was found to be least evident during proestrus and gradually increased until late metestrus when regression began. He also found that during pregnancy the fat content of the cell gradually increased.

Reese (33) found in rats that the granules were generally absent during estrus when the uterus was distended. As the animal passed out of the estrus period, a small group of granules appeared and as metestrus progressed, they reached a peak in size and number. Toward the last of diestrus, there was a gradual dissimulation in the fat content of the cell. Reese, like Reynolds, concluded that there was a correlation between the disappearance of fat granules and the distention of the uterus with fluids.

Effect of Various Secretions on the Level of Lipase Activity

In the previously considered references, the work has been directed toward a specific period of the reproductive cycle. It is now appropriate to consider the work which has been done in relating specific hormones to the lipase activity.

Effect of Estrogen on the Level of Lipase Activity. Most of the work relating estrogenic compounds to lipase activity level has been done with birds. However, definite relationships have been established, and the findings may apply to mammals as well as birds.

Estrogen has been shown to have a definite effect on the levels of activity of lipase in the blood. Riddle and Senum (36), working with birds, found that increased levels of lipids in the blood coincided with increased levels of estrogenic output by the ovary. This relationship was concluded to be a direct effect of estrogen. Lorenz et al. (26) found that in immature birds estrin injected intramuscularly almost doubled the blood lipid level. Landauer et al. (24) injected cocks with large amounts of estrogen and extreme lipemia developed. The blood serum was a deep canary yellow color, very trubid, and on standing quite a large amount of fat rose to the surface.

Heald and Rookledge (20), also working with birds, found that estrogen administration increased the levels of plasma lipids in the immature fowl. Entenman et al. (9) found that a rise in blood lipid level resulted from injections of estrogen into immature birds. Zondek et al. (55) also found that estrogen treatment of immature birds resulted in increased levels of blood lipids.

Aftergood and Alfin-Slater (1), while working with rats, concluded that a deficiency of estrogen resulted in a decrease of unsaturated fatty acids in the blood during treatments in which essential fatty acids were deficient in the diet. Fillos (11) observed that estrogens appear to favor higher serum cholesterol levels in rats.

From the work mentioned above, it can be concluded that increased levels of estrogen increases the blood lipid level and therefore must increase the level of activity of blood lipase.

Effect of the Thyroid Gland on the Level of Lipase Activity. There have been conflicting reports as to the relationship of the thyroid gland to blood lipid levels. Thyrotrophin, originating from the

pituitary gland, is known as the thyroid-stimulating hormone (TSH). As the levels of this hormone increase, the level of thyroid activity increases. There have been several reports concerning the effect of TSH on the blood lipid level. Engel and White (7) reported modest lipolytic activity by TSH. Vaughn and Steinberg (51) stated that the rate of lipolysis was significantly increased by the presence of TSH. Strand et al. (48) and Rudman et al. (41) found that lipase increased strikingly in activity when exposed to TSH. Frienkel (12) and Gilmour and McKerns (13) found that TSH stimulated the release of free fatty acids from adipose tissue.

Heald and Rookledge (20) reported that treatment with thyroxine decreased the levels of plasma lipids in the laying fowl, while plasma-free fatty acids were unaffected. Stamler at al. (46) found desiccated thyroid tissue highly effective as a lipotrophic agent against the cholesterol fatty liver induced in chicks chronically fed this sterol. From this latter report, it would seem that thyroid activity would stimulate increased serum lipase activity, while the former report would lead to the conclusion that thyroid activity would decrease serum lipase activity. It would seem, however, that increased thyroid activity coincides with estrus and increased estrogen output.

Effect of Progesterone on the Level of Lipase Activity. As with thyroxine, there have been seemingly conflicting reports concerning the effect that progesterone has on the lipid level of the blood. Okey et al. (31) reported that progesterone mobilized the fat in basal gland cells of the sow uterus. Alden (2) reported that in the pregnant rat uterus progesterone treatment resulted in a scarcity of fat in the cell.

Entenman et al. (9) concluded that progesterone alone had no

influence on the lipid level of the blood when injected into the immature bird. Gilmour et al. (13) reported that progesterone was without significant effect on synthesis of lipid from glucose in adipose tissue from the female rat. Black et al. (3) reported information which seems to clear up the situation. They concluded that progesterone alone will not lead to the appearance of basal fat in the menstruating women, but that basal fat cannot appear in the absence of progesterone. An optimum balance between estrogen and progesterone seems to be necessary before basal fat can be found in the uterine gland cells.

From previously reported data about the level of fatty tissue during the reproductive cycle, this latter report tended to agree with the expected deposition of fat granules in the basal gland cells during the progesterone-dominated part of the cycle and explains why Entenman et al. (9) and Gilmour and McKerns (13) obtained the reported results. Therefore, Rudzik and Miller (43) suggested that there is a decrease in serum lipase activity under the influence of progesterone. This may result from the decrease in estrogen and epinephrine levels in the portion of the cycle under the influence of progesterone.

Effect of Epinephrine on the Level of Lipase Activity. Epinephrine is secreted by the adrenal glands and is influential in uterine muscle motility. Although not directly related to the reproductive cycle, this hormone has been found to vary with the cycle and has a direct influence on the blood serum lipid levels. Rudzik and Miller (43) reported that the concentration of epinephrine was low during diestrus and increased with estrus. Cha et al. (4) concluded that the concentrations of epinephrine in the uterus were elevated during estrus in the rat and during the proliferation phase or the follicular phase of the menstrual cycle

in the human. Wurtman et al. (54), working with rats, reported that there was continuous increase in the capacity of the whole uterus to bind and retain epinephrine as the animal approached estrus. Green and Miller (16) reported similar results.

Working with rats, Cha et al. (4) reported that the level of epinephrine was reduced during pregnancy. Wurtman et al. (53) stated that when relaxin, an ovarian hormone thought to be elaborated during pregnancy, was administered subcutaneously to nonpregnant rats there was a pronounced decrease in uterine epinephrine. He further reported that the concentration of uterine epinephrine in rats, a few hours postpartum, was less than one fourth that of nonpregnant uteri and half that of pregnant uteri.

There have been many studies concerning the effect of epinephrine on the level of serum lipids. Gordon and Cherkes (15) found that the release of free fatty acids from adipose tissue was stimulated by a number of hormones, including epinephrine. Hollenberg et al. (22) reported that epinephrine increased free fatty acid accumulation in the rat blood stream by activating a lipase in adipose tissue. Leboeut et al. (25) concluded that epinephrine increased the release of free fatty acids into the blood stream from adipose tissue. Lynn et al. (27) found that incubation of rat adipose tissue in epinephrine resulted in an outpouring of fatty acids and glycerol into the medium. In a similar experiment, Rizack (37) stated that there was from 25% to 30% increased lipolytic activity in the presence of epinephrine.

Rudman et al. (41) included epinephrine in a list of natural occurring substances which stimulate mammalian adipose tissue to convert stored triglyceride into free fatty acid. Strand et al. (48) reported

that hormone-sensitive lipase increases strikingly in activity when exposed to the catecholamine class of compounds of which epinephrine is a member. Vaughn and Steinberg (51) and Engel and White (7) reported increased lipolysis in the presence of epinephrine.

It would seem from the above studies that the blood lipase level of activity would be influenced by the epinephrine levels in the body.

Effect of Other Hormones on the Level of Lipase Activity. There are other hormones that are active during the reproductive cycle and affect the lipase activity. Included in these are adrenocorticotropic hormone (ACTH), prolactin, and growth hormone (GH or STH).

Grollman (17) stated that ACTH stimulated the secretion of cortical hormone by the adrenal cortex. There are increased concentrations of ACTH at periods of the reproductive cycle involving increased activity of the animal (estrus) or increased body needs (pregnancy).

ACTH has been found to be very active in increasing serum lipid levels. White et al. (52) found that ACTH stimulated the release of free fatty acids from adipose tissue. Hirsch et al. (21), working with rabbits, reported that subcutaneous injections of ACTH mobilized free fatty acids from adipose tissue into the blood stream. Girolamo et al. (14), also working with rabbits, found ACTH highly effective in mobilizing free fatty acids. Hollenberg et al. (22) found a similar situation in rats.

Lynn et al. (27) stated that incubation of adipose tissue with ACTH resulted in an outpouring of fatty acids and glycerol into the medium. Rudman et al. (41) had similar results with hamsters.

Prolactin has been found active in prolongation of the functional life of the corpus luteum and the secretion of progesterone which is

necessary for the maintenance of pregnancy. Gilmour and McKerns (13) found prolactin active in lipolysis in rats.

Hafez (19) stated that growth hormone (GH or STH) has been shown to be concerned with general growth throughout the reproductive period of the animal, especially in the stimulation of uterine growth. It also has a synergistic relationship with estradiol and LH in stimulation of the ovary to release estrogen. There has also been some indication of increased release of STH during pregnancy.

Vaughn and Steinberg (51) found that the rate of lipolysis in vitro was significantly increased by the presence of STH.

Summary

In summarizing the evidence available, it must be concluded that there are various hormones which affect the blood lipase level of activity and thus affect the levels of plasma lipids. These hormones include estrogen, thyrotropin, thyroid gland secretions, progesterone, epinephrine, adrenocorticotropic hormone, prolactin, and growth hormone. Research to date indicates that the level of lipase activity varies in response to these hormones and their synergistic relationships.

The relationship of lipase activity in blood plasma with the estrous cycle of the cow has not been thoroughly investigated. Roussel and Stallcup (40), experimenting with cows, found significant differences ($P < 0.05$) among the phases of the estrous cycle in the level of lipase activity. These results indicated that the hormonal variations that occur during the estrous cycle are reflected to some degree in level of activity of blood plasma lipase.

CHAPTER III

EXPERIMENTAL PROCEDURE

The principle objective of this study was to determine the degree of relationship between blood plasma lipase activity and phases of the estrous cycle of postpartum cows.

Four cows, three Ayrshires and one Jersey, in the Oklahoma State University dairy herd with no previous history of reproductive problems, approximately the same age (4 to 5 years) and stage of lactation, and of comparable milk and fat production, were used in this study.

The cows on experiment received routine herd treatment except for the short (15 to 20 minutes) separation for blood collection. Animals were fed a ration of 23 lb medium quality alfalfa hay and 21 lb pelleted (1/4 in) concentrate per cow with the composition as given in Table I.

Blood was collected immediately after milking. Animals were tied and stanchioned with a maximum effort made to prevent undue excitation during the collection process. Following blood collection, the animals were immediately returned to the herd.

Blood sampling, by jugular puncture, commenced at the first estrus postpartum with samples being taken on the day of estrus and days one and two following estrus. The animals were then sampled twice weekly until the fifteenth day of the estrous cycle, with daily samples then taken until the following estrus commenced. The cows were artificially inseminated the third estrus following parturition. Blood samples were

taken every seven days from the bred cows to establish a general lipase pattern for the pregnancy period.

TABLE I

COMPOSITION OF CONCENTRATE RATION FED TO EXPERIMENT ANIMALS

Ingredient	Lb/ton
*Ground Milo	600
*Steam Rolled Barley	600
*Steam Rolled Oats	300
Wheat Bran	300
Cottonseed Meal (41% protein)	100
Urea C Feed Grade (42% nitrogen)	20
Trace Mineral Salt	20
Dicalcium Phosphate	20
Pellet Binder	40
Vitamin A added to provide 1000 IU/lb of feed	
Vitamin D_2 added to provide 100 IU/lb of feed	
	2000 lb

*Not less than number 2 grade.

Fifty ml of blood were collected in a centrifuge tube containing 5.0 ml of 3.5% sodium citrate (1 ml sodium citrate/9 ml blood). The blood was chilled immediately in an insulated ice water bath (5°C), then held in a refrigerator (5°C) for two days to conform to the laboratory schedule. Laboratory tests indicated no change in blood lipase values during this holding period.

Laboratory Procedure

After two days, the blood samples were centrifuged for 10 minutes at 3100 rpm and the plasma decanted, leaving the cellular debris in the centrifuge tube. The analysis, a modification of that used by Kern et al (23), was as follows: Duplicate 50 ml flasks were prepared, each containing 5 ml of blood plasma, 2 ml of substrate (an emulsion of 7.5% tributyrin and 15% bovine albumin in water),[1] and 5 ml of 0.2 M Tham buffer[2] (pH 9.6). The flasks, a blank and test for each sample, were then marked, capped, and agitated for 5 seconds. The pH of the blank was immediately reduced to 2.0 with 10% HCl and refrigerated. The other flask marked "test" was capped and incubated one hour in a 37°C "shaking" water bath. Following incubation, the pH of the test flask was reduced to 2.0 with HCl.

The samples were placed in separatory funnels, and the fat was extracted using the following procedure:

a. fifteen ml of 95% alcohol were added and the samples were agitated 10 seconds.
b. 60 ml of a 50-50 ethyl ether-petroleum ether mixture were added and the samples were shaken for 30 seconds.
c. the ether layer containing neutral lipids and free fatty acids was decanted into a clean, dry flask.

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[1] Weigh 7.5 g tributyrin and 15 g bovine albumin in separate beakers and gradually add albumin to about 75 ml of distilled H_2O. Then homogenize tributyrin with the albumin mixture and raise volume to 100 ml with distilled H_2O.

[2] Tham = Tris-hydroxy-methyl-amino-methane.

d. the ether extraction (steps b and c) was repeated and the ether extracts were combined. These combined extractions were then diluted, if necessary, to a final volume of 125 ml with the 50-50 ether solution.

The ether extracts were transferred into clean, dry flasks to eliminate the last traces of water in the samples. Twenty-five ml aliquots of the ether extracts were then titrated with alcoholic KOH (about 0.03 N) using thymolphthalein as the indicator.

Lipase activity was calculated by the following formula and expressed as %(by weight) of butyric acid produced per ml of blood plasma:

$$\frac{(\text{ml KOH sample} - \text{ml KOH blank}) \times \text{N-KOH} \times \frac{125^{a}}{25} \times .088^{b}}{5^{c}} \times 100 =$$

%(by weight) of butyric acid per ml of blood plasma.

This procedure allows the lipase present in the plasma to react with the tributyrin-albumin substrate to form free fatty acids. The extraction is a purifying process to remove compounds which would confound the obtained values, and titration is performed to measure the amount of butyric acid released per ml of blood plasma. This indirectly measures the amount of lipase activity in the blood plasma.

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[a] $\frac{\text{Total volume of sample}}{\text{Titrating sample size}}$

[b] Meq weight of butyric acid.

[c] Sample size in ml.

CHAPTER IV

RESULTS AND DISCUSSION

This study provides convincing evidence of differences in plasma lipase level of activity among cows, among periods of the cycle, and between cycles. The data obtained from this study (Tables V and VI) have been graphed for each cow for comparison with the other animals (Figures 1, 2, 3, and 4).

When comparisons of Figures 1, 2, 3, and 4 are made, several similarities are readily apparent from the data. First, if the period of time around "0" day (observed estrus) is examined, it is generally evident that the lipase values are at a low point during proestrus (1 to 2 days prior to estrus), reach a peak during observed estrus, and subside to a low level 2 or 3 days following estrus during the metestrus period. Second, after this drop during metestrus, the values increase as the animal passes into diestrus. Third, in general, larger values are observed in the second cycle; and fourth, there are obvious differences in plasma lipase levels among cows.

For analysis of the primary experimental data, it was necessary to select values considered representative of the four periods of the cycle (Table VII).

For proestrus, the lowest value within 2 days prior to estrus was selected. This theoretically would be that point just prior to rapid follicular growth and very near the end of luteal influence.

The highest value within one day of displayed estrus was used to represent the estrus period, because variations in length as well as intensity of estrus commonly occur in cows. This value should represent the blood picture near the peak of estrogenic influence.

The lowest value within 3 days after estrus was selected for metestrus. It is known that during metestrus ovulation occurs, estrogenic influence wanes, and the ruptured follicle is reorganized into the corpus luteum. The lowest lipase value in the metestrus period should represent the lowest point of both estrogenic and progesteronal influence.

An average value was calculated for diestrus from the lipase values obtained 5 days after estrus to 5 days prior to the following estrus. Considerable variation in level of activity was evident during the diestrus period, and no one value could be considered characteristic of the period. Therefore, an average value was considered to be more useful.

The selected data were statistically analyzed as outlined by Steel and Torrie (47) by application of a 4 x 4 x 2 factorial arrangement (4 cows x 4 periods x 2 cycles) with comparisons among cows and among periods (Table VIII).

A significant difference in plasma lipase level of activity among cows was found ($P < 0.01$). This is in agreement with the data obtained by Roussel and Stallcup (40), who found a highly significant difference ($P < 0.01$) in blood serum lipase activity among cows.

Further analysis of the data revealed cow 564 to be significantly ($P < 0.01$) higher in lipase activity values than 561 and 488 and higher than cow 477 ($P < 0.05$). Cow 477 was found to be higher than cow 561 ($P < 0.01$) and cow 488 ($P < 0.05$). No significant difference was found between cows 561 and 488.

It was observed that the cows with significantly higher lipase values (564 and 477) were more excitable and frequently became quite upset during the blood collection process, while cows 561 and 488 were very docile. From this observation, together with the significant differences in lipase values obtained, it is obvious that the individual animal's temperament may influence the level of plasma lipase activity to some extent, especially during periods of excitement or fear when epinephrine may be secreted into the blood stream.

The significant differences which were found among cows can likely be further attributed to the differing metabolic rates and hormone balances of the animals under study. Although all animals were given the same treatment, differences among animals were obvious and significant in their effect. It has been suggested that in times of high body needs the storage tissue is broken down to supply necessary nutrients. If this is true, then a period of time in which an animal undergoes stress (high milk production, disease, or high levels of body activity) should be coupled with high levels of lipase activity. This relationship should especially be true during reproductive stresses (estrus, pregnancy, nymphomania, or calving) in which the body's metabolism is altered to compensate for the stress.

It is fitting that the metabolic history and milk production of the experimental animals be briefly mentioned at this point. The animals with the lowest lipase activity values formed the extremes of both body condition and milk production, with cow 488 being the highest producer and having the poorest body condition and cow 561 being a fat, low-producing animal. Cows 564 and 477 may be categorized between these two animals in both milk production and body condition. No conclusions were

drawn from these observations because of the design of the experiment; however, the effects of these variables should be the subject of further study.

Analysis of lipase activity during the four periods of the cycle revealed a highly significant ($P < 0.01$) difference among periods. Additional analysis showed that the values obtained in estrus were significantly higher than those obtained in proestrus ($P < 0.01$) and metestrus ($P < 0.01$). Values obtained in diestrus were significantly higher than those in metestrus ($P < 0.05$). No significant differences were found between estrus and diestrus, proestrus and metestrus, or proestrus and diestrus.

The hormone balance appears to be directly related to the obtained lipase values. These values were found to be significantly higher in estrus, which is characterized by high levels of estrogen. They were also higher in diestrus, which is under the influence of progesterone. This hormone is secreted by the corpus luteum, the formation and control of which is under the influence of luteinizing hormone (LH). It appears from this that high levels of estrogen, progesterone, and LH are conducive to high lipase activity values. This agrees with the work done by Okey (31), Riddle and Senum (36), and Gilmour and McKerns (13) in their work with progesterone, estrogen, and LH, respectively. Proestrus and metestrus were found to be significantly lower in lipase activity values. Salisbury and VanDemark (45) stated that during proestrus follicle stimulating hormone (FSH) is present in substantial amounts; and from the results obtained in this study, it appears that the presence of FSH, in addition to the changing balance of estrogen, progesterone, and LH, provides a situation reflecting low levels of lipase activity. During

metestrus, the balance of the hormones shifts from FSH to LH and estrogen is undergoing replacement by progesterone and LH. This, too, apparently creates an environment in the animal's body resulting in a low level of lipase activity.

Additional support for this idea is provided by the pattern of plasma lipase during a false estrus in cow 561 (Table II). On day 15 of

TABLE II

PLASMA LIPASE LEVEL OF ACTIVITY VALUES OBTAINED FROM COW 561 WHILE SHOWING FALSE ESTRUS IN RESPONSE TO OTHER COWS SHOWING TRUE ESTRUS IN THE HERD

Day of Cycle	Lipase Value
0	.051
2	.010
6	.051
9	.051
13	.059
15[1]	.082
16	.028
17	.067
0[2]	.080

[1]False estrus observed.
[2]True estrus observed.

a normal cycle, the cow was in the presence of two estrus animals and was allowing limited mounting. This activity ceased in a few hours, and on day 18, the animal again came into estrus and demonstrated all the

characteristics of estrus. Blood samples were taken every 24 hours during this period; and from Table II, it is evident that the occurrence of the false estrus period was accompanied by a rise in lipase values. Apparently, this cow was in early proestrus, and the stimulus of other animals in estrus, plus rising estrogen levels characteristic of proestrus, was sufficient to induce psychological or false estrus. The cow went out of false estrus and came into normal estrus 3 days later.

To further test the estrus-estrogen-lipase relationships, two non-cycling cows were treated with hormones to determine what effects administered estrogen or luteinizing hormone would have on the lipase values. One, a non-cystic anestrous animal, was treated with estrogen and came into estrus one day after treatment. As evidenced by the values obtained (Table III), the lipase activity varied directly with observed

TABLE III

PLASMA LIPASE VALUES OF ANIMALS GIVEN HORMONE INJECTIONS

Animal Treated	Hormone Injected	DAY NUMBER			
		0[1]	+1	+2	+3
Non-cycling cow	Luteinizing hormone	.056	.092[2]	.070	.084
Cycling nonbreeding heifer	Estrogen	.064	.062	.080[2]	.067

[1]Day injection given, blood samples taken prior to injections.

[2]Day animal observed in estrus.

estrus showing a low value prior to estrus, a peak during estrus, and a low value afterwards. The other animal was treated with luteinizing

hormone and displayed estrus on the second day after treatment. Again, there was a direct relationship between a low value during proestrus, a high value during estrus, and a low lipase value during metestrus.

An additional experiment was performed on a normally cycling animal to determine the degree of variation in lipase values during a normal estrus. A cow showing the earliest indications of estrus, but not yet allowing mounting by other animals, was isolated from the herd and blood collected every 4 hours. As can be seen from the obtained data (Table IV), the lipase activity values increased as estrus intensity increased and decreased as the animal passed into the metestrus period.

TABLE IV

PLASMA LIPASE VALUES OBTAINED BY SAMPLING A COW IN ESTRUS EVERY FOUR HOURS

Time	Plasma Lipase Value
3 p.m.	.049
7 p.m.	.043
11 p.m.	.095
3 a.m.	.026
7 a.m.	.029
11 a.m.	.026

These data clearly demonstrate that the increased activity of estrus, caused by the high levels of estrogen, is accompanied by high lipase values. This clearly indicates that there is a real hormonal influence on plasma lipase activity; however, better definition has to await more information.

Cycle two was found to be significantly ($P < 0.01$) higher in plasma lipase activity values than cycle one. It is likely that this response was due to increased hormone concentrations in the blood of the animal during the second cycle caused by greater ovarian activity. It is known that during pregnancy the follicular activity of the ovary is suppressed and a length of time is necessary for the regeneration of the full potential level of hormone secretion. Further study is needed to determine if the lipase activity values continue to increase during the cycles following cycle two and to determine more completely the causes of the observed changes.

There were no interactions between cows and periods or between periods and cycles. However, a significant ($P < 0.05$) cow and cycle interaction was found. This is interpreted to mean that the expression of cyclical activity in a cow is dependent on the interrelationships of the many factors that are concerned in total reproductive function.

Because of the definite relationships found in this study, it is suggested that additional research be conducted with the goal of establishing a range of lipase activity for "normal" cows. This "normal" range could then be used as a basis for investigation of such problem areas as non-cycling cows, cystic animals, and other costly reproductive problems of the dairy industry.

It is proposed that the information found in this dissertation be used as a guide for deeper investigations into fertility problems among non-cycling heifers, mature cows, and aged cows. It is suggested that larger numbers of cows be used in these studies to take into consideration the individual cow variation found in this study. In addition, differences may exist among breeds, and this area should be considered as a source of variation.

Should further investigations prove conclusively that the differences found in this study are typical of dairy cows, research should be directed towards the development of diagnostic tools with which the dairy herdsman, or his veterinarian, could test his animals quickly and efficiently for deviations from the normal patterns of reproduction.

CHAPTER V

SUMMARY AND CONCLUSIONS

A study was conducted to determine the effects of the individual animal, the period of the estrual cycle, and the effect of recurring cycles on the level of activity of blood plasma lipase. The response of the four cows, three Ayrshires and one Jersey, was measured by the anount of butyric acid liberated in 1 hour at 37°C by the lipase activity in 1 ml of blood plasma and calculated in terms of %(by weight) of butyric acid per ml of blood plasma.

Blood was collected from each animal for two complete cycles, prior to breeding, starting approximately 45 days postpartum. The lipase values obtained during this period were statistically analyzed by the application of a 4 x 4 x 2 factorial arrangement.

A significant ($P < 0.01$) difference in plasma lipase activity among cows was found in the general statistical analysis. Further analysis revealed cow 564 to be significantly ($P < 0.01$) higher than 561 and 488 and higher than cow 477 ($P < 0.05$). Cow 477 was found to be higher than cow 561 ($P < 0.01$) and cow 488 ($P < 0.05$). No significant difference was found between cows 561 and 488.

Significant differences ($P < 0.05$) in plasma lipase activity among periods of the cycle were found. Additional analysis showed that estrus was significantly ($P < 0.01$) higher than proestrus and metestrus. Diestrus was found to be higher than metestrus ($P < 0.05$). No significant

differences were found between proestrus and metestrus; proestrus and diestrus; or estrus and diestrus.

Cycle two was found to be significantly ($P < 0.01$) higher in plasma lipase values than cycle one. No interaction between cows and periods or between periods and cycles was found; however, a significant ($P < 0.05$) interaction was found between cow and cycle.

It was concluded that there were significant variations in the plasma lipase level of activity among cows, periods of the cycle, and between cycles. Further research should be directed toward the expansion and practical application of the relationships found in this study.

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A P P E N D I X

TABLE V

PLASMA LIPASE VALUES OBTAINED FROM FOUR COWS ON FIRST CYCLE FOLLOWING PARTURITION

Day of Cycle	COW			
	564	561	488	477
-2[1]	.059		.049	.087
-1	.062	.067	.057	.085
0[2]	.085	.080	.059	.087
1	.080	.067	.072	.064
2	.082	.057	.064	.082
3		.067		.080
4		.075		
5	.064	.059	.072	.090
8	.080		.072	
9		.059		
10	.100		.080	.092
12	.080	.085	.064	
13				.082
14	.103		.059	
17	.123		.054	
18	.107			
19	.115			
20	.120			

[1]Two days prior to estrus.

[2]Day of estrus.

TABLE VI

PLASMA LIPASE VALUES OBTAINED FROM FOUR COWS ON SECOND CYCLE FOLLOWING PARTURITION

Day of Cycle	COW			
	564	561	488	477
-2[1]		.075	.067	.090
-1	.105	.062	.085	.102
0[2]	.087	.077	.090	.093
1	.112	.062	.077	.078
2		.077	.075	.075
3	.100		.065	
4		.072		.105
6	.126		.072	
9			.072	
10				
12				.077
13	.096	.080	.090	
16			.081	
17	.086		.050	
18	.074		.053	
19			.056	
20		.085	.080	
21			.050	

[1]Two days prior to estrus.

[2]Day of estrus.

TABLE VII

PLASMA LIPASE VALUES USED IN THE STATISTICAL ANALYSIS

Cycle Number	Period of Cycle	COW			
		564	561	488	477
1	proestrus[1]	.059	.067	.049	.085
1	estrus[2]	.085	.080	.072	.087
1	metestrus[3]	.080	.057	.064	.064
1	diestrus[4]	.085	.059	.069	.088
2	proestrus[1]	.105	.062	.067	.090
2	estrus[2]	.112	.077	.090	.093
2	metestrus[3]	.100	.062	.075	.075
2	diestrus[4]	.111	.080	.079	.077

[1]Lowest value within 2 days prior to estrus.

[2]Highest value within 1 day of displayed estrus.

[3]Lowest value within 3 days after estrus.

[4]Average of values between 5 days after estrus and 5 days prior to following estrus.

TABLE VIII

ANALYSIS OF VARIANCE

Source	df	Sum of Squares	Mean Squares	Cal. F	TABULATED F 0.05	TABULATED F 0.01
Total (uncorr.)	32	.203691				
Corr. factor	1	.196094				
Total (corr.)	31	.007597				
Cows[2]	3	.002982	.000994	16.85	3.86	6.99
564 x 561[2]	1	.002328	.002328	39.46	5.12	10.56
564 x 488[2]	1	.001849	.001849	31.34	5.12	10.56
564 x 477[1]	1	.000380	.000380	6.44	5.12	10.56
561 x 488	1	.000028	.000028	.47	5.12	10.56
561 x 477[2]	1	.000827	.000827	14.02	5.12	10.56
488 x 477[1]	1	.000552	.000552	9.36	5.12	10.56
Periods[1,3]	3	.001194	.000398	6.75	3.86	6.99
P x E[2]	1	.000784	.000784	13.29	5.12	10.56
P x M	1	.000003	.000003	.05	5.12	10.56
P x D	1	.000256	.000256	4.34	5.12	10.56
E x M[2]	1	.000885	.000885	15.00	5.12	10.56
E x D	1	.000144	.000144	2.44	5.12	10.56
M x D[1]	1	.000315	.000315	5.34	5.12	10.56
Cycles[2]	1	.001313	.001313	22.25	5.12	10.56
Cow x Period	9	.000624	.000069	1.17	3.18	
Cow x Cycle[1]	3	.000919	.000306	5.19	3.86	6.99
Period x Cycle	3	.000027	.000009	.15	3.86	
Error	9	.000538	.000059			

[1]Significant at ($P < 0.05$).

[2]Significant at ($P < 0.01$).

[3]Abbreviations: P = Proestrus, E = Estrus, M = Metestrus, and D = Diestrus.

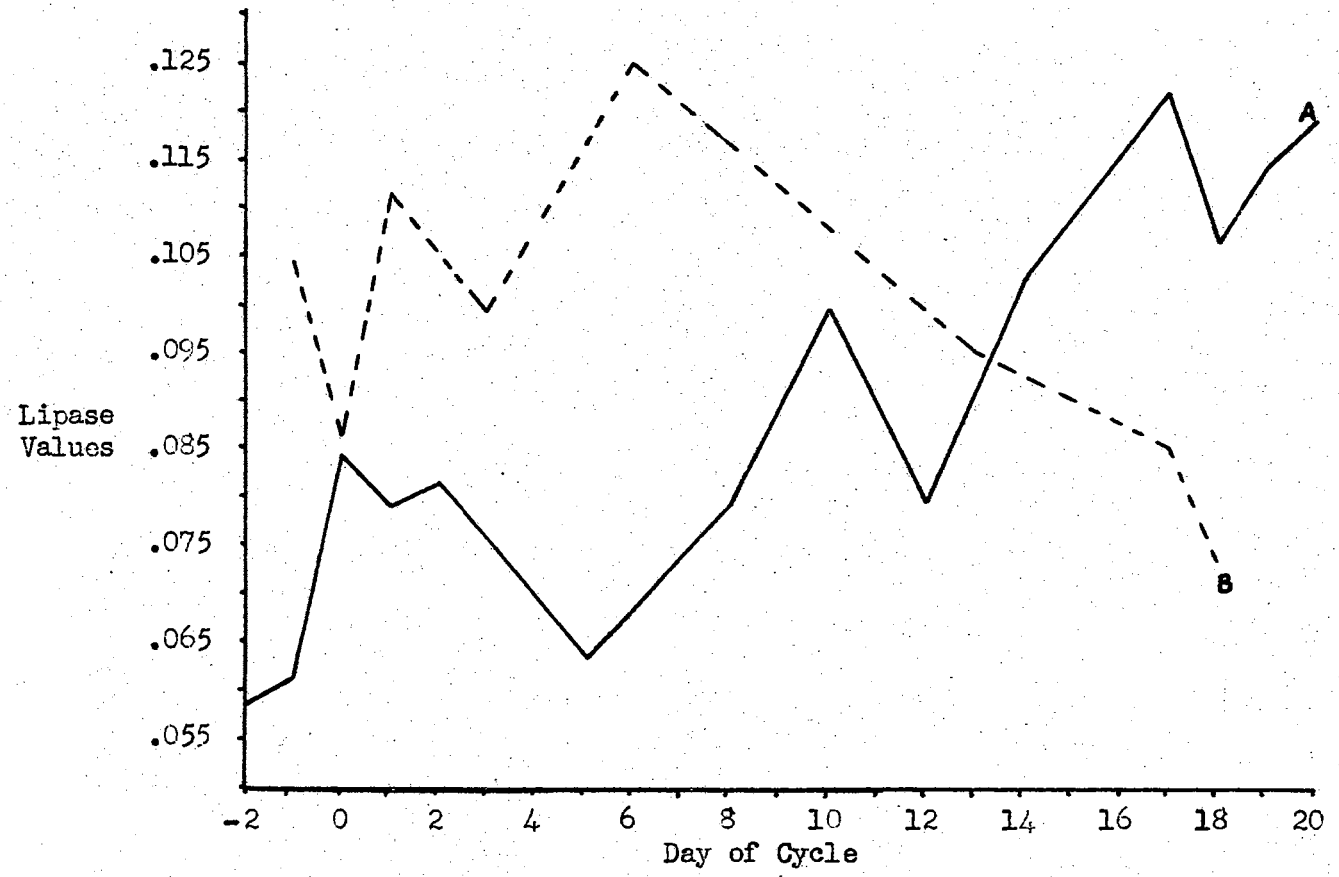

Figure 1. Lipase Values For Two Consecutive Estrous Cycles For Cow 564.

(A) Estrous cycle number one.
(B) Estrous cycle number two.

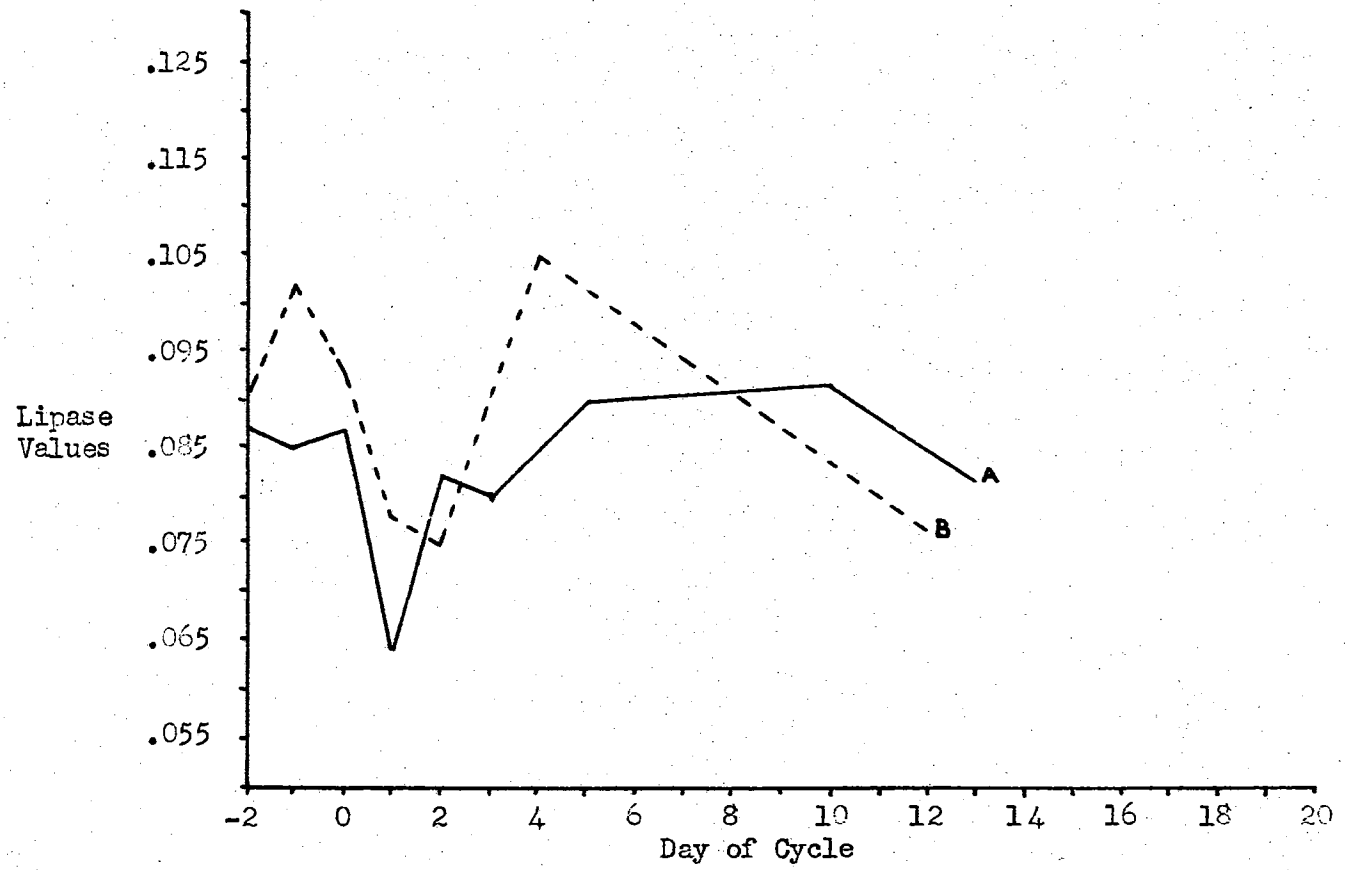

Figure 2. Lipase Values For Two Consecutive Estrous Cycles For Cow 477.

(A) Estrous cycle number one.
(B) Estrous cycle number two.

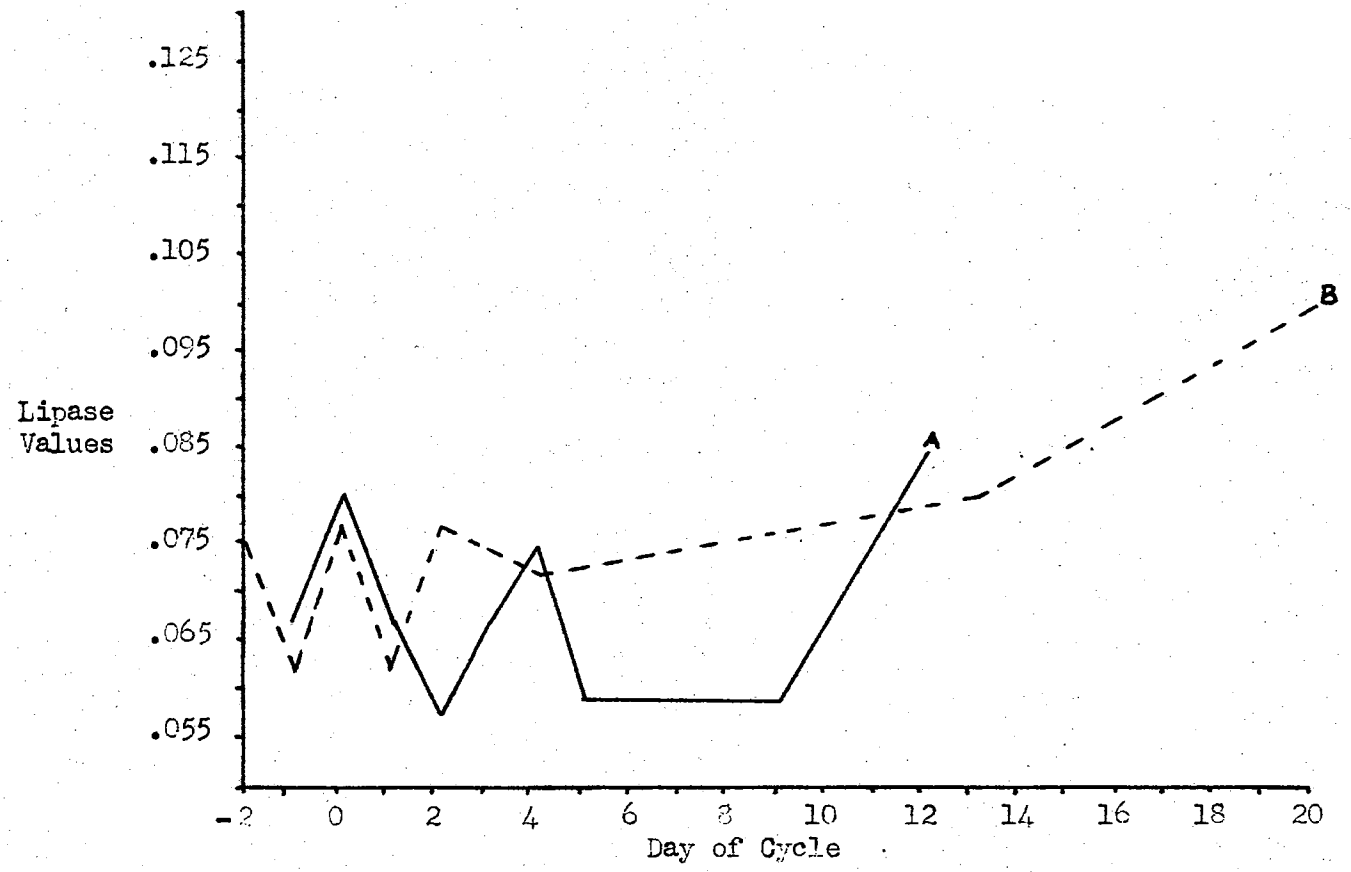

Figure 3. Lipase Values For Two Consecutive Estrous Cycles For Cow 561.

(A) Estrous cycle number one.
(B) Estrous cycle number two.

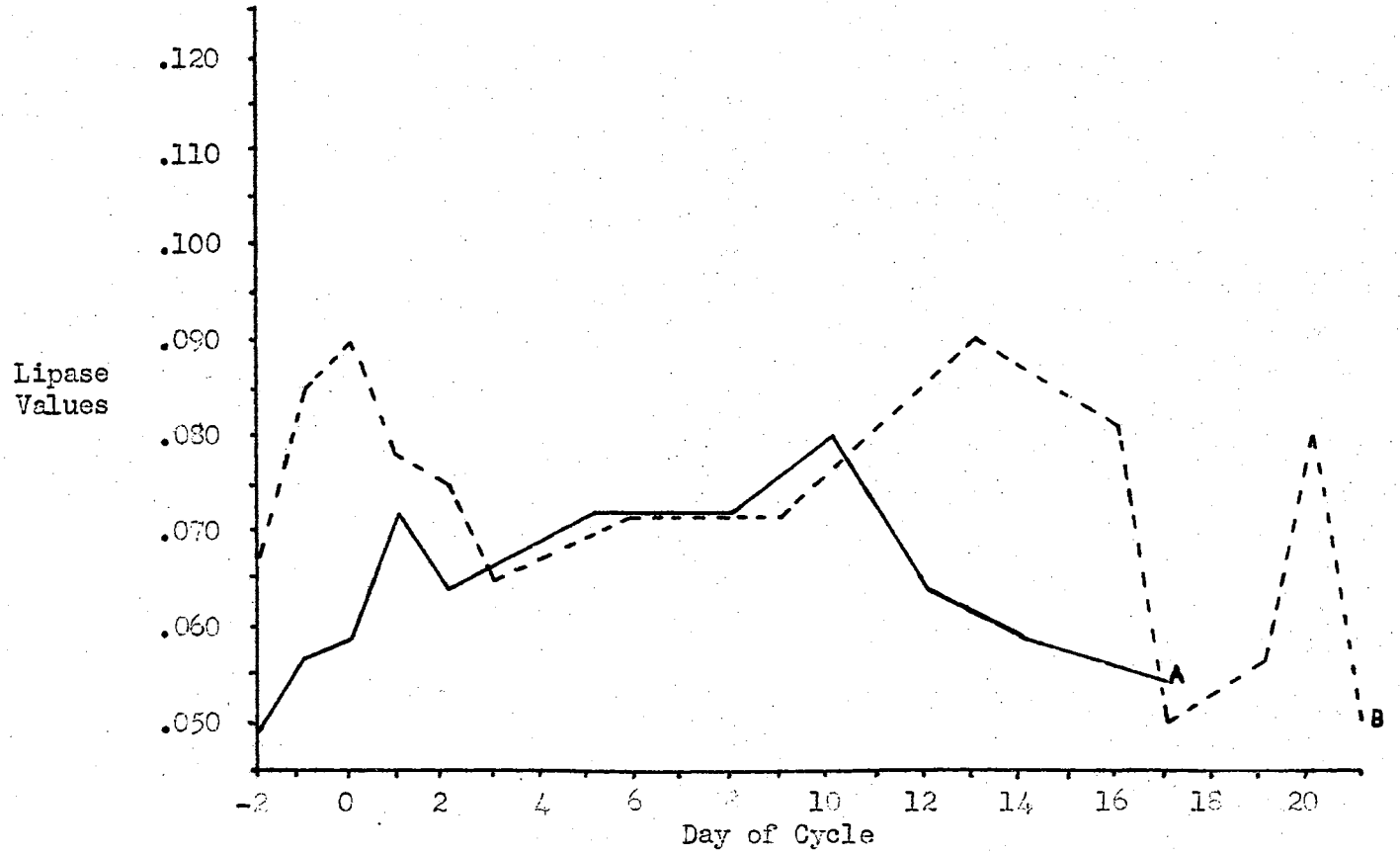

Figure 4. Lipase Values For Two Consecutive Estrous Cycles For Cow 488.

(A) Estrous cycle number one.
(B) Estrous cycle number two.

VITA

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