



Progesterone levels in the jugular vein and posterior vena cava of normal cycling Holstein heifers
by Dennis Paul Davis

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Animal Science
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Abstract:

Four normally cycling Holstein heifers were cannulated with Silicone rubber tubing in the jugular vein and posterior vena cava. The blood samples were analyzed for progesterone with a competitive proteinbinding assay which included a progesterone-internal standard. The study included five normal and four abnormal estrous cycles, ranging from 18 to 21 days and 14 to 30 days in length, respectively.

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A t-test of 10 progesterone sample-pairs (jugular -vs- vena cava) for the day of estrus found no significant differences; however, the ratio (jugular/vena cava) was 1.0 or greater for seven of the 10 pairs with a mean ratio of 7.29 ± 5.06 . Ratios for the three days before estrus starting with the day preceding estrus. were 0.81 ± 0.52 , 0.49 ± 0.12 and 0.47 ± 0.14 , respectively. The progesterone levels in the jugular vein exceeded that in the vena cava during four of the five surgery days. These data suggest that blood sampling from several sources within an animal may be useful in a compartmental analysis approach to. hormone binding and metabolism in vivo.

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CAVA OF NORMAL CYCLING HOLSTEIN HEIFERS

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DENNIS PAUL DAVIS

A thesis submitted to the Graduate Faculty in partial
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Approved:

R. W. Blackwell
Head, Major Department

E. L. Moody
Chairman, Examining Committee

R. Goring
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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ABSTRACT

Four normally cycling Holstein heifers were cannulated with Silicone rubber tubing in the jugular vein and posterior vena cava. The blood samples were analyzed for progesterone with a competitive protein-binding assay which included a progesterone-¹⁴C internal standard. The study included five normal and four abnormal estrous cycles, ranging from 18 to 21 days and 14 to 30 days in length, respectively.

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INTRODUCTION

The isolation of progesterone was achieved almost simultaneously by four groups of workers. The hormone was isolated in 1934 by the extraction of sows' ovarian tissue, and later the same year its structure was determined. It has been reported in bovine CL, ovaries, adrenals, placenta and blood.

Progesterone appears important in preparation of the uterus for implantation of the blastocyst, maintenance of pregnancy, as a conditioning agent in estrus, normal cyclic functions as a factor affecting tubal and uterine transport of sperm and ova, development of the alveolar system in the mammary gland, and hypophyseal-gonadal interrelationships.

Until recently studies relating to the metabolism and secretion of progesterone have been hindered by the lack of techniques sufficiently sensitive and specific enough for the quantitation of the hormone in small volumes of plasma. In the past it has been necessary to obtain reproductive tissue during surgery or slaughter for the estimation of progesterone.

There is not a great deal known about the hormone interrelationships involved in reproduction and any research providing basic information about these processes is worthwhile.

The present study was designed to quantitatively determine progesterone concentrations in the blood during the cycle to provide knowledge beneficial in future research. Some of the important phases of research

that may utilize conclusions drawn from this work are the estrus synchronization of cattle and attempted super ovulation leading to multiple births.

REVIEW OF LITERATURE

Cannulation Materials.-

A procedure using a medical grade of silicone rubber tubing for vascular cannulation was developed by Hull (1967). The tubing was physiologically inert, flexible and could be autoclaved many times. It was found that inherent rigidity was necessary for patency in blood collection. However, the tubing should have sufficient flexibility to not traumatize the vascular endothelium and induce thrombophlebitis as does the stiffer tubing. Christison and Curtin (1969) utilized silicone rubber for a catheter in the cranial vena cava of the pig. Venous blood samples could be drawn, or fluid injected into unanesthetized and unrestrained animals. Hamilton (1965) introduced silicone rubber catheters into the descending aorta of dogs and cats.

Robinson et al. (1969) devised an improved polyethylene cannulation technique. The method was developed to prevent the accidental removal of cannulae. A small bead was formed in the tubing which makes it possible to secure the cannula in place. A small section of the polyethylene tubing is heated momentarily which causes the plastic to contract lengthwise. This causes the formation of a small bead on the outside of the tube, but does not alter the lumen. Sutures can be secured immediately behind the bead or it can be passed into a vessel and the incision closed tightly around the tubing. Popovic and Popovic (1960) described a technique whereby the aorta and the vena cava of rats and ground squirrels are permanently intubated using polyethylene tubing. Shoemaker

et al. (1965) utilized polyethylene tubing for catheterization of the major hepatic vessels of dogs. Conner and Fries (1960) siliconized the inside and outside of polyethylene tubing for catheterization of portal and mesenteric veins of Holstein-Friesian calves. Conrad et al. (1958) fitted a polyethylene catheter in the gastrosplenic vein of a calf. Lumb et al. (1966) collected adrenal venous blood from cattle with polyethylene cannulae.

Blair-West et al. (1962) collected adrenal venous blood from sheep fitted with cannulae made of poly-vinyl tubing. Ralston et al. (1949) described a method whereby poly-vinyl chloride catheters were placed in the external jugular veins of cows. Katz and Bergman (1969) employed cannulae consisting of medical grade poly-vinyl tubing with attached "cuffs" for simultaneous collection of blood entering or leaving the liver and portal bed of the unanesthetized sheep.

Moodie et al. (1963) used two flexible polyamide (nylon) tubes, one of which fitted inside the other for the collection of portal and hepatic venous blood from conscious sheep. The authors found nylon to be less reactive than polyethylene and poly-vinyl chloride catheters, although within half an hour after insertion the nylon was coated with a fibrous coating. Ultimately, the presence of the nylon catheter resulted in the development of a low grade phlebitis.

Cannulation and Catheterization Sites in Various Animals.-

Ralston et al. (1949) inserted catheters in both external jugular

veins of cows. Radioactive substances were introduced into one jugular vein and blood samples were withdrawn from the other. The functional range of this catheter was 2.1 hours to 14.5 days. Popovic et al. (1968) surgically placed cannulae in the jugular veins of 50 mice. The polyethylene cannulae remained patent for drug injection as long as an animal lived. However, when blood samples were drawn, some cannulae were not functional longer than 10-15 days.

Popovic and Popovic (1960) exposed the right jugular vein of rats and ground squirrels. An incision was made in the anterior portion of the jugular to allow a cannula to be pushed into the body of the vein until the tip laid in the junction of the right superior and inferior vena cava. The cannulae would remain functional for 40 days with proper care. Christison and Curtin (1969) described a surgical technique whereby a catheter was inserted in the facial and external jugular veins of the pig and passed into the cranial vena cava. The catheters were placed in 11 pigs and remained patent for 0-47 days.

Popovic and Popovic (1960) made incisions in the neck of rats and ground squirrels in order to expose the left carotid artery. A small incision not larger than one-third the diameter of the carotid was made in the anterior part of the artery. A polyethylene tube was inserted through the incision and passed down the vessel until the tip reached the aorta. The cannula floating in the aortic blood stream seemed to have a longer life than those cannulae ending in the carotid. Hamilton

(1965) surgically implanted arterial catheters which allowed blood sampling and injection of fluids into unanesthetized and undisturbed dogs and cats. The silicone tubing is inserted into the descending aorta by way of the carotid artery and the free end passed through the skin at the back of the neck.

Conner and Fries (1960) described a technique for collecting portal vein blood from unanesthetized calves. The cannulae were used successfully for obtaining portal blood samples up to 73 days after an operation. Moodie et al. (1963) fitted catheters in the portal vein of sheep. The tip of the catheter laid in the vessel at the entrance to the liver (porta hepatis). Venous blood samples were collected from the region for an average period of 83 days (range 41-161 days). Katz and Bergman (1969) developed a procedure for cannulation of the hepatic vein of sheep. Cannulae remained patent up to five months, but the average functional period was five weeks. Shoemaker et al. (1965) described a surgical method for catheterization of the common hepatic vein, portal vein and splenic artery of dogs. The method allowed simultaneous blood sampling from all three major hepatic vessels in an unanesthetized dog.

Hume and Nelson (1954) developed a cannulation procedure for adrenal venous blood collection from conscious dogs. Blair-West et al. (1962) devised a surgical method for the successful cannulation of the adrenal vein in the sheep. Blood was allowed to flow from the left adrenal vein through a poly-vinyl tube, which passed outside the body and

was inserted in the jugular vein. Samples were collected from the conscious animal for a period of 2-4 days by breaking a Teflon joint inserted in the tubing. Lumb et al. (1966) reported a technique for obtaining adrenal venous blood from the conscious, as well as the anesthetized calf. The adrenal vein was cannulated and a loop of the polyethylene tubing brought through a stab wound in the skin and passed back into the body through another stab wound. Then the tip of the cannula was inserted in the posterior vena cava to maintain blood flow and allow free access to the cannula for venous effluent collection.

Hull (1967) described a procedure for the successful cannulation of the posterior vena cava of cattle. The cannulae were surgically placed in 12 Holstein-Friesian heifers and remained patent in blood sampling for 4 to 90 days (average functional life of 35 days).

Assay Methods for Progesterone.-

Studies concerned with the quantitative determination of progesterone levels in small volumes of plasma have been hindered in the past by the lack of sufficiently sensitive and specific chemical techniques. Gomes and Erb (1965) summarized all the available assay methods in a review of progesterone involvement in bovine reproduction.

Bush (1952) devised a paper chromatographic method for the effective isolation of small amounts of steroids in body fluids and biological media. Edgar (1953a) and Edgar (1953b) utilized ultra-violet absorption spectroscopy to determine progesterone content of body tissues and fluids.

Loy et al. (1957) developed a modified spectrophotometric technique which would permit estimation of the progesterone content of luteal tissue. Short (1958) described the successful quantitation of progesterone in large volumes of peripheral blood.

The general procedure for this widely used spectrophotometric method was an ether or hot alcohol extraction, column chromatography, countercurrent distribution, paper chromatography, ultra-violet absorption at 240 mμ and the use of progesterone -4-¹⁴C (Staples and Hansel, 1961 and Stormshak et al. 1961).

The spectrophotometric methods have a limit of sensitivity ranging from 0.5 to 1.0 μg if micro-cells are used in the spectrophotometer. Otherwise, five times as much hormone is required. Modification of the spectrophotometric techniques have been made to improve their sensitivity in progesterone measurements. Sommerville et al. (1963) quantitated progesterone in 5-10 ml of human plasma with a modification of the thiosemicarbazide reaction. The method was commonly used for 8-10 years in estimating progesterone content of tissues and yielded satisfactory progesterone determinations to 0.1 μg, but lacked the sensitivity necessary for blood analysis.

Short and Levitt (1962) described a fluoremetric procedure that was quite sensitive, but required purification and extreme care with reagents and paper to provide the necessary specificity. However, when the method was compared with the spectrophotometric method of Short

(1958), the fluorescence gave higher results at low concentrations of progesterone and lower results at high concentrations, indicating a lack of specificity in the fluorescence reaction. Riondel et al. (1962) applied the double-isotope-derivative procedure to the estimation of progesterone using progesterone³H with thiosemicarbazide³⁵S as the derivative forming agent. Woolever and Goldfein (1963) later reported quantitation by the conversion of progesterone to 20 B-ol using tritiated sodium borahydride and progesterone¹⁴C as the isotope sources. This method allowed effective measurements to slightly less than 0.01 ug of progesterone. Excellent techniques in gas chromatography have been reported by Yannone et al. (1964) and Stabenfeldt et al. (1970). Such methods afford sensitivity and specificity, but require a great deal of training to successfully apply them to steroid determination.

It has been proven recently that radio-immunoassays are sensitive and accurate in assaying the polypeptide hormones (Everett, 1969). The radio-immunoassay technique has been extended to steroid measurements and has the advantage that purification of the steroid is not necessary. However, Abraham (1969) does stress the importance of highly purified radio-labelled steroids.

Ferin et al. (1968) reported a radio-immunoassay technique for estradiol which employed an antisera raised in a ewe against estradiol-17B conjugated to bovine serum albumin. The assay has allowed detection of as low as 10 picograms estradiol, with dose response curves proven to

be linear between 10 and 200 picograms. Abraham (1969) has described a procedure for the radio-immunoassay of estradiol-17B. Plastic test tubes are coated with a standard amount of specific antibody and the quantity of radioactively labelled estradiol-17B that remains unbound or bound is determined in the presence of the test solution. Midgley et al. (1969) has demonstrated that the hapten-radio-immunoassay can be utilized for the quantitative determination of low molecular weight substances which can be made immunogenic by conjugation to a protein. This procedure has been employed for the quantitation of non-conjugated estrogenic steroids by the radioiodination of the protein portion of the conjugate.

Hendricks et al. (1969) employed gas-liquid chromatography and the protein-binding method to determine progesterone levels in systemic plasma collected from cows during the estrous cycle. Blood plasma analyzed with gas chromatography for Days 8, 12, 16 and 20 after estrus had progesterone levels at 4.4 ± 1.2 , 7.9 ± 1.2 , 7.1 ± 0.5 , 1.4 ± 0.2 ng/ml, respectively. Determinations made with the protein-binding assay were 0.6 ± 0.2 , 5.2 ± 0.7 , 4.9 ± 0.3 , 5.8 ± 0.9 , 1.6 ± 0.6 and 0.4 ± 0.1 ng progesterone per ml of plasma for 0, 10, 15, 17, 19 and 21 days after estrus. The results of this study indicated that plasma progesterone could be effectively measured with the less laborious protein-binding assay.

Competitive Protein-Binding Radioassay.-

The competitive protein-binding assay is a relatively simple, fast procedure for determining steroid levels in small volumes of plasma. The technique is specific in that the corticosterone-binding globulin (CBG, transcortin) has a binding affinity for only cortisol, corticosterone and progesterone..

This steroid binding protein was designated as CBG (cortisol-binding globulin) by Daughaday (1958) and transcortin by Slaunwhite and Sandberg (1959). Later, Murphy et al. (1963) termed the ~~α~~ globulin fraction as corticosteroid-binding globulin, which she used in a simple method for determining plasma corticoids. The assay employing this binding globulin has been named by Murphy (1964) as the "competitive protein-binding (PB) analysis" and by Barakat and Ekins (1961) as a "saturation analysis".

Murphy et al. (1963) utilized the steroid-binding properties of plasma to estimate levels of cortisol and corticosterone in human plasma. When increasing amounts of unlabelled cortisol were added to an equilibrium dialysis system containing plasma and a constant amount of cortisol-4-¹⁴C, there was a proportional decrease in the percentage of cortisol-4-¹⁴C bound to the plasma protein. These findings suggested that the binding depression induced by added steroid might provide a process for determining plasma levels of cortisol and corticosterone. Murphy (1967) found it possible to routinely measure cortisol in 0.1 ml of plasma or

cerebrospinal fluid and progesterone in 0.3 ml of plasma. She was also able to determine levels of compound S(11-desoxycortisol), corticosterone, cortisone, and 17-hydroxyprogesterone with the protein-binding assay. Neill et al. (1967a) analyzed the daily relationship between the concentrations of luteinizing hormone (LH) and progesterone in the peripheral plasma during the normal human menstrual cycle. Progesterone levels were determined in 0.5 ml aliquots of plasma by utilizing a modification of the protein-binding method reported by Murphy (1967). Neill et al. (1967b) determined the plasma progesterone concentrations throughout the menstrual cycle of normal Rhesus monkeys by employing the competitive protein-binding technique. Johansson et al. (1968) used the protein-binding assay to measure the peripheral plasma progesterone concentrations in normal cycling Rhesus monkeys at the day of ovulation.

Bassett and Hinks (1969) utilized the assay for the determination of corticoids in 0.1 ml or less of ovine plasma. Bassett et al. (1969) quantitated progesterone levels in the peripheral plasma of pregnant ewes throughout gestation with the protein-binding assay.

Before progesterone or other steroid levels can be quantitatively determined with the protein-binding assay they must be effectively isolated. Neill et al. (1967a) reported that progesterone could be extracted from 0.5-1.0 ml of plasma with 5 ml reagent grade Petroleum Ether (boiling 30°-60°C). This procedure extracted 90 percent of the progesterone from the plasma, and left behind over 99 percent of the

cortisol and corticosterone. After extraction, progesterone was isolated from its metabolites in a single chromatographic step. Samples were spotted on silica gel thin layer sheets and developed in ether-benzene 2:1 by ascending chromatography.

The manner in which the competitive protein-binding globulin is prepared is an important consideration in the assay. Murphy (1967) investigated the effectiveness of plasma from various species as a binding globulin in the competitive protein-binding assay. One ml of dog plasma could be used satisfactorily over the range 0.2-4 ng cortisol, monkey plasma for the range 0.2-4 ng corticosterone, cat plasma was effective over the range 0.2-4 ng 11-desoxycortisol, rabbit plasma over the range 0.2-4 ng cortisol and chicken plasma was satisfactory for 0.2-4 ng cortisol, 11-desoxycortisol, progesterone, or cortisone. Murphy (1967) concluded a 100-fold increase in the sensitivity of the assay could be obtained by selecting the proper species for the source of the binding protein and the use of tritiated steroids of higher specific activity than the previously used ^{14}C labeled steroids. Neill et al. (1967a) prepared CBG-corticosterone -1, 2- ^3H by diluting 2.5 ml of dog plasma to 100 ml with distilled water. Then 25 ng of corticosterone -1,2- ^3H (100 ng/ml ethanol) was added and the solution mixed gently. Johansson et al. (1968) enhanced the sensitivity of the assay by increasing the activity of the tritiated corticosterone from 33.4 to 57.2 c/mmole. The higher specific activity of the steroid allowed the mass of corticosterone

-1,2-³H to be reduced from 25 to 15 ng for a solution containing 2.5 ml of dog plasma dispersed in 97.5 ml of distilled water.

In order to quantitatively determine the steroid levels in plasma it is necessary to establish a standard curve for every group of samples analyzed. Neill et al. (1967a) pipetted a standard curve representing 1, 2, 5, and 10 ng of progesterone from aliquots of a progesterone solution in absolute ethanol (100 ng/ml). One ml of CBG saturated with corticosterone -1,2-³H was added to each sample tube containing isolated progesterone and every member of the standard curve. All tubes were placed in a 45°C water bath for five minutes to bring the progesterone into solution. The tubes were transferred to an ice bath and allowed to remain there for 10 minutes to increase the affinity of the CBG for the steroids. The steroids establish a dynamic equilibrium and compete for the binding sites on the protein.

A variety of procedures have been used to separate the unbound steroids from the fraction bound to the protein. Murphy et al. (1963) developed a dialysis method for estimating cortisol and corticosterone in human plasma. The chief disadvantage of dialysis from the clinical point of view is the 40 hours required for the process. Murphy and Pattee (1964) used corticosteroid-binding globulin in a gel filtration procedure to determine plasma corticoids. It was similar to the dialysis method employed earlier by Murphy et al. (1963) and retained the specificity, precision, and sensitivity. However, gel filtration could

be carried out in approximately two hours. De Moor et al. (1962) determined the amount of transcortin-bound corticoids in human plasma by using gel filtration on Sephadex columns. At a later time, De Moor et al. (1963) used this procedure to study the influence various steroids have on the cortisol-binding ability of human plasma in vitro. Murphy (1967) examined the effectiveness of several insoluble adsorbing agents (Fuller's earth, Florisil, coated charcoal) in separating protein-bound and unbound steroids. Murphy (1967) concluded that Fuller's earth, coated charcoal, and Lloyd's reagent were satisfactory for separating protein-bound and unbound cortisol, while Florisil and coated charcoal were suitable for the separation of protein-bound and unbound corticosterone. Neill et al. (1967a) reported that 80 mg of Florisil (activated magnesium silicate, 60-100 mesh) effectively adsorbed to all of the unbound labelled corticosterone in the solution when mixed for exactly 30 seconds in a Vortex mixer.

Neill et al. (1967a) had established that 0.5 ng of progesterone could be measured with a high degree of precision using the protein-binding assay. The lower limit of sensitivity in the technique appeared to be approximately 0.2 ng of progesterone. Johansson et al. (1968) found that saturation of the binding-globulin in dog plasma with high specific activity (57.2 c/mmole) corticosterone, in addition to the rigid standardization (exactly 30 seconds) of the shaking in the Vortex

mixer allowed detection of as little as 0.1 ng progesterone in 0.5 ml monkey plasma.

Progesterone Levels in the Non-pregnant Cow.-

Corpus Luteum and Ovary.- Several research workers concerned with various aspects of reproduction have measured progesterone levels in luteal tissue from cows and heifers at different stages of the estrous cycle.

Erb et al. (1962) collected 42 corpora lutea from 31 cows and heifers throughout the estrous cycle and analyzed them for progesterone content. Three cows had a small amount of progesterone in regressing corpora lutea (CL) during estrus or one day after, but there was no detectable progesterone at Day 2. However, from three to nine days after estrus, the average progesterone content increased from 16 to 158 ug per corpus luteum. The mean progesterone content of the corpus luteum reached a maximum of 570 ug at Day 14, which was followed by a sharp decline for Days 15 through 20. The considerable variation among animals in luteal tissue progesterone content was apparently related to the body weight and age of the animal. The mean concentration of progesterone was found to be 19 ug/g at Day 3 and reached a peak of 38 ug/g at Day 5, declining to 25 ug/g at Day 9, with a corresponding rapid increase in the concentration to a peak of 73 ug/g at Day 14.

Erb and Stormshak (1961) concluded the developing corpus luteum has a high progestin concentration (progesterone and Δ 4-pregnene-20B-ol-

3-one) per gram of luteal tissue on the second and third days of the cycle, but the total quantity was less than 100 ug until after Day 6. At a later stage of the estrous cycle (Days 14-16), the average progestin content rose to 251 ug. They also reported that progestins in the corpus albicans ranged from 0 to 24 ug before ovulation.

Armstrong and Hansel (1959) concluded that administration of natural or synthetic oxytocin to cycling heifers during the first week after estrus resulted in a shortened estrous cycle of 8 to 12 days. Corpora lutea formed during the oxytocin treatment period were smaller than the normal CL. The effect of exogenous oxytocin on the progestin content of the CL was investigated by Mares and Casida (1963). Normal cycling heifers were injected intravenously with (110 I.U.) oxytocin twice daily on Days 12 and 13, and the corpus luteum removed on Day 14, 24 hours after the last injection. The treated corpora lutea had a significantly ($P < .01$) greater content of both Δ^4 -pregnene-20B-ol-3-one) and progesterone than control CL.

Ovarian Vein.- The progestin levels in venous plasma collected through laparotomy and sectioning one of the veins draining the ovary containing the active CL or ripened follicle in three non-pregnant cows and heifers was analyzed by Gomes et al. (1963). The plasma levels were not detectable at estrus or one day following. However, there was a significant increase in the progestin concentrations in the ovarian venous plasma from Day 2 of the estrous cycle to a peak level of 6.2 ug/ml on

Day 15. Dobrowolski et al. (1968) determined the progesterone content of ovarian venous blood for nine cycling cows, three of which rose from 5.6 ug/100 ml plasma on Day 1 of the estrous cycle, to approximately 125 ug/100 ml at Day 8, and to about 180 ug/100 ml on Days 14 and 15 of the cycle. After this, there was a rapid decline (10 to 20 ug/100 ml) as the animals approached ovulation.

Jugular Vein.- Gomes et al. (1963) reported progesterone levels ranging from non-detectable to 0.0540 ug/ml of plasma in the jugular blood of 31 non-pregnant, cycling dairy cows and heifers. Stabenfeldt (in a discussion by Kaltenbach and Cook, 1968) reported a rapid rise in jugular vein plasma progesterone levels after estrus in four cows starting about Day 4 and continuing through about Day 16, followed by a marked decline at approximately Day 17 or 18.

Stabenfeldt et al. (1969) determined the daily progesterone levels in the jugular plasma of six cows during seven complete estrous cycles. The progesterone levels extended from less than 0.5 ng/ml plasma during the follicular phase to 6.6 ng/ml plasma at peak luteal phase. The study indicated that progesterone levels measured throughout 21-day cycles increased markedly from Day 3 to Day 8 (estrus = Day 1) with a much slower rate of increase from Day 8 to Day 17.

Progesterone Levels in the Pregnant Cow.-

Corpus Luteum and Ovary.- Gorski et al. (1958) determined the progesterone content of corpora lutea (five assays each containing three

CL) taken from pregnant cows. The luteal tissue contained an estimated 90 percent of the progesterone found in the ovaries of cows with levels ranging from 6.4 to 15.2 ug/g of tissue. The ovaries of a Jersey heifer pregnant 259 days contained an estimated 50 ug of progesterone.

The progesterone concentration in bovine corpora lutea throughout gestation were investigated by Melampy et al. (1959). They reported a mean of 2.3 ± 0.7 ug progesterone/g of luteal tissue for Days 10-49 of pregnancy, 3.6 ± 1.5 ug/g for Days 50-89, 5.0 ± 1.2 ug/g for Days 90-129, 3.8 ± 0.9 ug/g for Days 130-169, 3.9 ± 0.7 ug/g for Days 170-209, 1.5 ± 0.5 ug/g for Days 210-249, and 1.1 ± 0.3 ug/g for Days 250-280. The residual ovarian tissue was also investigated for progesterone content, and found to contain 1.1 ± 0.5 ug/g at Days 10-49 of gestation, 1.0 ± 0.4 ug/g for Days 50-89, 2.7 ± 0.6 ug/g for Days 90-129, 1.6 ± 0.5 ug/g for Days 130-169, 1.9 ± 0.5 ug/g for Days 170-209, 0.7 ± 0.1 ug/g for Days 210-249, and 0.8 ± 0.5 ug/g for Days 250-280 of the gestation period.

Erb et al. (1960) determined the progestin content of corpora lutea taken from 41 pregnant cows. The progestins (progesterone + Δ^4 -pregnene-20B-ol-3-one) averaged 25.5 ± 3.1 ug/g of luteal tissue for cows from 65 to 275 days of gestation. However, the concentration was greater ($P < 0.005$) for 11 cows between 50-109 days of gestation (49.0 ± 8.5 ug/g) than for cows in the late stages of pregnancy (23.5 ± 1.8 ug/g). The corpora lutea at the early stages of gestation were approximately 43

percent larger and did not change appreciably in concentration after 110 days of pregnancy. A study performed by Erb et al. (1961) indicated that the progesterone content in the CL of pregnancy was at a peak of about 270 ug at Days 13-15 and reached a low average of about 150 ug for Days 37-75, which was followed by an increase in content to Day 212 and a gradual decline to parturition.

Stormshak and Erb (1961) assayed 89 CL and 54 pairs of ovaries to determine progestin levels in cows pregnant 16 to 275 days. The progestin concentration was significantly higher ($P < 0.01$) in corpora lutea collected during Days 16 to 89 of gestation (33.6 ug/g) than at Days 90 to 179 (20.4 ug/g) or after 180 days (27.5 ug/g). There was significantly less progesterone per gram of luteal tissue at 90 to 179 days (12 ug/g) than after 180 days (18.7 ug/g) or for 16 to 89 days (20.4 ug/g). It was generally found that the progesterone content of the ovaries without corpora lutea was less than 0.1 ug/g.

Erb and Stormshak (1961) reported that seven pregnant cows had an average progestin (progesterone and 20B-ol) content of 161 ug/CL on Days 25-34, and six cows had 250 ug/CL at 37-42 days. Erb et al. (1968) reported the progesterone content ($P < 0.025$) of the pregnant bovine CL raised significantly as the age of the animal increased, but progesterone concentration did not. The average values for the study were: progesterone content of the CL 199 ± 9.9 ug and CL concentration,

31.1 ± 1.4 ug/g during Days 11 to 284 for cows which averaged 54 ± 2.2 months of age.

Staples and Hansel (1961) utilized the effectiveness of oxytocin injections in repressing corpus luteum development to study the relationship between embryo survival at 15 days after insemination and the progesterone content of luteal tissue. The 20 control heifers with normal embryos had a progesterone content in the CL of 270.2 ug. Eighteen of the heifers that received subcutaneous oxytocin (7 u.s.p. units per hundred pounds of body weight per day) had normal embryos and the average total progesterone content of their corpora lutea was 310.6 ug. Ten of the oxytocin treated heifers were not pregnant and had significantly less ($P < 0.05$) total progesterone (154.1 ug) than did the treated heifers having embryos. The data suggests that a threshold level of 100 ug progesterone in the CL is necessary for embryo survival at the 15th day.

Johnson and Erb (1962) reported that the progesterone concentration and the total progesterone content in the corpus luteum of pregnant dairy cattle was significantly increased ($P < 0.005$) by the administration of exogenous progesterone 1 to 12 days before ovariectomy. The results of the study indicated that pregnancy could be maintained if ovariectomy was performed at no earlier than 88 days after conception and exogenous progesterone treatment was begun at least eight days before surgery.

The progesterone levels in the corpora lutea of beef cattle bred at estrus after being treated with PMS on Day 16 and HCG at Day 20 were determined by Estergreen (1964). The progesterone content in luteal tissue differed significantly ($P < .01$) among those studied at three days, 30 to 45 days, and 60 to 90 days after ovulation occurred, with the highest levels being determined for the heifers pregnant 30-45 days.

Niswender et al. (1965) studied the effect low levels of estradiol-17B had on the ovarian activity of cycling beef heifers. Estradiol-17B injections were administered at various dosage levels on Day 6 of the estrous cycle. The CL weight, progestin content, progestin concentration and follicular fluid weight at Day 12 was generally lower in the heifers treated with higher levels of estrogen than in the control heifers. Estrogen injections given daily from Day 6 to 18 resulted in a reduction of luteal and follicular activity, with higher levels of estradiol showing the greatest decrease. It was concluded that six levels of estrogen (20, 40, 80, 160, 320 and 640 ug) administered to the heifers resulted in a reduction of all measures of ovarian activity.

Adrenal Gland.- The cortex of the mammalian adrenal gland had been found to contain nearly 50 steroids (Turner, 1966). Among these are androgens, progestogens and estrogens which are essential to reproductive processes.

Gorski et al. (1958) found the adrenal glands of a Jersey heifer pregnant 258 days to contain less than 10 ug of progesterone. The pro-

gesterone content of the left adrenal gland was investigated by Melampy et al. (1959). The steroid was non-detectable for Days 10-49 of pregnancy. However, the mean progesterone concentration was 1.5 ug/g of adrenal tissue for Days 50-89, 1.7 ± 0.04 ug/g for Days 90-129, 1.5 ± 0.09 ug/g for Days 130-169, 2.1 ± 0.09 ug/g for Days 170-209, 0.4 ug/g for Days 210-249 and 0.7 ug/g for Days 250-280.

Erb et al. (1960) reported the progestin content in adrenal glands collected during gestation to be 23.4 ug. Stormshak and Erb (1961) concluded that the progesterone content of 54 pairs of adrenal glands taken from pregnant cows was generally less than 1 ug/g. Erb and Stormshak (1961) described the adrenals of four cows 8-22 days post-partum to be large (average 46 g) and with progestin levels about four times greater than is generally observed (66 ug).

Placenta.- The placenta is a source of estradiol and progesterone in most farm animals (Hafez, 1968). However, the endocrine activity of the placental tissue varies a great deal among species. The progesterone concentrations in the placenta are low and are not always readily detected.

Gorski et al. (1958) found negative results for progesterone determination in six assays of cow placentae. Melampy et al. (1959) analyzed the bovine placenta during gestation for the presence of progesterone. The placental tissue contained an average of 0.08 ± 0.04 ug progesterone/g during Days 10-49 of pregnancy, 0.08 ± 0.03 ug/g for

Days 50-89, 0.16 ± 0.09 ug/g for Days 90-129, 0.20 ± 0.06 ug/g for Days 130-169, 0.20 ± 0.07 ug/g for Days 170-209, 0.05 ± 0.02 ug/g for Days 210-249 and 0.03 ± 0.01 ug/g for Days 250-280. Williams et al. (1964) characterized the distribution of progesterone in the uterine endometrium and myometrium. Centrifugal fractionation and chromatography on silica gel were used to study the presence of progesterone, 20B-ol and hydroxyprogesterone, in both uterine endometrium and myometrium.

Ovarian Vein.- Erb et al. (1961) reported that the progesterone concentration in the ovarian venous plasma from the CL bearing ovary was four times greater than the levels in the plasma from the opposite ovary ($P < 0.005$). Progesterone levels in the ovarian vein of cows pregnant 250 to 282 days were estimated by Gomes et al. (1962). Whole blood from ovaries (with corpora lutea) was obtained from the sectioned ovarian vein after laparotomy of cows pregnant 250-254 days averaged 2.2 ug progesterone/ml. However, during this same period, two plasma samples averaged 3.9 ug/ml. Blood collected from the ovarian vein of a cow pregnant 282 days had 1.2 ug/ml of whole blood and 1.4 ug/ml of plasma. Erb et al. (1968) described a significant ($P < 0.01$) decline in the progesterone values of ovarian venous plasma during pregnancy. The progesterone concentration declined from the 14 day level, 4.5 ± 0.77 ug/ml to 2.6 ± 0.29 ug/ml, during the second and third month of pregnancy. This was followed by a rise in progesterone level during the fourth

month only to decline to the lowest levels during 199 to 237 days of pregnancy.

Jugular Vein.- Short (1960) found that progesterone levels in the peripheral blood of pregnant cows remained relatively constant throughout the gestation period, but approximately 10 days before parturition it starts to decline. Gomes et al. (1962) reported that two cows pregnant 250 and 251 days had an average of 0.0092 ug progesterone per ml of peripheral plasma. Holm and Short (1962) examined progesterone levels in the peripheral blood of five cows pregnant up to 92 days beyond the normal gestation period (285 days). The levels during mid-pregnancy were the same as in normal cows, but there was no decline in progesterone level just prior to the expected date of parturition which is characteristic of normal cows. The progesterone content of jugular plasma was investigated by Erb et al. (1968) and found to increase significantly ($P < 0.05$) during pregnancy, with an average of 30.3 ± 1.7 ng/ml throughout gestation. Shemesh et al. (1968) concluded that the progesterone levels in jugular plasma were similar in cycling cows (mean = 0.43 ± 0.35 ug/100 ml) and inseminated cows during Days 10 to 18 after ovulation, regardless of whether the bred cows were pregnant (0.48 ± 0.43 ug/100 ml) or not. However, a significant difference between non-pregnant (cycling) and pregnant cows was observed 19 days after ovulation. Only two of 12 non-pregnant cows had measurable blood levels of progesterone (0.16 to 0.22 ug/100 ml); whereas, eight pregnant

cows had plasma progesterone levels greater than 0.34 ug/100 ml, on Day 19. The pregnant cows averaged 0.47 ± 0.03 ug/100 ml at that stage of gestation.

Erb et al. (1961) collected blood from the jugular vein of pregnant cows before and after ovariectomy to investigate the influence removal of the ovaries has on the concentration of progesterone in plasma. The levels in 115 plasma samples taken from intact cows, averaged 35 ± 1.1 ng/ml and did not alter significantly from 181 days of pregnancy through 12 hours post-partum.

A peak concentration of 44 ± 2.7 ng/ml was achieved at 261 days. In ovariectomized animals there was no apparent difference in the plasma progesterone levels following surgery in comparison with presurgery concentration until after seven days post-ovariectomy. These levels declined significantly thereafter and were maintained at a low concentration through one day post-partum. Four cows pregnant 250-254 days, and ovariectomized by Gomes et al. (1962), had no detectable progesterone in 1400 ml of peripheral plasma taken one hour after ovariectomy. Shemesh et al. (1968) detected a low concentration of progesterone (0.17 ug/100 ml) in jugular venous plasma collected from a cow at seven days post-ovariectomy, but was undetectable (≤ 0.15 ug/100 ml) three weeks after the surgery had been performed.

Stabenfeldt et al. (1970) determined progesterone concentrations in peripheral plasma of pregnant cows from Day 140 through parturition.

From Days 140-200 of gestation there was little increase in levels, with an average of 4.6 ng progesterone/ml plasma for the interval. The average progesterone concentrations had increased to 6.8 ng/ml plasma by Day 250. This was followed by a decline to 4 ng 10 days before parturition and an abrupt drop to less than 1 ng/ml approximately 24 hours prior to parturition. The authors (Stabenfeldt et al., 1970) concluded that the preparation for parturition begins 10 days pre-partum in the cow.

MATERIALS AND METHODS

Normal cycling, 600 pound Holstein heifers were selected from the Montana State University Dairy herd. Cannulations were made in the jugular vein and posterior vena cava of five heifers. The vena cava cannulae were surgically inserted by laparotomy and palpation, ventral and slightly lateral to the spinal column as described by Hull (1967).

Silastic rubber tubing manufactured by Dow Corning Corporation¹ was utilized as a cannulation material. The size of tubing was not the same for every cannula. Two sizes were introduced into the veins, the first had an inside diameter (I.D.) of 0.062" and an outside diameter (O.D.) of 0.095" and a thicker walled tubing, I.D. 0.062" and O.D. 0.125".

Prior to collection of the blood sample, the heparinized cannula was gently flushed with 2 to 3 ml of saline. Approximately 5 ml of saline, heparin, and blood was drawn into the syringe and the mixture discarded. A sample of 15 ml venous blood was drawn into the syringe and transferred to a heparinized centrifuge tube. Parafilm was placed securely over the tube and the blood mixed gently to distribute the heparin. Approximately 2 ml of heparin was injected onto each cannula to prevent clotting until the next blood collection.

Each morning after the blood samples had been collected, the heifers were watched to detect estrus. If an animal was due to come in estrus and no characteristic behavior was noticeable in the morning, observations were made in the afternoon, and all estrus dates recorded.

¹ Distributed by Biddle Crowther Co., 1801 Broadway, Seattle, Wash.

The blood samples were brought into the laboratory and immediately placed in refrigeration. Three to four hours later the samples were centrifuged at approximately 1200 RPM for two hours. This yielded 6-7 ml of straw-colored plasma. Disposable Pasteur pipettes were used to transfer the plasma to plastic Whirl-Pak containers. These containers were labelled and stored in the freezer at -10°C .

Progesterone Analysis.-

The quantitative determination of progesterone was made with the competitive protein-binding analysis of Murphy (1963). Specific developments for progesterone determination made by Neill et al. (1967a) were utilized in the assay.

The procedure is based on the ability of cortico-steroid binding globulin (CBG) in dog plasma to bind a select group of steroids, i.e., cortisol, corticosterone and progesterone. High specific activity (57.2 c/mmole) corticosterone $-1,2-^3\text{H}$ is used to saturate all the binding sites on the protein. When isolated progesterone is added to the system, a dynamic equilibrium is established with progesterone and corticosterone $-1,2-^3\text{H}$ competing for the binding sites. The amount of progesterone is quantitated by counting the ^3H labelled corticosterone which remains bound to the CBG. The greater the concentration of progesterone in the system, the lower the counts of ^3H due to a lower amount of bound corticosterone.

Plasma samples were allowed to thaw at room temperature prior to

extraction. In order to determine losses of endogenous progesterone incurred through extraction and isolation from the plasma, an interval standard of ^{14}C progesterone was prepared. The standard solution contained 1ng of ^{14}C progesterone (sp. act. 58.5 mc/mmmole) per 10 ul of ethanol. Before pipetting the plasma into the 12 ml extraction tubes, a 10 ul aliquot of the activity was added to each tube with a microliter syringe #725¹. At the same time, six standards of ^{14}C progesterone (for each group of 40 samples) were pipetted into 18 X 56 mm, low potassium counting vials.

Plasma volumes ranging from 0.25 to 2.5 ml were added to the extraction tubes. The plasma was extracted twice, with five times its volume of petroleum ether (30°-60° C Ligroine) as described by Neill et al. (1967a). The tubes were shook for approximately 30 seconds in each extraction and the extract transferred with Pasteur pipettes to 15 ml disposable culture tubes. They were placed in a 52°C salt bath under the hood and dried with a light stream of nitrogen. Two ml of pet ether were used to rinse the walls of each culture tube. After the extract had dried sufficiently under nitrogen, the rinsing procedure was repeated with 0.5 ml pet ether, further concentrating progesterone in the bottom of the tube.

In order to quickly and effectively isolate progesterone from other steroids and plasma pigments that were in the pet ether extract, a

¹ Manufactured by Hamilton Co., Inc., Whittier, Calif.

process of thin layer chromatography was adopted. Spotting single samples with a Pasteur pipette proved to be time consuming, so a spotting device was built to accommodate two chromatogram sheets at one time. (Figure 1).

A plexiglass overlay with seven evenly spaced slots was placed upon the 250 u 20 X 20 cm silica gel chromatogram¹. The metal tip of a dissecting pith was used to mark six lanes into the silica gel utilizing the overlay as a guide (Figure 2). Then the sheets were placed on the spotting device in preparation for spotting.

Two hundred ul of chloroform was added to each culture tube to serve as a spotting medium. The solution was taken up in Pasteur pipettes and added to the plastic syringes. The tip of each 1", 30 guage needle was suspended approximately 1.0 cm above the center of every lane and 2.2 cm from the bottom of the chromatogram. As the chloroform solution dripped from the needles onto the sheets, a stream of warm air was passed across the silica gel with an electric hair dryer². The warm air hastened the drying of the chloroform spot and thus prevented excessive spreading of the solution. When the spotting was complete, syringes and needles were thoroughly rinsed to cleanse them of any progesterone residue. First 2 ml of anhydrous methanol were pipetted into each syringe and expelled through the tip of the needle. This procedure was then repeated with 1-2 ml of chloroform.

¹ Eastman Chromatogram sheet No. 6060 with fluorescent indicator.

² Model 38100, McGraw-Edison Co., Bersted Mfg. Div., Boonville, Mo.

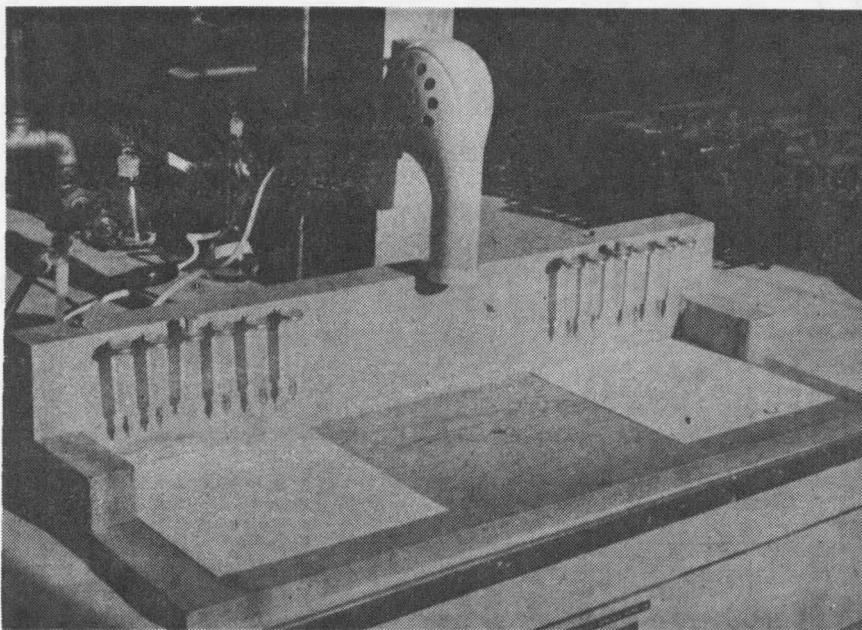


Figure 1. Chromatogram sheets on the thin layer spotting device.

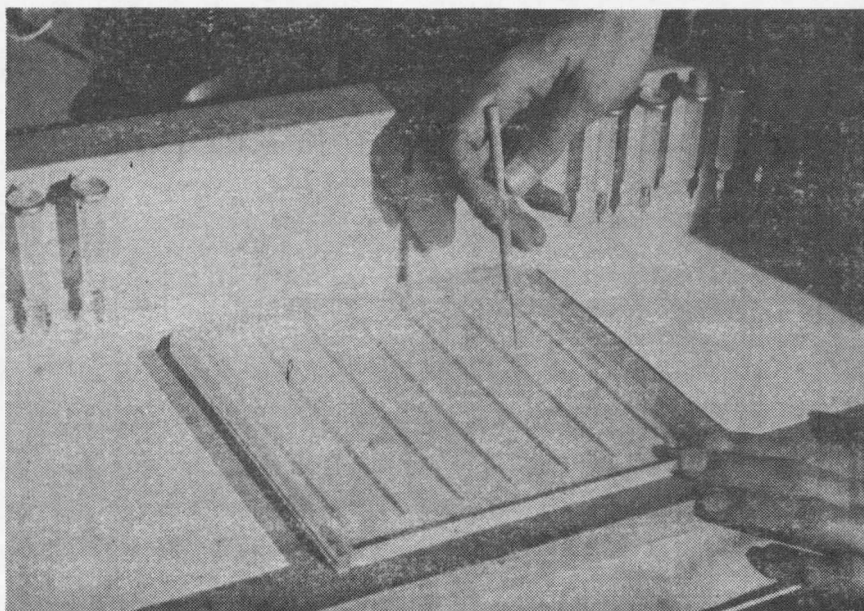


Figure 2. Plexiglass overlay used as a guide for marking the chromatogram.

In order to determine the effectiveness of chromatography in isolating progesterone, a standard progesterone reference was spotted in one of the two center lanes on the chromatogram. The standard contained 40 ug progesterone per ml of a benzene: methylene chloride (1:1) solution. Approximately 100 ul. of the standard was allowed to drip from a Pasteur pipette into a small, single spot on the sheet. The disposable pipette was used in this spotting process to avoid progesterone contamination of the needles and plastic syringes.

The chromatograms were placed in a glass thin layer chromatography tank containing an ether:benzene (2:1) solvent. Each plate was allowed to remain in the tank for 55 minutes, which brought the solvent front to within approximately 2 cm of the upper edge. The compounds present in the spots on the chromatogram separate according to their adsorbing affinity for the solvent and the silica gel. The sheets were viewed under ultra-violet light in the Chromato-Vue¹; by measuring the distance the standard reference spot had moved, it was found to have an Rf value of 0.50 to 0.55.

The progesterone in the sample spots was not of sufficient concentration to be seen in ultra-violet light. However, the sample progesterone progresses on the chromatogram at the same Rf as the standard reference. Therefore, the area containing the sample progesterone could be determined and segregated by drawing the tip of a dissecting pith

¹ Chromato-Vue, Model C-3, manufactured by Ultra-Violet Products, Inc., San Gabriel, Calif.

across the silica gel immediately above and below the reference spot. This process isolated five squares of silica gel containing sample progesterone and one square with the standard reference progesterone on every chromatogram.

An elution system was developed, whereby progesterone adsorbed to the silica gel could be collected in culture tubes. (Figure 3). Holes just wide enough to accommodate the barrel of a 2½ ml plastic syringe were countersunk and drilled in the wood. Twelve syringes with 25 gauge 5/8" needles were suspended through these holes. The needle tips were bent slightly upward to facilitate holding the chromatogram squares. Anhydrous methanol pipetted into the syringes passed through the needles and slowly dispersed across the silica gel serving as a solvent for the progesterone.

The chromatograms were prepared for elution by placing them upon clean paper towels. This avoided contaminating the sheets with any progesterone that might be on the surface of the counter. The square containing the concentrated progesterone reference was cut out and discarded to avoid spreading silica gel and progesterone to the sample lanes. The tip of a dissecting pith was used to form a small hole in the corner of each remaining square. The individual squares were then cut away from the chromatogram and held with clean forceps. Each square was transferred to the elution device and suspended from an upturned needle point. A test tube rack containing appropriately marked

culture tubes was positioned about 3 inches below the chromatogram squares. A glass funnel (5 cm in diameter and 9 cm long) was placed in each tube directly below the lower corner of the square. Two and one-half ml of anhydrous methanol was pipetted into each syringe and allowed to saturate the silica gel and drip into the funnel. When the elution was complete, the tubes were transferred to a warm salt bath (52°C) in the hood and dried under a light stream of nitrogen. In order to further concentrate the progesterone in the bottom of the tubes, 1 ml of methanol was rinsed down the walls of each culture tube. A stream of nitrogen gas was passed into the culture tube to facilitate evaporation of the methanol.

Dog plasma necessary for the quantitative determination of progesterone with the competitive protein-binding technique was purchased from the Colorado Serum Company¹. To avoid repeated freezing and thawing, the heparinized, frozen plasma was thawed at room temperature and 3 ml aliquots were pipetted into culture tubes. All of the tubes were sealed with parafilm, frozen and stored at -12°C.

The CBG - corticosterone -1,2-³H was prepared according to the procedure outlined by Neill et al. (1967a). Two and one-half ml of dog plasma were diluted to 100 ml with double distilled water. Next, two hundred and fifty μ l of ethanol containing 25 ng of corticosterone -1,2-³H (sp. act. 30 c/mmole) was drawn into a micro-pipette and added

¹ Colorado Serum Co. Laboratories, Denver, Colorado.

to the dog plasma solution. The solution was then mixed gently to disperse the labelled steroid in the plasma. Neill et al., (1967a) found the solution to remain stable for at least two weeks when stored at 5-8°C.

A standard curve was prepared from a solution containing 100 ng of progesterone per ml of absolute ethanol. Aliquots corresponding to 0.2, 0.6, 1.0, 2.0, 3.0 and 4.0 ng of progesterone were pipetted in duplicate into 14 X 100 mm culture tubes and evaporated to dryness. Also, two tubes were marked to serve as controls, but no progesterone was added to them.

When all samples and members of the standard curve were dried sufficiently, they were placed in test tube racks. One ml of CBG-corticosterone -1,2-³H was added to each sample tube and every tube of the standard curve. The pipette tip was touched near the mouth of the culture tube and the solution expelled in a circular motion, rinsing the walls of the tubes.

Meanwhile, a water bath was being prepared in a Metabolic Shaking Incubator¹. The dial for regulating temperature was set at 4.5 to raise the water temperature to 45°C. The system had a movable carriage in the bath and a shaking speed of 80 complete motions per minute when a dial setting of 4.75 was used. The tubes were placed in the bath and shaken for 5 minutes which brought the progesterone into solution (Figure 4).

¹ Dubnoff, Precision Scientific Co., Subsidiary of GCA Corp. Chicago, Ill.

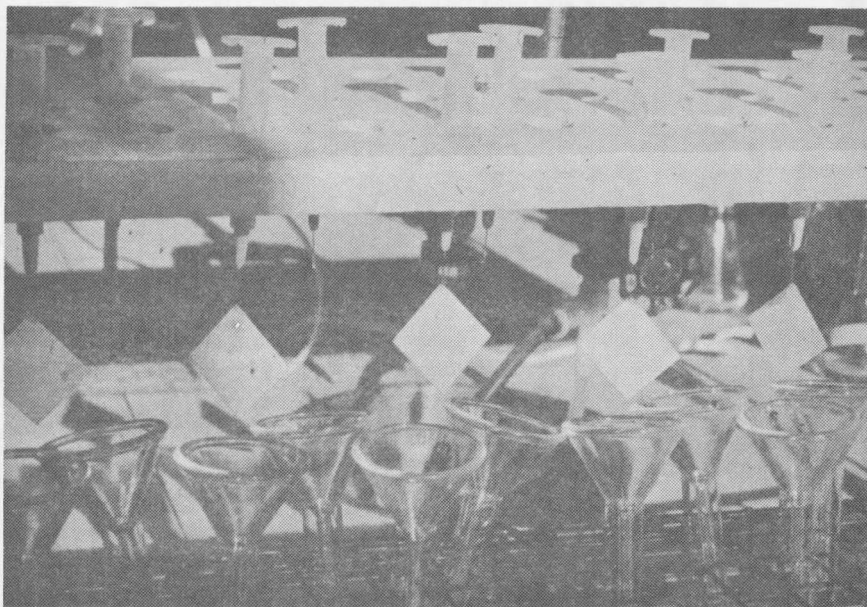


Figure 3. Elution device.

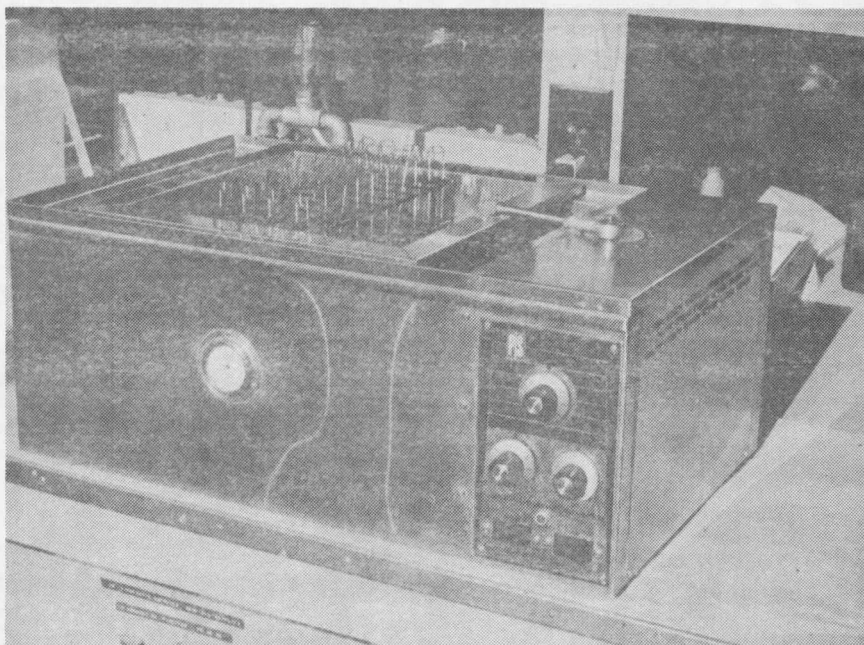


Figure 4. Culture tubes in the metabolic shaking incubator.

Then the tubes were transferred to an ice bath to increase the affinity of the CBG for the steroids. After remaining there 10 minutes, a small plastic scoop was used to add 80 mg of Florisil¹ to each tube. Prior to this, Florisil had been washed 12-15 times in distilled water and allowed to dry in an oven at 100°C. Starting with the unknowns the Florisil was poured into a glass funnel (5 cm in diameter and 9 cm long) 5-10 seconds before mixing in a Vari-Whirl² mixer. Each tube was held in the mixer for exactly 30 seconds at a dial setting of 2. After shaking, the tube was immediately returned to the ice bath. The standard curve was the last group of tubes to which Florisil was added beginning with the higher concentrations of progesterone and progressing toward the lower concentration. This sequence was necessary because the same pipette was used throughout to make 0.5 ml aliquots of the supernatant. The amount of ³H labelled corticosterone bound to the CBG would be lower in the tubes containing 4 ng progesterone than in the controls. Therefore, there would be less influence from ³H steroid residue as the pipette was transferred from one tube to another, than there would be if pipetting was begun at the greater amounts of protein bound corticosterone -1,2-³H in the controls.

One-half ml aliquots of the supernatant were pipetted into 18 X 56 mm, low potassium counting vials³. Fifteen ml portions of scintillation

¹ Activated magnesium silicate, 50-100 mesh, Purchased from Fisher Scientific Co.

² Catalog No. 58810, distributed by Van Waters & Rogers.

³ Purchased from Packard Instrument Co., Inc.

fluid (100 g naphthalene, 8 g PPO, 0.3 g POPOP and 1 liter 1,4-Dioxane) were poured from a 500 ml Packard fluid dispenser (Pyrex, No. 4984) into the vials containing the supernatant. Five ml aliquots of scintillation fluid were dispensed three times into the balance of the supernatant and Florisil remaining in the culture tubes. After each aliquot of counting fluid was poured, the tube was placed in the Vari-Whirl mixer for 10-20 seconds. When the speed was set at 4.0, suspensions were readily formed and immediately poured into scintillation vials. Fifteen ml aliquots of scintillation fluid were added to each vial containing a ^{14}C progesterone standard and two empty vials which were to serve as indicators of background ^{14}C counts.

The vials were placed in a Beckman liquid scintillation counter¹. Channels 2 and 3 were programmed with iso-sets representing the ^3H and ^{14}C spectra above ^3H , respectively. The counter was set to count for 10 minutes or a 1 percent error, whichever was arrived at first. Vials comprising the standard curve were counted for CPM of ^3H to determine the depression in counts with a specific amount of progesterone. Sample vials were analyzed for ^3H so that the progesterone bound to the transcortin could be quantitated with the standard curve. The ^{14}C was counted for a determination of the ^{14}C progesterone recovery in the 0.5 ml aliquot of the supernatant. The vials containing the balance of the supernatant and Florisil were counted to determine the remainder of the

¹ Model LS-100 Liquid Scintillation System.

^{14}C progesterone recovered. The ^{14}C progesterone standards were analyzed to determine the ^{14}C counts equivalent to 1 ng of the isotope added to each of the extraction vials prior to extraction, isolation and quantitation of the progesterone. The vials which represent a background analysis were counted for any ^{14}C radioactivity which might be present in the scintillation fluid or an empty vial.

All data recorded on the printed tape from the counter was transferred to data paper and recorded on a Tele-Type¹ punch output tape. The Hewlett-Packard computer was programmed with a punched program tape in preparation for an analysis of the data tape as it was fed through the Tele-Type recorder. The computer statistically analyzed the standard curve and printed analysis of variance listing degrees of freedom, sum of squares, mean square and F value for regression, deviation from regression, between dose and within dose response. In addition to this, a regression equation and an index of precision (r) was calculated for the standard curve. The sample data was analyzed for levels of progesterone (ng/ml) in any particular sample and the ^{14}C progesterone recovered. Likewise, the computer printed back the ml of plasma extracted for each sample. The data was statistically analyzed according to a procedure described by Finney (1964).

¹ Tele-Type Corporation, Bell System.

RESULTS

Protein-binding Assay.-

The progesterone concentrations in the posterior vena cava and jugular vein of four Holstein heifers were determined with the competitive protein-binding radio-assay (Neill et al., 1967a). The minimum level of sensitivity in plasma was 0.2 ng progesterone. A 0.2 ng aliquot of progesterone was the lowest concentration included in the standard curve. This amount of progesterone was detectable in 67 percent of the assays.

A regression equation was computed for every standard curve. An F test on the data from each standard indicated that regression was always highly significant ($P < 0.005$). The linearity of the regression line on each standard curve was another point of consideration in each assay. A curve that deviated significantly from linearity ($P < .01$) was not used in the quantitation of progesterone in the samples. The linearity of a standard curve generally ranged from 0.2 to 3.0 ng progesterone. However, 7 percent of the time the linearity of the curve extended to 4.0 ng progesterone. An index of precision (γ) was computed for every standard curve, and for the last 12 assays the mean index of precision (γ) was 0.1515 ± 0.0289 .

The recovery of the internal standard, comprised of 1 ng ^{14}C progesterone averaged 51 percent in all determinations. This value was improved to 64 percent recovery in six of the better analyses. If the

progesterone recovered from a sample of plasma was less than 30 percent, the sample was re-analyzed.

Progesterone Levels in the Jugular Vein and Posterior Vena Cava of Cycling Holstein Heifers.-

The progesterone levels in the jugular vein and posterior vena cava of four cycling Holstein heifers were determined for nine estrous cycles. The study included five normal cycles and four abnormal cycles, ranging from 18 to 21 days and 14 to 30 days in length, respectively.

A t-test evaluating the difference between progesterone concentrations in the posterior vena cava and the jugular vein was calculated (Snedecor, 1956) (Table I). The comparison included all days on which both progesterone concentrations were detected, and demonstrated that the progesterone concentrations in the posterior vena cava were higher ($P < .001$) than the jugular.

Another t-test was performed to determine if there was a significant difference between progesterone concentrations in the two veins at estrus (Table II) and no significant difference ($P < .05$) in the vena cava and jugular vein was observed. However, there were six of the 10 estrus dates which had higher jugular than vena cava progesterone concentrations. It should be recognized that this evaluation included estrus dates at the beginning and end of abnormal cycles, as well as estrus during normal cycles. All of the estrus days that occurred between two normal cycles had higher jugular values (Table XVIII).

TABLE I. DIFFERENCE BETWEEN PROGESTERONE CONCENTRATION IN THE POSTERIOR VENA CAVA AND THE JUGULAR VEIN OF FOUR CYCLING HOLSTEIN HEIFERS.

Comparison Number	Heifer Number	Progesterone (ng/ml plasma)		Difference D=X ₁ -X ₂
		Post. V.C.X ₁	X ₂ Jugular	
1	200	3.8	6.1	-2.3
2	200	4.1	0.7	3.4
3	200	1.0	1.2	-0.2
4	200	3.8	0.8	3.0
5	200	1.1	3.0	-1.9
6	200	0.6	1.6	-1.0
7	200	0.7	0.2	0.5
8	200	4.6	1.6	3.0
9	200	4.3	2.2	2.1
10	200	4.2	4.0	0.2
11	200	6.5	4.4	2.1
12	200	7.1	1.8	5.3
13	200	13.8	2.0	11.8
14	200	8.3	1.3	9.6
15	200	11.9	2.1	9.7
16	200	9.0	1.0	8.0
17	200	6.6	3.6	3.0
18	200	9.4	4.6	4.8
19	200	3.0	6.0	3.0
20	200	2.9	2.5	0.4
21	200	3.4	1.9	1.5
22	200	3.6	1.6	2.0
23	200	14.7	2.4	12.3
24	200	0.4	8.6	-8.2
25	200	15.6	1.3	14.3
26	200	14.2	6.1	8.1
27	200	10.0	2.9	7.1
28	200	5.8	6.3	-0.5
29	200	14.4	3.0	11.4
30	200	11.1	11.8	-0.7
31	200	18.2	7.2	11.0
32	200	1.0	0.9	0.1
33	200	5.1	3.4	1.7
34	200	2.8	1.8	1.0
35	200	1.9	2.3	-0.4
36	200	3.0	1.3	1.7

Table I. Continued.

Comparison Number	Heifer Number	Progesterone (ng/ml plasma)		Difference $D = X_1 - X_2$
		Post V.C.X ₁	X ₂ Jugular	
37	200	2.2	0.7	1.5
38	200	0.8	2.7	-1.9
39	200	4.1	2.3	1.8
40	200	3.4	1.0	2.4
41	200	2.3	1.1	1.2
42	200	7.0	3.5	3.5
43	200	4.3	7.4	-3.1
44	200	0.4	6.0	-5.6
45	200	0.5	0.4	0.1
46	200	1.1	0.04	1.06
47	200	1.5	0.8	0.7
48	200	1.5	1.3	0.2
49	200	3.2	1.3	1.9
50	200	4.3	0.7	3.6
51	200	1.8	0.10	1.7
52	200	0.5	3.6	-3.1
53	200	2.3	1.1	1.2
54	200	3.1	1.7	1.4
55	200	1.6	2.1	-0.5
56	200	3.4	2.3	1.1
57	200	0.2	0.2	0.0
58	200	0.9	0.8	-0.1
59	233	7.3	12.6	-5.3
60	233	6.0	3.5	2.5
61	233	6.9	1.7	5.2
62	233	0.6	1.6	-1.0
63	233	0.2	1.4	-1.2
64	233	0.1	0.5	-0.4
65	233	0.1	2.6	-.25
66	233	1.6	1.3	0.3
67	233	2.2	0.01	2.19
68	233	3.4	0.3	3.1
69	233	2.0	1.5	0.5
70	233	4.6	2.1	2.5
71	233	2.0	0.4	1.6
72	233	4.9	0.2	4.7
73	233	3.1	1.2	1.9
74	233	10.0	2.3	7.7

Table I. Continued

Comparison Number	Heifer Number	Progesterone (ng/ml plasma)		Difference $D = X_1 - X_2$
		Post V.C. X_1	X_2 Jugular	
75	233	4.2	1.1	3.1
76	233	4.6	2.6	2.0
77	233	2.9	2.4	0.5
78	233	2.5	1.4	1.1
79	233	6.6	2.1	4.5
80	233	2.3	0.9	1.4
81	250	3.1	8.6	-5.5
82	250	3.2	2.9	0.3
83	250	2.5	2.3	0.2
84	250	3.0	4.6	-1.6
85	250	1.1	3.2	-2.1
86	250	5.6	0.7	4.9
87	250	2.4	0.7	1.7
88	250	1.7	1.8	0.1
89	250	0.4	0.4	0.0
90	250	0.4	0.1	0.3
91	250	0.5	0.9	0.4
92	250	0.2	0.2	0.0
93	250	0.9	0.8	0.1
94	250	2.5	0.4	2.1
95	250	0.02	0.5	-0.48
96	250	1.5	0.8	0.7
97	250	3.0	1.3	1.7
98	250	3.6	3.0	0.6
99	250	4.0	0.7	3.3
100	250	3.7	1.7	2.0
101	250	7.3	2.5	4.8
102	250	6.0	3.6	2.4
103	250	5.8	3.6	2.2
104	250	2.4	1.0	1.4
105	250	2.2	0.6	1.6
106	250	0.9	0.2	0.7
107	259	4.8	2.7	2.1
108	259	7.0	2.4	4.6
109	259	5.5	3.7	1.8
110	259	8.4	2.8	5.6
111	259	0.03	1.4	-1.37
112	259	1.2	1.7	-0.5

TABLE I. Continued

Comparison Number	Heifer Number	Progesterone (ng/ml plasma)		Difference D=X ₁ - X ₂
		Post V.C.X ₁	X ₂ Jugular	
113	259	14.9	6.3	8.6
114	259	5.6	6.3	-0.7
115	259	19.6	6.2	13.4
116	259	16.9	7.4	9.5
117	259	5.0	2.2	2.8
118	259	2.5	4.1	-1.6
119	259	5.9	0.3	5.6
120	259	4.3	3.0	1.3
121	259	7.5	9.2	-1.7
122	259	0.8	2.7	-1.9
123	259	5.9	0.3	5.6
124	259	1.4	0.3	1.1
125	259	1.4	0.4	1.0
126	259	1.6	0.5	1.1
127	259	3.0	1.1	1.9
128	259	0.4	0.6	-0.2
129	259	3.3	1.0	2.3
130	259	1.8	3.4	-1.6
131	259	2.6	2.2	0.4
132	259	1.5	0.6	0.9
133	259	1.5	0.8	0.7
134	259	0.7	1.9	-1.2
135	259	1.0	0.5	0.5
136	259	5.0	0.3	4.7
137	259	0.8	2.0	-1.2
138	259	4.4	2.1	2.3
139	259	1.4	0.2	1.2
140	259	2.2	1.0	1.2
141	259	0.4	2.0	-1.6
142	259	0.5	0.2	0.3
TOTAL		578.95	329.25	259.10
MEAN		4.08	2.32	d=1.82
(P < 0.001)				

TABLE II. DIFFERENCE BETWEEN PROGESTERONE CONCENTRATION IN THE POSTERIOR VENA CAVA AND THE JUGULAR VEIN AT ESTRUS IN FOUR CYCLING HOLSTEIN HEIFERS.

Comparison Number	Heifer Number	Progesterone (ng/ml plasma)		Difference D=X ₁ -X ₂
		Post V.C.X ₁	X ₂ Jugular	
1	200	0.6	1.6	-1.0
2	200	3.0	6.0	-3.0
3	200	14.7	3.4	12.3
4	200	7.0	3.5	3.5
5	200	0.4	6.0	-5.6
6	200	0.2	0.2	0.0
7	233	0.6	1.6	-1.0
8	250	0.5	0.8	-0.4
9	259	0.03	1.4	-1.37
10	259	3.0	1.1	1.9
TOTAL		30.03	24.70	5.33
MEAN				d=0.53
Non-significant				

Heifer No. 200.- The progesterone concentrations (ng/ml plasma) were determined in blood samples collected over a period of 104 days from the posterior vena cava of Holstein heifer No. 200 (Table III, Figure 5). Approximately one month after the vena cava cannula lost patency, another cannulation was made in the vein.

The functional life of the jugular vein cannula was considerably shorter than the vena cava and three cannulations were performed to allow venous sampling for progesterone analysis. After the last of these cannulae became non-functional, the vessel was penetrated with an 18 guage needle and samples collected in a heparinized centrifuge tube.

Daily progesterone levels were estimated for three complete normal estrous cycles before the heifer cycled abnormally (Table III, Figure 5). There appeared to be a rise in the progesterone levels (ng/ml plasma) for both the posterior vena cava and the jugular vein from one cycle to the next, until Days 20-30 of the erratic cycle. However, a jugular sample was not obtained every day throughout the three normal cycles, and it was not until the beginning of the abnormal cycle (30 days) that progesterone determinations were made every day. Low progesterone values were measured in both vessels throughout this prolonged period.

A marked decline in jugular and vena cava progesterone levels was apparent just prior to estrus. Three of the estrous dates with complete information had greater concentrations of progesterone in the jugular than was estimated in the vena cava. At the last detected estrus

TABLE III. PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 200.

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
3-28	15	3.8	6.1
3-29	16	1.0	0.17 ¹
3-30	17	4.1	0.7
3-31	18	1.0	1.2
4-1	19	2.1	*
4-2	20	3.8	0.8
4-3	21	1.1	3.0
4-4	0	0.6	1.6
4-5	1	1.1	*
4-6	2	0.7	0.2
4-7	3	4.6	1.6
4-8	4	4.3	2.2
4-9	5	4.2	4.0
4-10	6	*	0.6
4-11	7	*	1.3
4-12	8	0.8	*
4-13	9	6.5	4.4
4-14	10	7.5	*
4-15	11	2.5	*
4-16	12	10.2	*
4-17	13	*	*
4-18	14	8.9	*
4-19	15	2.0	*
4-20	16	3.4	*
4-21	17	11.4	*
4-22	18	*	*
4-23	19	0.6	*
4-24	0	0.2	*
4-25	1	1.6	*
4-26	2	*	*
4-27	3	*	*
4-28	4	2.7	*
4-29	5	7.1	1.8
4-30	6	13.8	2.0
5-1	7	8.3	1.3
5-2	8	11.8	2.1
5-3	9	9.0	1.0
5-4	10	6.6	3.6

TABLE III. Continued

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
5-5	11	9.4	4.6
5-6	12	9.3	*
5-7	13	10.1	*
5-8	14	10.0	*
5-9	15	10.9	*
5-10	16	*	*
5-11	17	*	*
5-12	18	*	2.5
5-13	0	3.0	6.0
5-14	1	2.9	2.5
5-15	2	3.4	1.9
5-16	3	3.6	1.6
5-17	4	1.8	1.5 ¹
5-18	5	2.2	*
5-19	6	1.4	*
5-20	7	13.1	*
5-21	8	9.5	*
5-22	9	8.5	*
5-23	10	10.8	*
5-24	11	17.5	*
5-25	12	12.5	*
5-26	13	6.2	*
5-27	14	5.7	*
5-28	15	18.3	*
5-29	16	12.7	*
5-30	17	12.9	*
5-31	18	15.3	*
6-1	19	20.2	*
6-2	20	6.4	*
6-3	0	14.7	2.4
6-4	1	0.25 ¹	0.4
6-5	2	0.02	0.12
6-6	3	*	3.1
6-7	4	0.5 ¹	0.3 ¹
6-8	5	0.4	8.6
6-9	6	15.6	1.3
6-10	7	0.5 ¹	*
6-11	8	14.2	6.1
6-12	9	11.8	*

TABLE III. Continued.

1969 Date	Days of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
6-13	10	10.0	2.9
6-14	11	5.8	6.3
6-15	12	11.6	*
6-16	13	10.4	*
6-17	14	14.4	3.0
6-18	15	11.1	11.8
6-19	16	18.2	7.2
6-20	17	1.0	0.9
6-21	18	15.6	0.5 ¹
6-22	19	5.1	3.4
6-23	20	2.8	1.8
6-24	21	1.9	2.3
6-25	22	3.0	1.3
6-26	23	4.3	*
6-27	24	*	1.6
6-28	25	2.2	0.7
6-29	26	0.8	2.7
6-30	27	4.1	2.3
7-1	28	3.4	1.0
7-2	29	2.7	*
7-3	30	2.3	1.1
7-4	0	7.0	3.5
7-5	1	4.3	7.4
7-6	2	0.9	*
7-7	3	2.4	*
7-8	4	2.6	*

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

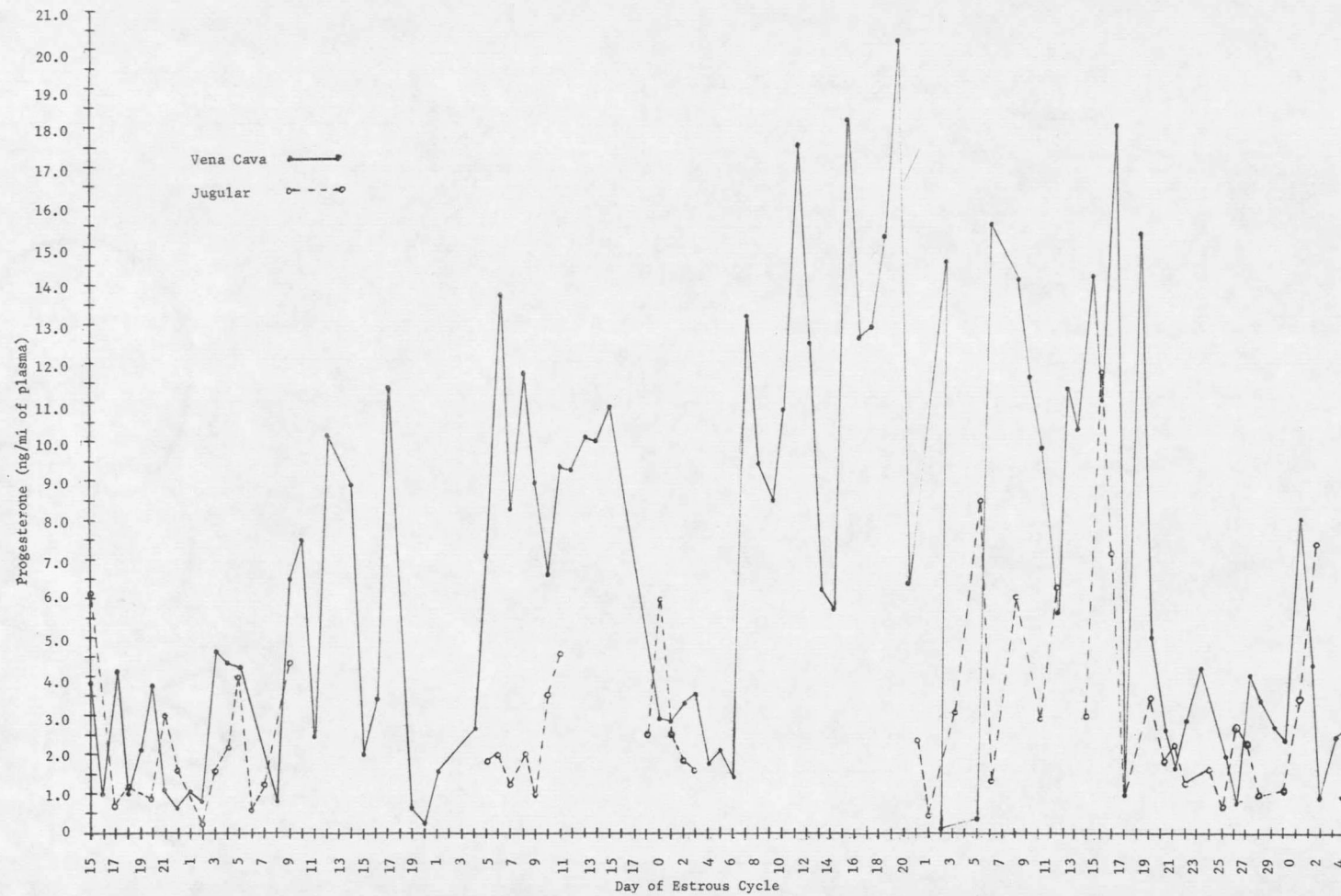


Figure 5. Progesterone levels in the jugular vein and posterior vena cava of Holstein No. 200.

(Table IV, Figure 6), both vessels contained 0.2 ng progesterone/ml plasma. Generally, the progesterone levels started to increase noticeably during Days 4-6. For the most part, vena cava levels surpassed those in jugular plasma by Days 2-3. The progesterone content of the vena cava began to decrease sharply during Days 20-21 of a 21 day cycle, Days 17-19 of a 19 day cycle, Days 15-16 during an 18 day cycle, and Days 19-20 of a 20 day cycle (Figure 5).

Heifer No. 233.- Progesterone levels in posterior vena cava and jugular venous plasma were determined for heifer No. 233 from cannulation at Day 14 through Day 20 and the following 21 day estrous cycle (Table V, Figure 7). Between Days 17 of the first cycle and the day of estrus of the second cycle, the progesterone value for the vena cava declined markedly (6.9 ng/ml to 0.6 ng/ml plasma).

The jugular concentration did not drop as low as the vena cava at Day 0. The jugular levels remained higher until Day 4 when the vena cava values exceeded those in the jugular and tended to increase steadily to a peak of 10.1 ng/ml plasma at Day 13. Thereafter, the progesterone content of the vena cava decreased to 2.3 ng/ml at Day 19. Jugular vein progesterone levels reached a peak of 2.6 ng/ml at Day 15 and declined to 1.0 ng/ml at Day 20.

Heifer No. 250.- The progesterone levels in the jugular vein and posterior vena cava of heifer No. 250 were analyzed from the day of surgery (Day 11) to Day 21 and throughout the ensuing 20 day estrous

TABLE IV. PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 200 (Second Cannulation).

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
8-8	0	0.4	6.0
8-9	1	0.5	0.4
8-10	2	0.03	1.0 ¹
8-11	3	1.1	0.04
8-12	4	1.5	0.8
8-13	5	1.5	1.3
8-14	6	3.2	1.3
8-15	7	4.3	0.7
8-16	8	1.8	0.1
8-17	9	0.5	3.6
8-18	10	2.3	1.1
8-19	11	3.1	1.7
8-20	12	1.6	2.1
8-21	13	3.4	2.3
8-22	14	0.4	1.0 ¹
8-23	0	0.2	0.2
8-24	1	0.9	0.8
8-25	2	0.75	0.2

¹ Not detectable in this amount of plasma.

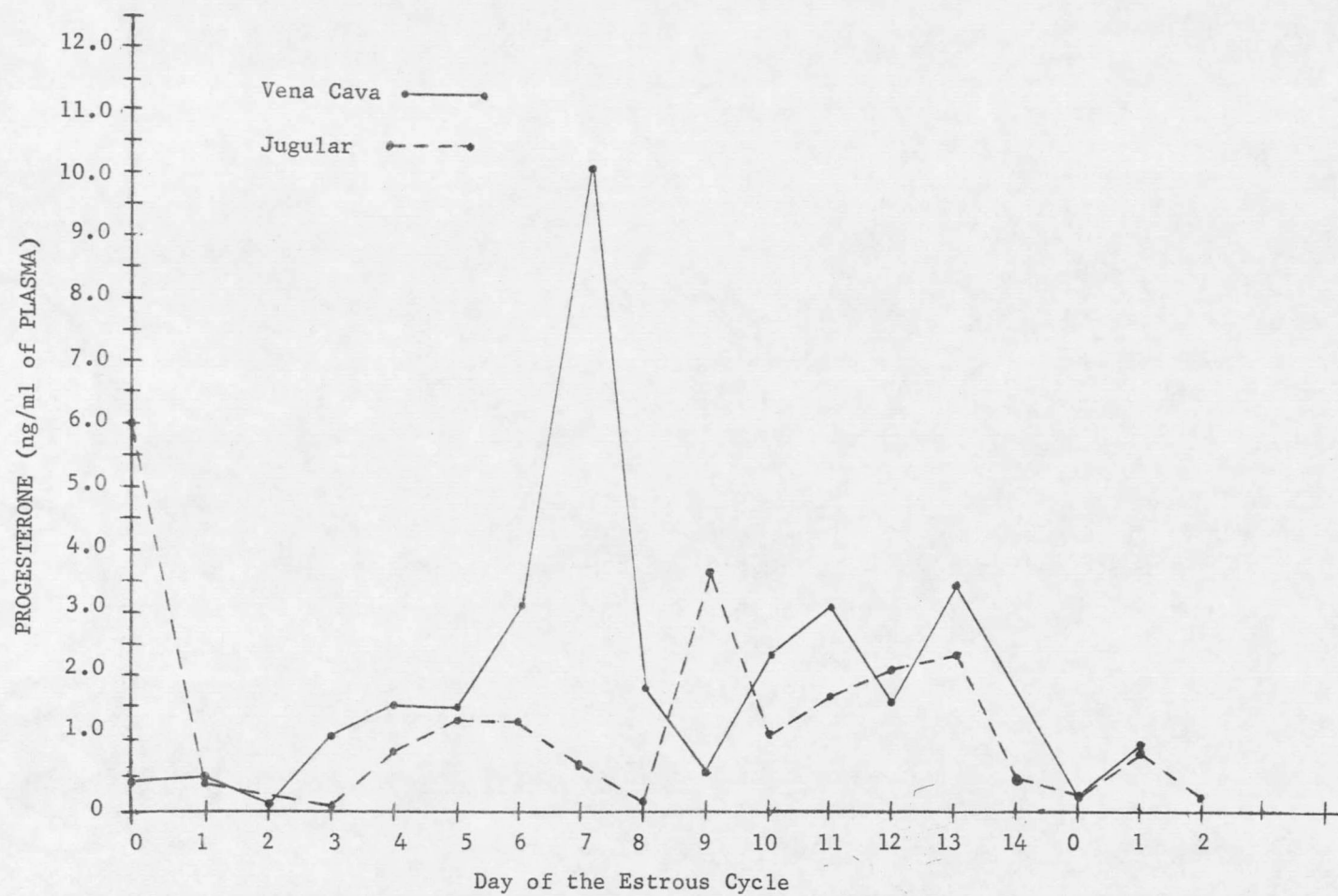


Figure 6. Progesterone levels in the jugular vein and posterior vena cava of Holstein heifer No. 200 (second cannulation).

TABLE V. PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 233.

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
7-24	14	7.3	12.6
7-25	15	1.6	*
7-26	16	6.0	3.5
7-27	17	6.9	1.7
7-28	18	4.4	*
7-29	19	2.1	*
7-30	0	0.6	1.6
7-31	1	0.2	1.4
8-1	2	0.1	0.5
8-2	3	0.1	2.6
8-3	4	1.6	1.3
8-4	5	2.2	0.01
8-5	6	3.4	0.3
8-6	7	0.2	0.5 ¹
8-7	8	2.0	1.5
8-8	9	4.6	2.1
8-9	10	2.0	0.4
8-10	11	4.9	0.2
8-11	12	3.1	1.2
8-12	13	10.0	2.3
8-13	14	4.2	1.1
8-14	15	4.6	2.6
8-15	16	2.9	2.4
8-16	17	2.5	1.4
8-17	18	6.6	2.1
8-18	19	2.3	0.9
8-19	20	*	1.0
8-20	21	*	0.9
8-21	0	*	1.5 ¹

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

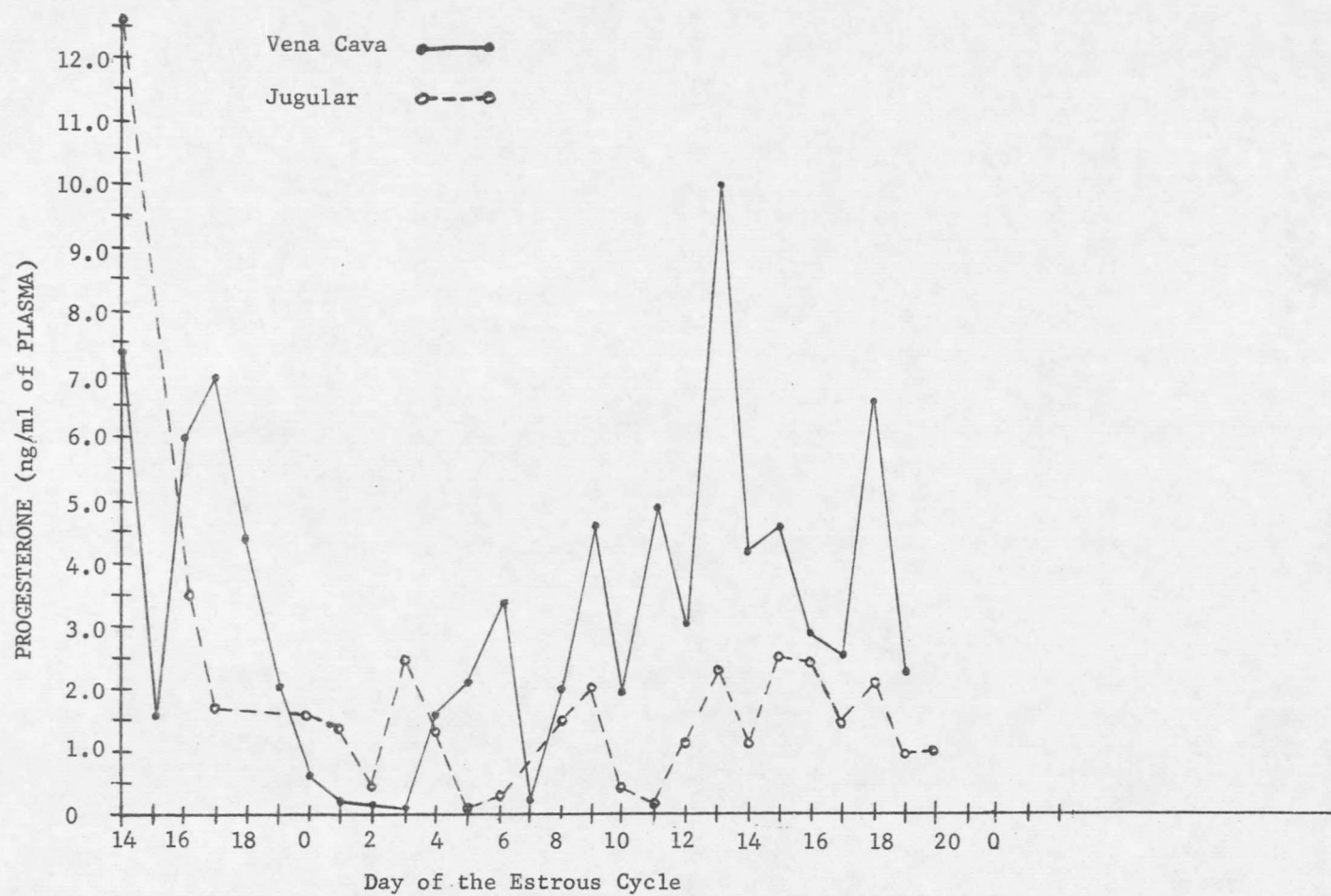


Figure 7. Progesterone levels in the jugular vein and posterior vena cava of Holstein heifer No. 233.

cycle (Table VI, Figure 8). The vena cava concentration trended slightly downward from 3.1 ng progesterone/ml plasma at Day 11 to 1.1 ng/ml at Day 15. Then there was a sharp increase to 5.6 ng/ml at Day 16, which was followed by a decline to 0.4 ng/ml during Days 20 and 21 to a progesterone concentration of 0.5 ng/ml at estrus. The vena cava level remained low through Day 6 (0.7 ng/ml) of the following cycle. Then there was a noticeable increase to 2.5 and 2.7 ng/ml at Days 7 and 8, respectively. A precipitous decline on Day 9 was followed by a steadily increasing progesterone concentration to a peak of 7.3 ng/ml progesterone at Day 15. Then levels dropped each following day to 2.2 ng/ml at Day 19 and 1.7 ng/ml at estrus.

The progesterone concentration in the jugular vein dropped noticeably from the day of surgery (Day 11) to a level slightly below that in the posterior vena cava at Day 12 of the estrous cycle. The values declined on Day 13, followed by a rise in progesterone concentration (4.6 ng/ml) at Day 14 surpassing the vena cava levels on Days 14-15. The following days of the cycle showed a decrease in the jugular venous level to a low of 0.1 ng/ml (Day 21) and 0.9 ng progesterone/ml (Day 0).

The progesterone concentration in the jugular plasma was 0.9 ng/ml at estrus, whereas the vena cava level (0.5 ng/ml) was noticeably lower. The jugular progesterone levels were maintained approximately the same as those in the posterior vena cava up to Day 6. Then the jugular progesterone concentration increased gradually, but at a lower level than the

TABLE VI. PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 250.

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
7-28	11	3.1	8.6
7-29	12	3.2	2.9
7-30	13	2.5	2.3
7-31	14	3.0	4.6
8-1	15	1.1	3.2
8-2	16	5.6	0.7
8-3	17	2.4	0.7
8-4	18	0.5 ¹	1.2
8-5	19	1.7	1.8
8-6	20	0.4	0.4
8-7	21	0.4	0.1
8-8	0	0.5	0.9
8-9	1	0.1	1.25 ¹
8-10	2	0.2	0.2
8-11	3	1.1	1.0 ¹
8-12	4	0.5	1.5 ¹
8-13	5	0.9	0.8
8-14	6	0.7	0.5 ¹
8-15	7	2.5	0.4
8-16	8	2.7	0.5 ¹
8-17	9	0.02	0.5
8-18	10	1.5	0.8
8-19	11	3.0	1.3
8-20	12	3.6	3.0
8-21	13	4.0	0.7
8-22	14	3.7	1.7
8-23	15	7.3	2.5
8-24	16	6.0	3.6
8-25	17	5.8	3.6
8-26	18	2.4	1.0
8-27	19	2.2	0.6
8-28	0	1.7	*
8-29	1	0.9	0.2

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

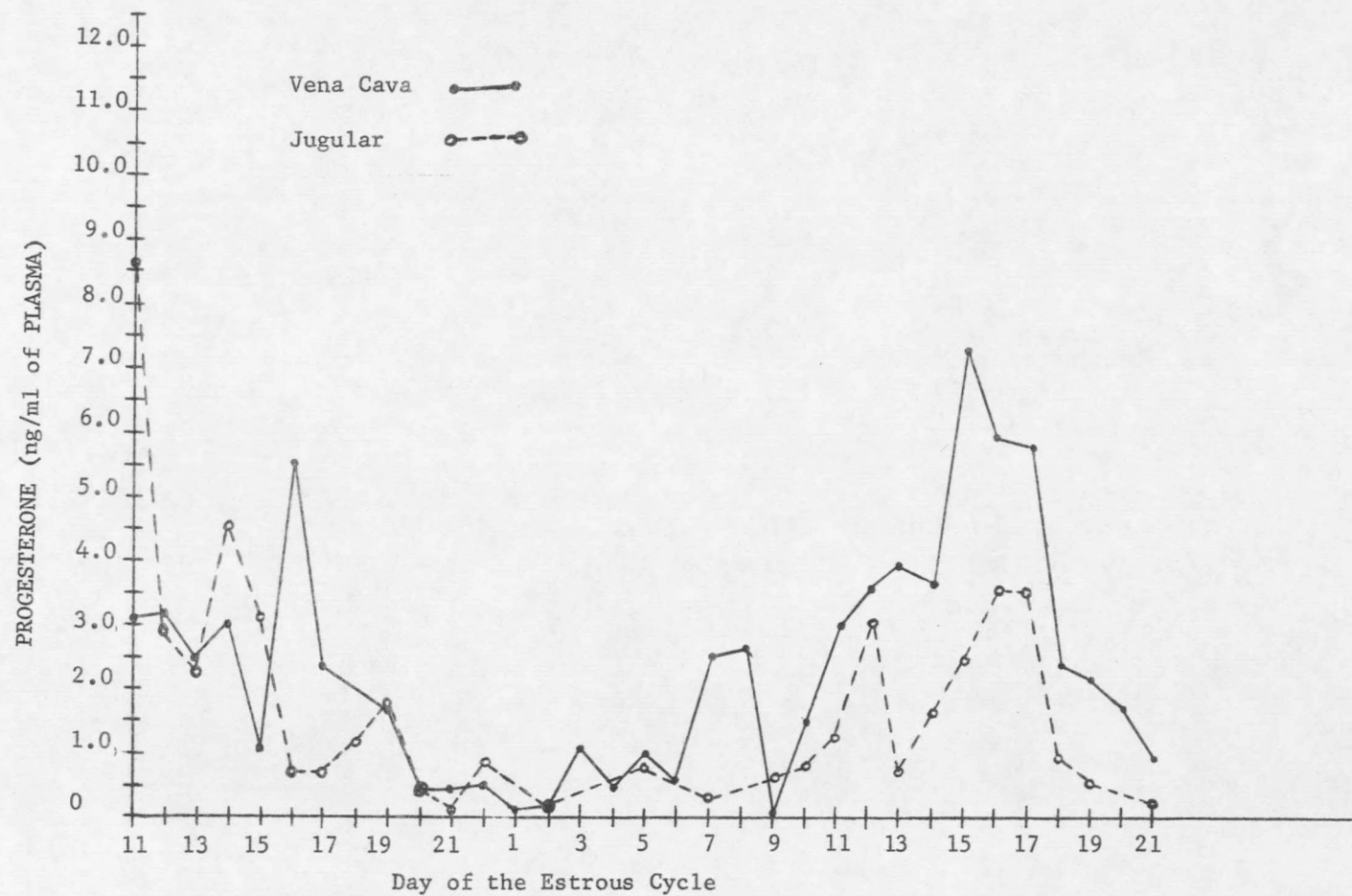


Figure 8. Progesterone levels in the jugular vein and posterior vena cava of Holstein heifer No. 250.

vena cava. A maximum jugular vein concentration for the second estrous cycle was reached at Day 16 (3.6 ng progesterone/ml plasma) and maintained at 3.6 ng/ml through Day 17, after which there was a marked decline to the last day of the estrous cycle.

Heifer No. 259.- The progesterone levels in the jugular vein and posterior vena cava were quantitated daily for heifer No. 259 (Table VII, Figure 9). The vena cava cannula remained functional, allowing blood sample collection for 62 days. However, the cannula inserted in the jugular vein retained its patency for only six days. A recannulation of the vessel several days later in the second estrous cycle remained operational six days, also. Thereafter, jugular venous samples were obtained for progesterone determination by inserting an 18 gauge bleeding needle in the vessel and the blood sample collected in a heparinized centrifuge tube.

The progesterone level in the posterior vena cava dropped distinctly from 8.4 ng/ml plasma at Day 11 of the first abnormal cycle to 0.4 ng/ml at Day 12. The vena cava progesterone concentration was found to be at a low of 0.03 ng/ml plasma at Day 0. However, the jugular level was considerably higher (1.4 ng/ml) at estrus.

The vena cava values raised daily to 1.8 ng/ml progesterone (Day 3) and were maintained at this concentration with slight daily fluctuations until Day 9, when a sharp rise in progesterone concentration was appar-

TABLE VII. PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 259.

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
6-30	8	4.8	2.7
7-1	9	7.0	2.4
7-2	10	5.5	3.7
7-3	11	8.4	2.8
7-4	12	0.4	*
7-5	0	0.03	1.4
7-6	1	0.6	*
7-7	2	1.5	*
7-8	3	1.8	*
7-9	4	0.9	*
7-10	5	1.6	*
7-11	6	1.3	*
7-12	7	1.8	*
7-13	8	0.9	*
7-14	9	1.2	1.7
7-15	10	14.9	6.3
7-16	11	5.6	6.3
7-17	12	19.6	6.2
7-18	13	16.9	7.4
7-19	14	5.0	2.2
7-20	15	5.7	*
7-21	16	19.7	*
7-22	17	13.8	*
7-23	18	14.3	*
7-24	19	2.5	4.1
7-25	20	5.9	0.33
7-26	21	4.3	3.0
7-27	22	7.5	9.2
7-28	23	*	1.8
7-29	24	0.8	2.7
7-30	25	5.9	0.3
7-31	26	1.4	0.3
8-1	27	1.4	0.4
8-2	28	1.6	0.5
8-3	0	3.0	1.1
8-4	1	0.4	0.6
8-5	2	3.3	1.0
8-6	3	1.8	3.4

TABLE VII. Continued.

1969 Date	Day of Estrous Cycle	Progesterone (ng/ml of plasma)	
		Posterior Vena Cava	Jugular Vein
8-7	4	1.7	*
8-8	5	2.6	2.2
8-9	6	5.6	*
8-10	7	1.5	0.6
8-11	8	1.6	*
8-12	9	1.5	0.8
8-13	10	3.4	*
8-14	11	0.7	1.9
8-15	12	1.0	0.5
8-16	13	0.4	*
8-17	14	0.4	*
8-18	15	5.0	0.3
8-19	16	*	*
8-20	17	0.8	2.0
8-21	18	4.4	2.1
8-22	19	1.4	0.2
8-23	20	2.2	1.0
8-24	21	0.4	2.0
8-25	22	*	2.5
8-26	23	1.2	1.0 ¹
8-27	24	1.3	1.25 ¹
8-28	25	*	*
8-29	0	0.1	1.0 ¹
8-30	1	0.5	0.2

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

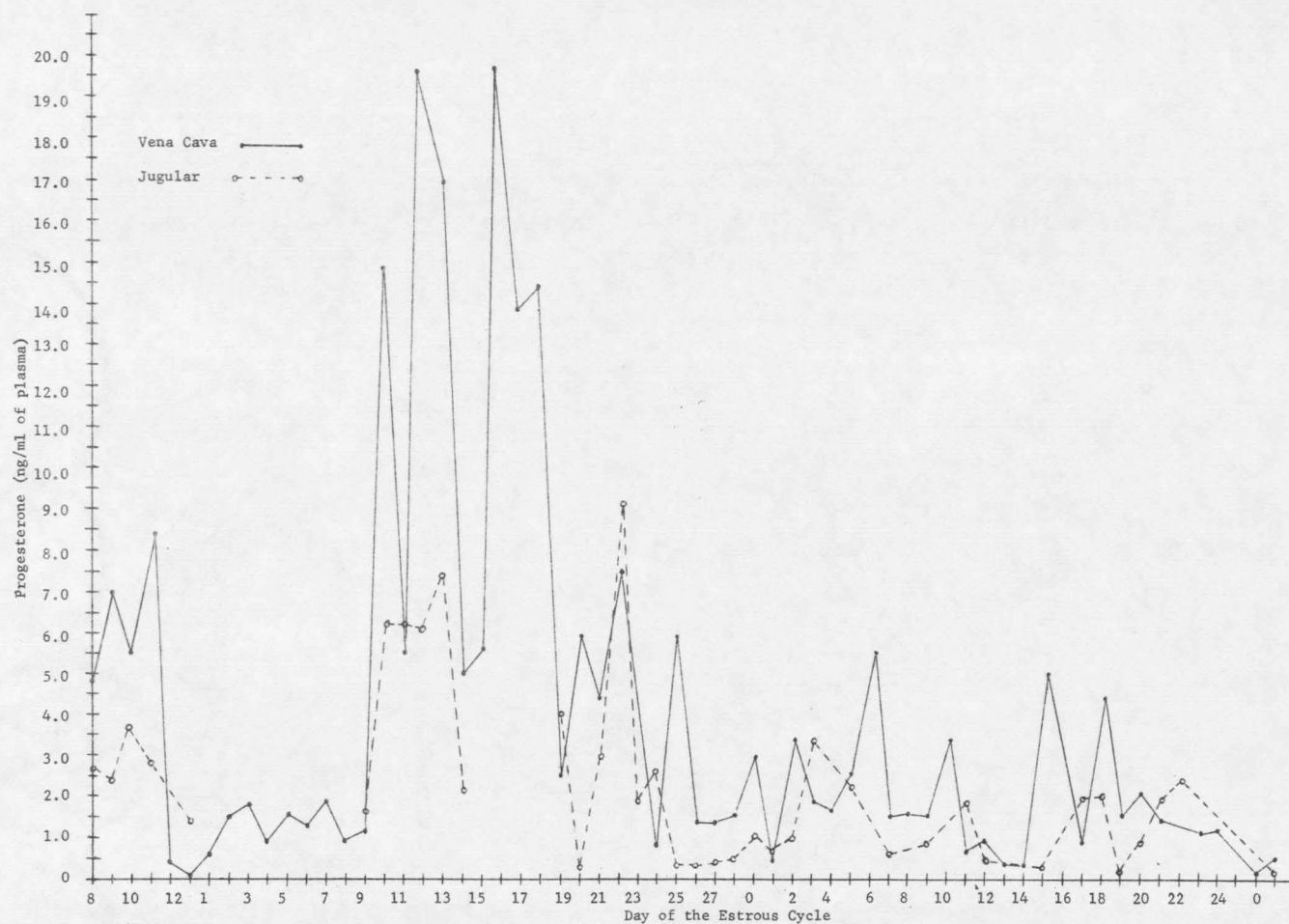


Figure 9. Progesterone levels in the jugular vein and posterior vena cava of Holstein No. 259.

ent. The jugular values also increased noticeably at this time, but remained at a lower concentration than the vena cava.

The vena cava reached two peak progesterone values, 18.6 ng/ml (Day 12) and 19.7 ng/ml (Day 16) in the second estrous cycle. The levels declined to 2.5 ng/ml on Day 19 and then rose and fell repeatedly for nine days before the heifer came in estrus for the second time (28 day cycle). At this estrus, the progesterone content in the vena cava was noticeably greater than at the previous one. However, there was a drop from 3.0 ng/ml (Day 0) to 0.4 ng/ml progesterone at Day 1.

At estrus, the jugular plasma level (1.1 ng/ml) was lower than that found in the vena cava, but at Day 1 of the third estrous cycle, the jugular progesterone value was estimated to be 0.6 ng/ml which was greater than the vena cava progesterone concentration (0.4 ng/ml).

The progesterone level in the vena cava increased noticeably on Day 2 (3.3 ng/ml) followed by a rising jugular progesterone concentration. Thereafter, the progesterone content in both vessels rose and declined throughout the remainder of the 25 day cycle, with a marked drop in progesterone concentration from Days 22 to 25.

Comparison of Daily Progesterone Levels in the Posterior Vena Cava During Normal Estrous Cycles.-

A comparison was made of the average daily progesterone levels (ng/ml plasma) in the vena cava of three Holstein heifers during five normal estrous cycles (Table VIII). The values were determined during

TABLE VIII. AVERAGE DAILY PROGESTERONE LEVELS (ng/ml of plasma) IN THE POSTERIOR VENA CAVA OF THREE HOLSTEIN HEIFERS DURING FIVE NORMAL ESTROUS CYCLES.

Day of Estrous Cycle	200	200	200	233	250	n	Mean ng/ml ± S. E.
-3	2.1	11.4	*	6.9	1.7	4	5.52 ± 2.23
-2	3.8	*	*	4.4	0.4	3	2.87 ± 1.25
-1	1.1	0.6	*	2.1	0.4	4	1.05 ± 0.29
0	0.6	0.2	3.0	0.6	0.5	5	0.98 ± 1.30
1	1.1	1.6	2.9	0.2	0.1	5	1.18 ± 0.51
2	0.7	*	3.4	0.1	0.2	4	1.10 ± 0.78
3	4.6	*	3.6	0.1	1.1	4	2.35 ± 1.05
4	4.3	2.7	1.8	1.6	0.5	5	2.18 ± 0.64
5	4.2	7.1	2.2	2.2	0.9	5	3.32 ± 1.08
6	*	13.8	1.4	3.4	0.7	4	4.82 ± 3.04
7	*	8.3	13.1	0.2	2.5	4	6.02 ± 2.91
8	0.8	11.8	9.5	2.0	2.7	5	5.36 ± 2.21
9	6.5	9.0	8.5	4.6	0.02	5	5.72 ± 1.62
10	7.5	6.6	10.8	2.0	1.5	5	5.68 ± 1.75
11	2.5	9.4	17.5	4.9	3.0	5	7.46 ± 2.79
12	10.2	9.3	12.5	3.1	3.6	5	7.74 ± 1.87
13	*	10.1	6.2	10.0	4.0	4	7.58 ± 1.50
14	8.9	10.0	5.7	4.2	3.7	5	6.50 ± 1.26
15	2.0	10.9	18.3	4.6	7.3	5	8.62 ± 2.83
16	3.4	*	12.7	2.9	6.0	4	6.25 ± 2.26
17	11.4	*	12.9	2.5	5.8	4	8.15 ± 2.42
18	*	*	15.3	6.6	2.4	3	8.10 ± 3.80
19	0.6		20.2	2.3	2.2	4	6.32 ± 4.34
20			6.4	*		1	6.40
21				*			

* It was not possible to obtain a blood sample.

three complete estrous cycles for heifer No. 200, one cycle for No. 233 and one complete normal cycle for heifer No. 250. However, it was not possible to collect a blood sample every day of each cycle and some days progesterone was not detectable in the amount of plasma available.

The mean progesterone concentrations in the vena cava were lowest at estrus (0.98 ± 1.30 ng/ml plasma). There were comparable average values determined for Day 1 and 2 (1.18 ± 0.51 ng/ml and 1.10 ± 0.78 ng/ml, respectively). However, a rapid increase in concentration was apparent at Day 3 (2.35 ± 1.05 ng/ml). This was followed by an overall increase in progesterone concentration to a peak of 8.62 ± 2.83 ng/ml at Day 15 and a decline in mean concentration to 6.32 ± 4.34 ng/ml at Day 19. Near the end of the cycle there was a noticeable increase in the standard error calculated for each day.

The results in Table IX represent the average daily progesterone levels in the posterior vena cava of heifer No. 200 during three normal estrous cycles. It was possible to make vena cava blood sample collections and progesterone determinations on 21 consecutive days during the third cycle,

The mean progesterone concentration was lowest at estrus (1.27 ± 0.92 ng/ml plasma) and increased to 10.70 ± 2.40 ng/ml at Day 7. The mean progesterone levels lowered slightly through Day 11 and rose again at Day 12 for another peak concentration (10.67 ± 0.06 ng/ml). Thereafter, the vena cava progesterone levels were maintained at a relatively

TABLE IX. AVERAGE DAILY PROGESTERONE LEVELS (ng/ml of plasma) IN THE POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 200 DURING THREE NORMAL ESTROUS CYCLES.

Day of Estrous Cycle	1969 Dates			n	Mean ng/ml ± S. E.
	4-4 to 4-23	4-24 to 5-12	5-13 to 6-2		
0	0.6	0.2	3.0	3	1.27 ± 0.92
1	1.1	1.6	2.9	3	1.87 ± 0.54
2	0.7	*	3.4	2	2.05 ± 1.35
3	4.6	*	3.6	2	4.10 ± 0.50
4	4.3	2.7	1.8	3	2.93 ± 0.73
5	4.2	7.1	2.2	3	4.50 ± 1.42
6	*	13.8	1.4	2	7.60 ± 6.20
7	*	8.3	13.1	2	10.70 ± 2.40
8	0.8	11.8	9.5	3	7.37 ± 3.35
9	6.5	9.0	8.5	3	8.00 ± 0.76
10	7.5	6.6	10.8	3	8.30 ± 1.28
11	2.5	9.4	17.5	3	9.80 ± 4.43
12	10.2	9.3	12.5	3	10.67 ± 0.95
13	*	10.1	6.2	2	8.15 ± 1.95
14	8.9	10.0	5.7	3	8.20 ± 1.28
15	2.0	10.9	18.3	3	10.40 ± 4.71
16	3.4	*	12.7	2	8.05 ± 4.65
17	11.4	*	12.9	2	12.15 ± 1.10
18	*	*	15.3	1	15.30 ±
19	0.6		20.2	2	10.40 ± 9.80
20			6.4	1	6.40 ±

* It was not possible to obtain a blood sample.

high concentration until 2-3 days prior to estrus, at which time there was a sharp decline in the progesterone concentration.

Average Daily Progesterone Concentration in the Jugular Vein of Two Normal Heifers.-

The progesterone levels in the jugular vein of two heifers were quantitated daily during two normal estrous cycles (Table X). There were some days when the volume of plasma was not sufficient enough to allow detection of the steroid. However, on most days there was a value for each heifer permitting calculation of a mean concentration. There were no striking differences between mean progesterone values at one day of the estrous cycle as compared to another day. However the first 11 days of the cycle had an overall mean of 1.01 ng/ml, and the last 10 days of the cycle had an average of 1.72 ng/ml.

Daily Ratio of Jugular Vein to Posterior Vena Cava Progesterone Levels.-

A ratio of daily progesterone concentrations in the jugular vein to those levels found in the posterior vena cava were determined for each heifer.

There were several days in the 30 day cycle of heifer No. 200 (Table XI) when both vena cava and jugular progesterone concentrations were available. There were no set patterns in the ratios calculated during the abnormal cycle. The ratio of jugular to vena cava progesterone concentration was 0.16 at the first estrus and increased markedly to 21.50 at Day 5 and dropped to 0.08 on Day 6. Thereafter, no marked

TABLE X. AVERAGE DAILY PROGESTERONE LEVELS IN THE JUGULAR VEIN OF TWO HOLSTEIN HEIFERS DURING TWO NORMAL ESTROUS CYCLES.

Day of Estrous Cycle	Heifer Number		n	Mean ± S. E.
	233	250		
0	1.6	0.9	2	1.25 ± 0.35
1	1.4	1.25 ¹	1	1.40
2	0.5	0.2	2	0.35 ± 0.15
3	2.6	1.0 ¹	1	2.60
4	1.3	1.5 ¹	1	1.30
5	0.01	0.8	2	0.40 ± 0.39
6	0.3	0.5 ¹	1	0.30
7	0.5 ¹	0.4	1	0.40
8	1.5	0.5 ¹	1	1.50
9	2.1	0.5	2	1.30 ± 0.80
10	0.4	0.8	2	0.60 ± 0.20
11	0.2	1.3	2	0.75 ± 0.55
12	1.2	3.0	2	2.10 ± 0.90
13	2.3	0.7	2	1.50 ± 0.90
14	1.1	1.7	2	1.40 ± 0.30
15	2.6	2.5	2	2.55 ± 0.05
16	2.4	3.6	2	3.00 ± 0.60
17	1.4	3.6	2	2.50 ± 1.10
18	2.1	1.0	2	1.55 ± 0.55
19	0.9	0.6	2	0.75 ± 0.15
20	1.0		1	1.00
21	0.9		1	0.90

¹ It was not possible to detect progesterone in this amount of plasma.

TABLE XI. DAILY RATIO OF PROGESTERONE LEVELS IN THE JUGULAR VEIN TO THOSE IN THE POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 200.

Day of Estrous Cycle	Progesterone (ng/ml of plasma)		Ratio of Jug- ular to Vena Cava Levels
	Posterior Vena Cava	Jugular Vein	
0	14.7	2.4	0.16
1	0.25 ¹	0.4	--
2	0.02	0.12 ¹	--
3	*	3.1	--
4	0.5 ¹	0.3 ¹	--
5	0.4	8.6	21.50
6	15.6	1.3	0.08
7	0.5 ¹	*	--
8	14.2	6.1	0.43
9	11.8	*	--
10	10.0	2.9	0.29
11	5.8	6.3	1.09
12	11.6	*	--
13	10.4	*	--
14	14.4	3.0	0.21
15	11.1	11.8	1.06
16	18.2	7.2	0.40
17	1.0	0.9	0.90
18	15.6	0.5 ¹	--
19	5.1	3.4	0.67
20	2.8	1.8	0.64
21	1.9	2.3	1.21
22	3.0	1.3	0.43
23	4.3	*	--
24	*	1.6	--
25	2.2	0.7	0.32
26	0.8	2.7	3.38
27	4.1	2.3	0.56
28	3.4	1.0	0.29
29	2.7	*	--
30	2.3	1.1	0.48
0	7.0	3.5	0.50
1	4.3	7.4	1.72

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

change in the ratios was apparent until Day 25, at which time the progesterone concentration in the jugular vein was 2.7 ng/ml as compared to 0.8 ng/ml in the vena cava (ratio=3.38). The ratio values then declined and remained low until the second estrus.

A quantitative analysis of the vena cava progesterone concentration in blood samples collected from the second cannula placed in the vena cava (heifer No. 200) was compared with the jugular levels (Table XII). At the first recorded estrus, the ratio was 15.00, but dropped to 0.80 at Day 1 and remained below 1.00 until Day 9 of the 14 day cycle, when a value of 7.20 was recorded. The concentrations of progesterone were relatively comparable through Day 13. It was not possible to detect progesterone in 1.0 ml of jugular plasma on the last day of the estrous cycle, and as a result no ratio could be calculated. The following day (estrus) the progesterone concentrations were equivalent in both veins (ratio=1.00).

The ratios were calculated for 19 days of a normal 21 day estrous cycle reported for heifer No. 233 (Table XIII). The progesterone concentrations in the jugular vein were noticeably higher than vena cava values during the first four days of the cycle ranging from ratios of 2.67 (Day 0) to 26.00 (Day 3). At Day 4, the vena cava concentration increased markedly, with a resulting drop in ratio to 0.81 (Jugular/Vena Cava). The ratios remained less than 1.00 throughout the remainder of the days which were compared (included through Day 19).

TABLE XII. DAILY RATIO OF PROGESTERONE LEVELS IN THE JUGULAR VEIN TO THOSE IN THE POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 200 (Second Cannulation).

Day of Estrous Cycle	Progesterone (ng/ml of plasma)		Ratio of Jug- ular to Vena Cava Levels
	Posterior Vena Cava	Jugular Vein	
0	0.4	6.0	15.00
1	0.5	0.4	0.80
2	0.03	1.0 ¹	--
3	1.1	0.04	0.04
4	1.5	0.8	0.53
5	1.5	1.3	0.87
6	3.2	1.3	0.41
7	10.1	0.7	0.07
8	1.8	0.1	0.06
9	0.5	3.6	7.20
10	2.3	1.1	0.48
11	3.1	1.7	0.55
12	1.6	2.1	1.31
13	3.4	2.3	0.68
14	0.4	1.0 ¹	--
0	0.2	0.2	1.00
1	0.9	0.8	0.89
2	0.75 ¹	0.2	--

¹ No detectable in this amount of plasma.

TABLE XIII. DAILY RATIO OF PROGESTERONE LEVELS IN THE JUGULAR VEIN TO THOSE IN THE POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 233.

Day of Estrous Cycle	Progesterone (ng/ml of plasma)		Ratio of Jug- ular to Vena Cava Levels
	Posterior Vena Cava	Jugular Vein	
0	0.6	1.6	2.67
1	0.2	1.4	7.00
2	0.1	0.5	5.00
3	0.1	2.6	26.00
4	1.6	1.3	0.81
5	2.2	0.01	0.004
6	3.4	0.3	0.09
7	0.2	0.5 ¹	--
8	2.0	1.5	0.75
9	4.6	2.1	0.46
10	2.0	0.4	0.20
11	4.9	0.2	0.04
12	3.1	1.2	0.39
13	10.0	2.3	0.23
14	4.2	1.1	0.26
15	4.6	2.6	0.56
16	2.9	2.4	0.83
17	2.5	1.4	0.56
18	6.6	2.1	0.32
19	2.3	0.9	0.39
20	*	1.0	--
21	*	0.9	--

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

The daily ratio was calculated for 15 days of a 25 day abnormal cycle on heifer No. 259 (Table XV). The ratios for this cycle did not follow the same pattern as was evident during the normal cycles. Ratios greater than 1.00 which indicated a higher progesterone concentration in the jugular vein than the vena cava were recorded on Days 1, 3, 11, 17 and 21. The mean ratio of progesterone concentrations in the veins was computed for four heifers at each day of the estrous cycle (Table XVI). The greatest mean ratio was 8.04 ± 5.06 at estrus, which was followed by 2.15 ± 1.20 (Day 1), 1.43 ± 0.93 (Day 2) and 5.74 ± 5.08 (Day 3). The mean ratios calculated for the remainder of the data was markedly greater than 1.00 on three occasions; whereas 16 of the mean ratios were 1.00 or less.

A least squares analysis of variance was computed for the progesterone levels in the jugular vein and posterior vena cava of heifer No. 233 and heifer No. 250 (Table XVII). There was not a significant difference between the progesterone levels for the heifers. A highly significant ($P < 0.005$) difference was found between the progesterone sources (jugular vein and vena cava). Likewise, there was a highly significant ($P < 0.005$) variation in progesterone levels among the days of the estrous cycle. However, no significant variance was found in the heifer-source interaction, heifer-day (day of the estrous cycle) interaction or the progesterone source-day interaction.

TABLE XIV. DAILY RATIO OF PROGESTERONE LEVELS IN THE JUGULAR VEIN TO THOSE IN THE POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 250

Day of Estrous Cycle	Progesterone (ng/ml of plasma)		Ratio of Jug- ular to Vena Cava Levels
	Posterior Vena Cava	Jugular Vein	
0	0.5	0.9	1.80
1	0.1	1.25 ¹	--
2	0.2	0.2	1.00
3	1.1	1.0 ¹	--
4	0.5	1.5 ¹	--
5	0.9	0.8	0.89
6	0.7	0.5 ¹	--
7	2.5	0.4	0.16
8	2.7	0.5 ¹	--
9	0.02	0.5	25.00
10	1.5	0.8	0.53
11	3.0	1.3	0.43
12	3.6	3.0	0.83
13	4.0	0.7	0.18
14	3.7	1.7	0.46
15	7.3	2.5	0.34
16	6.0	3.6	0.60
17	5.8	3.6	0.62
18	2.4	1.0	0.42
19	2.2	0.6	0.27

¹ Not detectable in this amount of plasma.

TABLE XV. DAILY RATIO OF PROGESTERONE LEVELS IN THE JUGULAR VEIN TO THOSE IN THE POSTERIOR VENA CAVA OF HOLSTEIN HEIFER NO. 259.

Day of Estrous Cycle	Progesterone (ng/ml of plasma)		Ratio of Jug- ular to Vena Cava Levels
	Posterior Vena Cava	Jugular Vein	
0	3.0	1.1	0.37
1	0.4	0.6	1.50
2	3.3	1.0	0.30
3	1.8	3.4	1.89
4	1.7	*	--
5	2.6	2.2	0.85
6	5.6	*	--
7	1.5	0.6	0.40
8	1.6	*	--
9	1.5	0.8	0.53
10	3.4	*	--
11	0.7	1.9	2.71
12	1.0	0.5	0.50
13	0.4	*	--
14	0.4	*	--
15	5.0	0.3	0.06
16	*	*	--
17	0.8	2.0	2.50
18	4.4	2.1	0.48
19	1.4	0.2	0.86
20	2.2	1.0	0.45
21	0.4	2.0	5.00
22	*	2.5	--
23	1.2	1.0 ¹	--
24	1.3	1.25 ¹	--
25	*	*	--

* It was not possible to obtain a blood sample.

¹ Not detectable in this amount of plasma.

TABLE XVI. AVERAGE DAILY RATIO OF PROGESTERONE LEVELS IN THE JUGULAR VEIN TO THOSE IN THE POSTERIOR VENA CAVA OF FOUR HOLSTEIN HEIFERS.

Day of Estrous Cycle	Heifer Number											n	Mean + S. E.		
	200						233		250		259				
-3	0.29	1.31					0.25	0.32	0.11	0.62		0.67	0.21	8	0.47 ± 0.14
-2	0.21	0.68							1.00	0.42		0.33	0.29	6	0.49 ± 0.12
-1	2.73	0.48							0.25	0.27		0.31		5	0.81 ± 0.52
0	2.67	2.00	0.16	0.50	15.00	1.00	2.67		1.80			46.67	0.37	10	7.29 ± 5.06
1	0.86	0.80	0.89				7.00					1.50		5	2.15 ± 1.20
2	0.29	0.56					5.00		1.00			0.30		5	1.43 ± 0.93
3	0.35	0.44	0.04				26.00					1.89		5	5.74 ± 5.08
4	0.51	0.53					0.81							3	0.62 ± 0.10
5	0.95	0.25	21.50	0.87			0.004		0.89			0.85		7	3.62 ± 2.98
6	0.15	0.08	0.41				0.09							4	0.18 ± 0.08
7	0.16	0.07							0.16			0.40		4	0.20 ± 0.07
8	0.18	0.43	0.06				0.75					0.56		5	0.40 ± 0.12
9	0.68	0.11	0.14				0.46		25.00			0.34	1.42	8	3.58 ± 3.12
10	0.54	0.29	0.48				0.20		0.53			0.67	0.42	7	0.45 ± 0.06
11	0.49	1.09	0.55				0.04		2.77	0.43		0.33	1.12	9	1.06 ± 0.31
12	1.31						0.39		0.91	0.83		0.32	0.50	6	0.71 ± 0.16
13	0.68						0.23		0.92	0.18		0.44		5	0.49 ± 0.14
14	0.21						1.73	0.26	1.53	0.46		0.44		6	0.77 ± 0.27
15	1.60						0.56		2.91	0.34		0.06		5	1.09 ± 0.52
16	1.06	0.40					0.58	0.83	0.12	0.60				6	0.60 ± 0.13
17	0.17	0.90					0.25	0.56	0.29	0.62		2.50		7	0.76 ± 0.34
18	1.20						0.32		0.42			0.48		4	0.60 ± 0.20
19	0.67						0.39		1.06	0.27		1.64	0.86	6	0.82 ± 0.20
20	0.21	0.64							1.00			0.06	0.45	5	0.47 ± 0.17
21	2.73	1.21							0.25			0.70	5.00	5	1.98 ± 0.87
22	0.43											1.23		2	0.83 ± 0.40
23														0	
24												3.38		1	3.38

TABLE XVII. LEAST SQUARES ANALYSIS OF VARIANCE BETWEEN PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HEIFERS NOS. 233 AND 250.

Source	D.F.	Sum of Squares	Mean Squares	F Value	Level of Significance
Cows	1	5.99	5.99	3.61	.100
Source	1	34.82	34.82	21.00	.005
Days	20	124.34	6.22	3.75	.005
Cows X Source	1	0.70	0.70	0.42	NS
Cows X Days	20	59.53	2.98	1.80	NS
Source X Days	20	44.65	2.23	1.35	NS
Error	19	31.50	1.66	--	

TABLE XVIII. CLASSIFICATION OF ESTROUS DAYS AND THEIR RELATIONSHIP TO PROGESTERONE LEVELS IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF HOLSTEIN HEIFERS¹.

Heifer Number	Progesterone (ng/ml plasma)		Ratio of Jugular to Vena Cava Progesterone	Estrus Class- ification ¹
	Posterior Vena Cava	Jugular Vein		
200	0.6	1.6	2.67	I
200	3.0	6.0	2.00	I
200	14.7	3.4	0.16	II
200	7.0	3.5	0.50	IV
200	0.4	6.0	15.00	IV
200	0.2	0.2	1.00	IV
233	0.6	1.6	2.67	I
250	0.5	0.8	1.80	I
259	0.03	1.4	46.67	III
259	3.0	1.1	0.37	III

¹ Estrus Classification:

- I - Estrus occurs between two normal estrous cycles.
- II - Estrus occurs between a normal and an abnormal estrous cycle.
- III - Estrus occurs between two abnormal estrous cycles.
- IV - Estrus occurs at the beginning or termination of an abnormal cycle. No additional information is available.

DISCUSSION

Blood Sample Collection.-

In order to quantitate venous progesterone levels, it is important that functional cannulae be placed in the vessels. This is especially critical in collecting blood samples from the posterior vena cava, which is relatively inaccessible and requires surgery in the cannulation procedure.

Silastic rubber tubing, a cannulation material described by Hull (1967) was found to be satisfactory for drawing blood samples daily from the vena cava for as long as 104 days (Table III).

Hull (1967) reported that posterior vena cava cannulae placed in cattle had remained patent for 90 days. Earlier, Conner and Fries (1960) had maintained portal vein cannulae for successful blood collection up to 73 days. The inherent flexibility of the silastic material is helpful in reducing vessel irritation and the incidence of thrombophlebitis caused by stiffer tubing. It was found that the size of the tubing used was an important consideration. The tube had to be small enough to lie freely in the vein and yet large enough to allow 20-25 ml of blood to be drawn readily. It was apparent that great care had to be taken in the amount of negative pressure applied on the thinner walled tubing, because it readily collapsed. The use of a thicker walled tubing, but with the same inside diameter as the thin walled tubing, seemed to aid the ease with which samples could be collected. Silastic tubing

with an inside diameter of 0.062" and an outside diameter of 0.125" was most satisfactory.

The functional life of the jugular cannulae was considerably shorter than the vena cava cannulae in most cases. There are several reasons why this may have occurred. First, the vena cava cannula was more fixed in the vessel than the jugular tubing. The movement of the animal did not cause the tubing to slide back and forth in the vein; whereas, the jugular cannula was in a vulnerable position on the neck of the animal. As the heifer turned her head from one side to the other, the tubing moved up and down in the vein enhancing the chance of vessel wall irritation. Also, as the heifer reached into the hay manger to feed, the cannula was rubbed, further provoking irritation and eventual phlebitis.

Competitive Protein-Binding Radio-assay.

The competitive protein-binding assay was developed by Murphy et al. (1963) as a simple method for the determination of plasma corticoids. Later, Neill et al. (1967a) made some alterations in the procedure and utilized it as a rapid effective means of determining minute amounts of progesterone in small volumes of plasma. It was critical that a quantitation method be used that would detect progesterone in small amounts of plasma, because if large volumes of blood were drawn daily from the animals this would cause undue stress and possibly result in an acyclic condition (Young, 1961).

The assay is not an involved procedure and it was generally possi-

ble to analyze a group of 40 samples every two or three days. A rapid determination such as this eliminates the storage of plasma samples for a long period of time before analysis, thus decreasing the chance of metabolic degradation of progesterone. Young (1961) has suggested that certain bacteria may degrade steroids.

In order to account for the losses of endogenous progesterone that occurred in extraction and chromatographic isolation, a ^{14}C progesterone internal standard was incorporated in each sample at the beginning of the analysis.

The internal standard method used in this work does have a distinct advantage over the external standard method used by Neill et al., (1967a) as it provides each sample with an individual recovery factor. This does improve the repeatability of the assay as the error due to sample group recovery corrections is eliminated.

Neill et al. (1967a) accounted for procedural losses in progesterone estimated during the normal human menstrual cycle by recovery of ^3H progesterone from plasma which had been subjected to identical extraction and chromatographic procedures as the sample plasma.

The internal standard (^{14}C progesterone) used in this system has some advantages over the ^3H progesterone standard (Neill et al., 1967a). The ^{14}C recovery standard was dispersed in the sample plasma and therefore underwent all the extraction, isolation and quantitation procedures with the endogenous progesterone. Whereas, the ^3H standard used by

Neill et al. (1967a) was mixed with blood plasma and treated in the same manner as the samples, but was not actually a part of the sample plasma. Also, if ^3H labelled progesterone were used as an internal recovery standard with the protein-binding method, it would not be possible to segregate the recovery standard ^3H counts from the counts per minute of corticosterone -1,2- ^3H bound to the binding globulin.

Progesterone Levels in the Jugular Vein and Posterior Vena Cava of Cycling Heifers.

It would be meaningful to discuss the progesterone concentration in the jugular vein and posterior vena cava of each heifer individually and account for the values found before a worthwhile investigation of the collective data for all heifers is undertaken.

Heifer No. 200.- The regression of the corpus luteum was responsible for the marked drop in progesterone concentrations, especially in the vena cava about two days prior to estrus (Figure 5). This lowered level permitted maturation of the developing follicle and the sequential ovulation of the egg. Mares and Casida (1963) conducted a study to determine the time of ovulation in cattle. The results of this work indicated that ovulation occurred about 24 hours after the termination of estrus. After ovulation, a corpus begins to form at the ovulatory site (Hafez, 1968). Figure 5 illustrates a rise in progesterone concentrations during Days 2 to 3 of each estrous cycle as the development of the CL begins.

Although no statistical analysis was made, the vena cava progesterone levels appeared to increase from the first complete estrous cycle to each successive one through the third cycle. This trend was not apparent in the cycles of other heifers, nor were the other progesterone values generally as high as were found for Heifer No. 200 (Figure 5).

There are several plausible conclusions for the pattern this figure illustrates. The most realistic reason would seem to be that the blood samples collected during the first 40-50 days the cannula was functional were stored for a long period of time before an analysis was made. Therefore, there was more opportunity for the progesterone in these samples to undergo a metabolic degradation of progesterone than those samples assayed soon after collection.

Secondly, the results recorded during the first few estrous cycles were made at a time when the protein-binding assay was being made operational. As a result, the details involved in the methodology had not been well established at that time.

The third justification of the rising progesterone levels from one cycle to the next is a consideration of maturity. Erb et al., (1968) has established that the progesterone content ($P < 0.025$) of the pregnant bovine CL raised significantly as the age of the animal increased, but progesterone concentrations did not. It is not probable, however, that advancing maturity was the most important factor in these data.

During most cycles in Figure 5, the progesterone values in both jugular and vena cava are highest for the period Days 6-18 of a 20-21 day cycle. This is indicative of active corpus luteum progesterone production through this period.

The fourth complete cycle recorded for Heifer No. 200 was abnormally long (Figure 5). This was probably the result of a foot rot infection the animal contracted in the damp pen where she was being kept. The rapid decline in vena cava progesterone concentration on Day 18 of this fourth cycle is indicative of normal luteal regression. However, the infection must have been sufficient to inhibit any estrus until 12 days later. However, the heifer did not exhibit a distinct estrus behavior, as she had shown on the four previously observed estrus dates. The small daily fluctuations in progesterone levels in both vessels through Day 30 indicate that ovulation and the subsequent formation of new corpora lutea did not occur. These fluctuations were probably due to the release of progesterone from the adrenal glands.

Heifer No. 233.- The data presented in Figure 7 is a daily comparison between progesterone concentrations in the vena cava and jugular vein through the latter part of one estrous cycle and through Day 19 of the following 20-day estrous cycle.

It is interesting to speculate about the relatively high progesterone levels measured in the jugular vein and vena cava when the vessels were cannulated on Day 14 of the first cycle. Likewise, when the

surgery dates for all heifers are considered there are greater jugular than vena cava concentrations during four of the five surgery days. The average of all vena cava determinations for the five days was 3.9 ng progesterone per ml of plasma as opposed to 7.2 ng in the jugular vein of the heifers.

There are several feasible reasons why this reversal occurred. First, the tip of the vena cava cannula was anterior to the region where the adrenal veins enter the posterior vena cava. Therefore, adrenal venous blood was a part of the vena cava sample. If the adrenals do release progesterone during a stress condition as concluded by Turner (1966), but if the concentration was not high when the vena cava sample was collected, then this could indicate that the steroid was secreted for a short while during the cannulation operation. However, when the sample was collected after the surgery, the effluent blood had reached the head region and was flowing down the jugular vein. Then in the following days the hormone level in the jugular vein dropped below that in the vena cava as the progesterone rich blood became more dilute and was utilized or passed out of the animal's body.

In considering the flow of adrenal venous blood into the vena cava, there might be what one could term a laminating effect. If the adrenal veins enter in the lateral portion of the vena cava, then adrenal blood may flow along in the dorsal region of the vessel rather than mixing with all the blood flowing in the vena cava. Therefore, if the tip of

the cannula (anterior to the entrance of the adrenal veins) was lying on the ventral surface of the posterior vena cava, the adrenal venous blood could flow past the tubing with very little of it being collected from the cannula.

Another possible explanation for the greater jugular than vena cava concentrations on the day of surgery could have been the release of progesterone from the hypothalamus. The existence of progesterone in the female rat brain was substantiated in a study by Bidder (1968). If this progesterone was released by the hypothalamus in response to the stress created during the surgery, this could account for the high progesterone levels in the jugular vein.

The progesterone concentration in the vena cava dropped significantly from Day 17 to Day 19 of the first cycle (Figure 7), which is indicative of luteal regression prior to estrus. The noticeable rise in progesterone levels on Day 4 of the second cycle and the increasing concentration to a peak at Day 13 indicates that the corpus luteum has formed. Although determinations were not made throughout the remainder of the cycle, the decline in levels beginning at Day 18 was probably due to luteal regression before the onset of the next estrus.

Heifer No. 250.- The regression of the CL causing a gradual decline in vena cava progesterone concentrations on Days 17-19 of the first estrous cycle was followed by a sharp drop from Day 19 to Day 20.

The low progesterone levels allowed the onset of estrus two days later (Figure 8).

If the progesterone levels for all of the heifers are studied for the day of estrus a distinct pattern becomes apparent. On six of the 10 estrus dates, the jugular progesterone levels were greater than the vena cava; and during one of the other four days the concentrations in the two vessels were the same.

Even though the mean progesterone concentration in the vena cava (3.0 ng/ml) was greater than that in the jugular vein (2.47 ng/ml), it should be recognized that only three of 10 estrus days had vena cava values greater than 3.0 ng/ml. Also, Heifer No. 200 was not always observed in standing estrus and the days when these high vena cava levels were measured had been calculated from the time when metestrual blood first appeared around the vulva and under the heifer's tail. In these instances the following day might have been the day of estrus, as a marked decline in vena cava progesterone is seen from Day 0 to Day 1 (14.7 ug/ml and non-detectable in 0.25 ml plasma, respectively) of the fourth cycle analyzed for Heifer No. 200 (Table III). Also, the progesterone levels dropped from 7.0 ng/ml (Day 0) to 4.3 ng/ml of plasma (Day 1) during the onset of the last estrous cycle (Table III). Likewise, it should be recognized that the cycle prior to the last estrus date was longer than normal (30 days). Early in this abnormal cycle Heifer No. 200 contracted foot rot and this infection was probably the

principal reason for the extended cycle length. Every estrus that occurred between two normal cycles had greater jugular than vena cava progesterone concentrations.

The luteal production of progesterone is minimal at estrus, therefore it would be expected that the vena cava progesterone levels be low. The higher jugular concentration might be the result of residual progesterone flowing in the vein. Williams (1962) has concluded that 1.2-5.4 percent of the progesterone passed out of the body was excreted in the urine and approximately 40 percent was lost in the feces. Therefore, some of the blood drawn from the vena cava cannula has been filtered through the kidney before it passes the tip of the silastic tubing. However, intestinal venous blood that could have lost progesterone in the fecal contents of the intestine flows from the portal vein to the liver from which the hepatic veins pass to confluence with the posterior vena cava. These branches enter the vena cava anterior to the region where blood samples are collected. Then it can be concluded that an active kidney filtration of progesterone at a period when luteal progesterone production is at a minimum could contribute in a small way to the lower vena cava concentrations at estrus, but the fecal progesterone loss would not affect it.

However, when the days of estrus and the last day of the previous cycle were compared in Figures 5-9, it was found that in five out of seven comparisons the progesterone concentration in the jugular vein

increased at estrus. A feasible explanation for this rise and the relatively high jugular progesterone level could be the release of the hormone from the hypothalamus at the onset of estrus and subsequent ovulation.

Callantine and Humphrey (1965) have made observations which indicate that progesterone cannot influence the response of a target organ to placental (or pituitary) gonadotropins, but rather inhibits the release of endogenous FSH. Spies et al. (1969) reported that electrical excitation of the basal tuberal hypothalamus induced ovulation in rabbits, however, these animals failed to ovulate when 2 mg of progesterone was injected 16-18 hours prior to stimulation. Earlier, Kobayashi et al. (1967) had shown that the secretion of FSH and LH from the rat anterior pituitary was regulated by the specific hypothalamic FSH-RF (FSH-releasing factor) and LH-RF, respectively. In another study Watanabe and McCann (1968) concluded that the FSH-releasing factor is found in the stalk-median eminence area in the rat. They studied the in vitro effect of rat hypothalamic extracts on male rat anterior pituitaries. After a six hour incubation period, a noticeable increase in FSH was apparent.

Heifer No. 259. The progesterone concentrations in the vena cava and jugular vein of heifer No. 259 (Figure 9) have been presented for the latter part of one abnormal estrous cycle and two cycles of longer than normal duration. Turner (1966) has stated that menstrual cycles

can be altered appreciably by environmental changes and mental upsets. If this is the case in the menstrual cycle, then perhaps the abnormal length of the first estrous cycle could be caused by such a disturbance.

The high progesterone values on Days 9 and 12 of the first cycle are greater than has generally been detected in the other heifers at this stage of the cycle. This increase may be due to an adrenal response during the cannulation operation.

The rapid increase in progesterone concentration on Day 10 of the second cycle and the high levels through Day 18 indicate an adrenal progesterone production during this time. This is plausible because on Days 1 and 2 a large amount of heparin had been added to the heifer's circulatory system in an attempt to draw venous blood samples from the jugular vein. As a result the animal became sensitized and an injection of heparin into the cannula on Day 3 of the first complete cycle caused an anaphylactic shock reaction. In response to this stress condition the adrenal glands will produce adreno-cortical steroids (Turner, 1966).

At Day 19, luteal regression was evident as the vena cava progesterone concentration dropped. However, the heifer did not come in heat within the next few days and it was apparent that some adrenal response occurred as the progesterone levels in both veins rose at Day 22. Perhaps the use of heparin for flushing the cannula caused this response. In light of this reaction, thereafter the cannula was flushed with a 0.9 percent saline solution.

Average Progesterone Levels in the Posterior Vena Cava During Normal Estrous Cycles.-

The mean progesterone levels in the posterior vena cava during five normal estrous cycles were calculated for each day of the cycle to permit a comparison of composite progesterone concentration among days (Table VIII). The vena cava progesterone values for the last two recorded cycles for Heifer No. 200 and all cycles for Heifer No. 259 were not included in this table because the cycles were abnormal in length. There were 11 days among the five normal cycles when a temporary or permanent loss of patency in the cannulae did not allow blood sample collection.

The lowest mean progesterone concentration (0.98 ± 1.30 ng/ml) was recorded at estrus. Estrus was not always detectable in each heifer. However, several times blood was found under the tail of a heifer two to three days after she was expected to come in estrus. In order to make the comparison of data meaningful, it was important to determine Day 0 for every estrous cycle. Hafez (1968) reported that metestrus bleeding begins two days after the onset of estrus. Thus, the day of estrus was readily determined by counting back two days from the time when fresh blood first appeared.

The progesterone concentration rose slightly (1.18 ± 0.51 ng/ml and 1.10 ± 0.78 ng/ml) on Days 1 and 2. No noticeable increase in progesterone concentration is to be expected on these days. Ovulation gener-

ally occurs 24 hours after the termination of estrus, which would be about Day 1. Donaldson and Hansel (1965) reported that granulosa derived luteal cells develop into functioning luteal cells by about Day 4, but they do not undergo any further mitotic division after this time. The theca interna cells, on the other hand, contain smaller luteal cells which respond to plasma luteotropin. These cells multiply and grow into large luteal cells which are primarily responsible for the CL growth after Day 4.

The data indicated development of the CL and active production of progesterone with the rise in concentration at Day 3 (2.35 ± 1.05 ng/ml). The following day (Day 4) the vena cava progesterone value was only slightly lower (2.18 ± 0.65 ng/ml) than the mean recorded at Day 3. This would indicate that luteal progesterone production was fairly constant during Days 3 and 4. In the days following an increase in progesterone secretion from luteal tissue was evident in the rising progesterone concentration.

The progesterone values in the vena cava averaged 7.74 ± 1.87 ng/ml at Day 12, but did not increase markedly until Day 15 when a peak concentration of 8.63 ± 2.83 ng/ml was reached. On Day 15 the progesterone levels were high in four estrous cycles, which accounted for the high mean concentration. However, the low progesterone value (2.0 ng/ml) found for heifer No. 200 (19 day cycle) accounted for the relatively large standard error.

The vena cava progesterone levels declined to Day 19 (6.32 ± 4.34 ng/ml) as the corpora lutea began to regress and decrease progesterone production (Donaldson and Hansel, 1965). The variation in the length of estrous cycles and consequently the difference in the time of luteal regression among the cycles dictate the large standard error of the mean. For two of the cycles, Day 19 was the day prior to estrus; whereas heifer No. 200 (third estrous cycle) and No. 233 had 20 and 21 day cycles, respectively.

The three normal estrous cycles investigated for heifer No. 200 (Table IX) demonstrate a minimum of luteal progesterone production at Day 0 (mean = 1.27 ± 0.92 ng/ml) and a slight increase for Days 1 and 2 (1.87 ± 0.54 ng/ml and 2.05 ± 1.35 ng/ml, respectively). However, at Day 3 there was a noticeable rise in progesterone secretion from the corpora lutea (4.10 ± 0.50 ng/ml). The vena cava progesterone levels decreased on Day 4 (2.93 ± 0.73 ng/ml) and then rose again and remained high through one day prior to the termination of the estrous cycles.

Average Progesterone Levels in the Jugular Vein of Normal Cycling Heifers.-

The progesterone concentrations in the jugular vein of heifer No. 233 and No. 250 were compared and averaged daily for the duration of two normal cycles whenever both values were detectable (Table X). Only the two heifers were used in this composite because these were the normal cycles with the most complete jugular data. However, there were several

days during the early part of the estrous cycle analyzed for heifer No. 250 when progesterone levels were undetectable in the amount of plasma available.

The lowest progesterone level in the jugular vein was observed on Day 4 (heifer No. 250) when there was not any detectable progesterone in 1.5 ml of plasma. The greatest concentration of progesterone was measured on Day 16, when 3.00 ± 0.60 ng/ml was quantitated for the two heifers. The first five days of the cycle for heifer No. 233, the jugular progesterone concentrations were relatively high (1.6 ng/ml, Day 0; 1.4 ng/ml, Day 1; 0.5 ng/ml, Day 2; 2.6 ng/ml, Day 3; and 1.3 ng/ml, Day 4), but the progesterone levels for heifer No. 250 were undetectable in the available plasma on three of these five days. The progesterone levels for both heifers declined during the next three days only to rise and fall up to Day 11. Thereafter, the concentrations remained high until the last day of the cycles (2.10 ± 0.90 ng/ml, Day 12; 1.50 ± 0.90 ng/ml, Day 13; 1.40 ± 0.30 ng/ml, Day 14; 2.55 ± 0.05 ng/ml, Day 15; 3.00 ± 0.60 ng/ml, Day 16; 2.50 ± 1.10 ng/ml, Day 17; 1.55 ± 0.55 ng/ml, Day 18; 0.75 ± 0.15 ng/ml, Day 19; 1.00 ng/ml, Day 20; and 0.90 ng/ml, Day 21.

These results are not in agreement with conclusions made by Gomes et al. (1963). That study indicated that jugular progesterone levels were not correlated with the stages of the estrous cycle. In more recent work, Stabenfeldt et al. (1969) analyzed the jugular progesterone

concentration in cows each day of normal 21 day estrous cycles. During the follicular phase of the cycle the cows averaged less than 0.5 ng progesterone per ml of plasma. The maximum cyclic luteal activity was evident at Day 13, when 6.6 ng/ml was quantitated. However, Gomes et al. (1963) reported progesterone levels in cycling dairy cows and heifers that ranged from non-detectable (near estrus) to 0.054 ng/ml. This value is much higher than the peak concentration reported by Stabenfeldt et al. (1969).

Daily Ratio of Jugular Vein to Posterior Vena Cava Progesterone Levels.-

The ratio of progesterone values in the jugular vein to those in the posterior vena cava was computed daily for the heifers. This permitted a contrast to be made between the levels in the two veins at various stages of the estrous cycle.

Before a discussion of the changes in the mean ratio is undertaken, it would be beneficial to consider the general trends in the progesterone ratios and what implication they have. It is apparent that the jugular concentrations are generally greater than the vena cava progesterone values near estrus. This was a period when luteal tissue formation and progesterone production was at a minimum. Some of the blood collected from the vena cava was filtered through the kidney prior to collection, thus progesterone can pass into the urine (Williams, 1962). However, the jugular blood was not filtered through the kidney before sampling. Also, it was possible that near estrus, progesterone which

had accumulated in the hypothalamic area was released and enhanced the progesterone concentration in the jugular vein. The higher jugular progesterone may have been due to one of these factors or both of them.

Most of the ratios calculated for normal cycles at or near estrus were greater than 1.00 (Tables XIII and XIV). These high ratios resulted from greater jugular than vena cava concentrations. However, as the progesterone levels in the vena cava increased (Days 3 and 4) there was a corresponding drop in the ratio of jugular to vena cava progesterone concentrations.

It should be mentioned that the ratio of estrus was 0.16 at Day 0 of an abnormal cycle for heifer No. 200 (Table XI). The heifer did not show characteristic signs of estrus that day, but two days later fresh blood was passed as she began metestrus bleeding. Therefore, this was the criteria used to calculate estrus. The progesterone level in the vena cava was not detectable in 0.25 ml of plasma on Day 1 and the concentration was only 0.02 ng/ml at Day 2. It is possible that during this normal cycle the metestrus bleeding began earlier than reported by Hafez (1968).

All the ratios of progesterone levels in the two veins of four heifers during nine estrous cycles were averaged for each day of the cycle (Table XVI). This permitted all of the ratios determined for each normal or abnormal cycle to be included in the calculation of a mean ratio.

Seven of the 10 ratios considered at Day 0 (Table XVI) were 1.00 or greater, which indicates that the jugular concentration was greater than the vena cava level in seven estrous cycles. The 0.16 ratio (No. 200 heifer) was also considered in this determination. It is possible that estrus in that cycle occurred on the following day. At that time there was a marked decline in progesterone levels in the vena cava, which would have raised the ratio considerably. The 0.50 ratio determined for heifer No. 200 was calculated for an estrus at the conclusion of a 30 day cycle. The third ratio less than 1.00 was calculated for heifer No. 259 at the termination of a 28 day cycle and the beginning of another abnormally long cycle (25 days). Estrus detection during these cycles was difficult and the heifers were never seen in "standing estrus".

As the heifers approached mid-cycle there was a noticeable drop in the mean ratio of jugular progesterone to vena cava levels because of an increase in progesterone production in the newly formed CL and the rising progesterone concentration in the vena cava.

The nearly complete data for the jugular as well as the daily vena cava concentration throughout two normal estrous cycles (heifer Nos. 233 and 250) permitted a meaningful least squares analysis of variance in the results. The least squares analysis was useful because even though all progesterone values were not detectable, the data could be tested to determine statistical differences between the progesterone concentrations in the heifers and the two vessels.

The highly significant differences ($P < 0.005$) between the progesterone sources (jugular vein and posterior vena cava) substantiated the findings of the t-test which had taken into account progesterone values for all the heifers (Table I). The least squares analysis was meaningful because it tested normal cycles; whereas Table I included values from abnormal as well as normal cycles. The apparent variation in progesterone concentration among the days of the estrus cycle (Figures 5-9) was proven to be significant ($P < 0.005$) for heifers 233 and 250. This type of variation would be expected with the low values at estrus and the increasing progesterone concentrations as the heifers approached mid-cycle.

The analysis (Table XVII) indicated that there was not an important difference between progesterone levels measured in the two heifers ($P < 0.10$). The heifer by source interaction suggests that there was not a significant variation from a particular vein in one heifer to that same vessel in the other heifer. The non-significance of the heifer by day interaction indicates there was no statistical difference between the daily progesterone concentrations determined for heifer No. 233 and the values quantitated for heifer No. 250. Likewise, the lack of significant variation in the progesterone source and the day interaction implies that there was not any statistically important difference between the progesterone levels measured in a vein on a particular day and those detected for that vessel in the other animal.

SUMMARY

This study was conducted to determine the daily progesterone concentrations in the jugular vein and posterior vena cava of Holstein heifers throughout the estrous cycle. Blood samples were collected daily from cannulae surgically placed in the vessels and then analyzed for progesterone content with a modification of the competitive protein-binding technique.

The regression of the standard curve used to calculate the progesterone concentrations in the plasma was always highly significant ($P < 0.005$). A curve that deviated significantly from linearity ($P < .01$) was not used in the quantitation of progesterone.

Samples collected from four heifers were assayed throughout nine complete estrous cycles, four of which were abnormal in length. The progesterone concentration in the posterior vena cava was greater ($P < 0.001$) than the jugular concentration in 142 comparisons.

A least squares analysis of variance for two heifers during two normal estrous cycles demonstrated a difference between progesterone concentrations in the jugular vein and posterior vena cava ($P < 0.005$). Also, a statistical difference in the daily progesterone levels existed among the days of the estrous cycles ($P < 0.005$).

The major findings of this work are as follows;

1. The progesterone levels in both vessels were low near the time of estrus. Generally, the progesterone levels started to increase noticeably during Days 4-6 as the CL developed. The vena cava surpassed

those in the jugular plasma by Days 2-3. The progesterone values in both veins were generally highest for the period, Days 6-18 of a 20-21 day cycle. The levels declined markedly 1-2 days prior to estrus.

2. The results suggest that excessive stress due to foot rot and an anaphylactic shock reaction resulted in extended estrous cycles for two heifers. However, the decline in progesterone levels indicated the CL regressed at the normal termination time of the cycles.

3. On seven out of 10 days of estrus the progesterone concentration in the jugular vein was equal to or greater than the vena cava levels. This suggests that any of one or a combination of events may have occurred.

First, the luteal production of progesterone was minimal at that time and very little progesterone was flowing in the vena cava. Consequently, the greater jugular progesterone might have been due to residual progesterone remaining in the vein. Second, kidney filtration of the low progesterone could have caused lower vena cava than jugular values at estrus. Third, a hypothalamic release of progesterone may occur near the time of estrus.

4. The progesterone levels in the jugular vein exceeded that in the vena cava during four of the five surgery days. These greater levels were probably the result of progesterone secretion from the adrenal glands during surgery. The lower vena cava concentrations might result from sampling after the progesterone secreted by the adrenal had

passed the tip of the cannula. Another possible explanation could be a "laminated blood flow" in the posterior vena cava. The adrenal venous blood may flow in a certain region of the vein instead of dispersing throughout the vena cava; therefore, the adrenal blood could pass by the vena cava cannula tip without being collected when samples were taken. Whereas, the adrenal flow would be sufficiently mixed with the remainder of the blood after passing through the heart, lungs and the head, so as to be detected in the jugular sampling.

APPENDIX

TABLE I. SUPPLEMENT. DIFFERENCE BETWEEN PROGESTERONE CONCENTRATION IN THE POSTERIOR VENA CAVA AND THE JUGULAR VEIN OF FOUR CYCLING HOLSTEIN HEIFERS.

Comparison No. 1-36		Comparison No. 37-72		Comparison No. 73-108	
Deviation	Squared	Deviation	Squared	Deviation	Squared
$d = D - \bar{d}$	d^2	$d = D - \bar{d}$	d^2	$d = D - \bar{d}$	d^2
-4.12	16.97	-0.32	0.10	0.08	0.01
1.58	2.50	-0.72	13.84	5.88	34.57
1.62	2.62	-0.02	0.0004	1.28	1.64
1.18	1.39	0.58	0.34	0.18	0.03
-3.72	13.84	-0.62	0.38	-1.32	1.74
-2.82	7.95	1.68	2.82	-0.72	0.52
-1.32	1.74	-4.92	24.21	2.68	7.18
1.18	1.39	-7.42	55.06	-0.42	0.18
2.28	5.20	-1.72	2.96	-7.32	53.58
-1.62	2.62	-0.76	0.58	-1.52	2.31
0.28	0.08	-1.12	1.25	-1.62	2.62
3.48	12.11	-1.62	2.62	-3.42	11.70
9.98	99.60	0.08	0.01	-3.92	15.37
7.78	60.53	1.78	3.17	3.08	9.49
7.88	62.09	-0.12	0.01	-0.12	0.01
6.18	38.19	-4.92	24.21	-1.72	2.96
1.18	1.39	-0.62	0.38	-1.82	3.31
2.98	8.88	-0.42	0.18	-1.52	2.31
1.18	1.39	-2.32	5.38	-1.42	2.02
-1.42	2.02	-0.72	0.52	-1.82	3.31
-0.32	0.10	-1.82	3.31	-1.72	2.96
0.18	0.03	-1.92	3.69	0.28	0.08
10.48	109.83	-7.12	50.69	-2.30	5.29
-10.02	100.40	0.68	0.46	-1.12	1.25
12.48	155.75	3.38	11.42	-0.12	0.01
6.28	39.44	-2.82	7.95	-1.22	1.49
5.28	27.88	-3.02	9.12	1.48	2.19
-2.32	5.38	-2.22	4.93	0.18	0.03
9.58	91.78	-4.32	18.66	2.98	8.88
-2.52	6.35	-1.52	2.31	0.58	0.34
9.18	84.27	0.37	0.14	0.38	0.14
-1.72	2.96	1.28	1.64	-0.42	0.18
-0.12	0.01	-1.32	1.74	-0.22	0.05
-0.82	0.67	0.68	0.46	-1.12	1.25
-2.22	4.93	-0.22	0.05	0.28	0.08
-0.12	0.01	2.88	8.29	2.78	7.73

TABLE I. SUPPLEMENT (Continued).

Comparison No. 109-125		Comparison No. 126-141	
Deviation $d = D - \bar{d}$	Squared Deviation d^2	Deviation $d = D - \bar{d}$	Squared Deviation d^2
-0.02	0.0004	-0.72	0.52
3.78	14.29	0.08	0.01
-3.19	10.18	-2.02	4.08
-2.32	5.38	0.48	0.23
6.78	45.97	-3.42	11.70
-2.52	6.35	-1.42	2.04
11.58	134.10	-0.92	0.85
7.68	58.98	-1.12	1.25
0.98	0.96	-3.02	9.12
-3.42	11.70	-1.32	1.74
3.78	14.29	2.88	8.29
-0.52	0.27	-3.02	9.12
-3.52	12.39	0.48	0.23
-3.72	13.84	-0.62	0.38
3.78	14.29	-0.62	0.38
-0.72	0.52	-3.42	11.70
-0.82	0.67	0	1835.49
		$s_p^2 = 13.02$	

$$s_D = \sqrt{13.02} = 3.61 = \text{Standard Deviation} = s$$

$$s_d = \frac{13.02}{142} = 0.092 = \text{Mean of the variance}$$

$$s_d = \frac{3.61}{\sqrt{142}} = 0.30 = \text{Standard error of the Mean} \left(SE = \frac{s}{\sqrt{n}} \right)$$

$$t = \frac{d - uD}{s_d} = \frac{1.82 - 0}{0.30} = 6.07,$$

$$t = 6.07, d.f. = n - 1 = 141$$

$$1\% \text{ Level } t_{0.001, 141} = 3.73$$

$$(P < 0.001)$$

The progesterone concentration in the posterior vena cava is significantly greater than the concentration in the jugular vein at the 0.1% level of significance.

TABLE II, SUPPLEMENT. DIFFERENCE BETWEEN PROGESTERONE CONCENTRATION IN THE POSTERIOR VENA CAVA AND THE JUGULAR VEIN AT ESTRUS IN FOUR CYCLING HOLSTEIN HEIFERS.

No. of Estrous Dates	Heifer Number	Deviation $d = D - \bar{d}$	Squared Deviation d^2
1	200	-1.53	2.34
2	200	-3.53	12.46
3	200	11.77	138.53
4	200	2.97	8.82
5	200	6.13	37.58
6	200	-0.53	0.28
7	233	-1.53	2.34
8	250	-0.93	0.86
9	259	-1.90	3.61
10	259	1.37	1.88
			208.70
			S_d^2 23.19

$$S_d = \sqrt{23.19} = 4.82 = \text{Standard Deviation}$$

$$S_d^2 = \frac{23.19}{10} = 2.32 = \text{Mean of the Variance}$$

$$S_{\bar{d}} = \frac{4.82}{\sqrt{10}} = 1.52 = \text{Standard Error of the Mean}$$

$$t = \frac{d - \text{up}}{S_{\bar{d}}} = \frac{0.53 - 0}{1.52} = 0.35$$

$$t = 0.35, \text{ df} = n - 1 = 9$$

$$t_{0.05; 9} = 2.262$$

The progesterone concentrations do not differ significantly at the 5% level during estrus.

BASIC COMPUTER PROGRAM FOR COMPUTATION OF PROGESTERONE CONCENTRATIONS
MEASURED IN THE JUGULAR VEIN AND POSTERIOR VENA CAVA OF CYCLING HOLSTEIN
HEIFERS.

```
10 LET X1=0
15 LET Y1=0
20 LET C1=0
25 LET B1=0
30 LET X2=0
35 LET Y3=0
40 LET I=0
50 READ N,M
60 FOR K=1 TO M
65 LET Y[K]=0
66 LET P[K]=0
68 NEXT K
70 READ L,X,Y4
80 LET I=I+1
90 LET X1=X1+X
95 LET Y1=Y1+Y4
100 LET Y L =Y [L]+Y4
105 LET P L =P [L]+1
110 LET C1=C1+X*Y4
120 LET X2=X2+X2
125 LET Y3=Y3+Y42
130 IF I<N THEN 70
140 LET B=(N*C1-X1*Y1)/(N*X2-X12)
150 LET A=(Y1-B*X1)/N
155 PRINT
160 PRINT "Y(P) = ";A;"+";B;"X"
161 LET R1=(C1-(X1*Y1)/N)2/(X2-X12/N)
162 FOR K=1 TO M
163 LET B1=B1+(Y[K]2)/P[K]
164 NEXT K
165 LET B1=B1=Y12/N
166 LET B2=B1-R1
167 LET T=Y3-Y12/N
168 LET W=T-B1
169 LET D3=M-2
170 LET D4=M-1
171 LET D5=N-M
172 LET D6=N-1
175 LET O1=R2/D3
179 LET O2=W/D5
```

BASIC COMPUTER PROGRAM (Continued).

```
180 LET R8=R1/Q2
181 LET R9=Q1/Q2
182 PRINT
183 PRINT "NATURE OF VARIATION          DF          SS          ";
184 PRINT "MEAN SQUARE          R"
185 PRINT "REGRESSION          1          "R1;R1;F8
186 PRINT "DEVIATION FROM REGRESSION          ";D3;R2;Q1;F9
187 PRINT "BETWEEN DOSE          "D4;B1
188 PRINT "WITHIN DOSE          "D5;W;Q2
189 PRINT "TOTAL          "D6;T
190 LET L2=SQR(W/D5)/B
191 PRINT
192 PRINT "LAMBDA =" ;L2
193 PRINT
194 PRINT
195 READ C2,C3,E1,E2,E3,E4,E5,E6
196 LET C2=(C2+C3)/2
197 LET D2=(E1+E2+E3+E4+E5+E6)/6
198 READ J,Y2,A2,B2,F2
199 IF J=99999. THEN 999
200 LET X3=(Y2-A)/B
201 LET X3=(X3*(D2-C2)-(A2+B2-2*C2))/(F2*(A2+B2-2*C2))
202 LET Z=(A2+B2-2*C2)/D2
203 PRINT "CORRECTED X";J;"=";X3;Z;F2
204 GO TO 210
205 DATA 99999.,0,0,0,0
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D292 Progesterone levels in
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posterior vena cava of
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NAME AND ADDRESS

DATE	NAME AND ADDRESS
March 8, '71	Kelly Fibpatrick 1306 Jones St #3 Box 207

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