DETERMINATION OF CALCIUM, SODIUM, AND POTASSIUM VARIATIONS IN THE BLOOD AND ESTRUAL SECRETIONS OF THE DOMESTIC COW (BOS TAURUS) DURING THE NORMAL ESTROUS CYCLE

by

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INTRODUCTION

This study was undertaken to supply, if possible, certain basic information regarding the normal changes occurring in the biochemistry of the estrual secretions of the normally cycling cow. More attention will be given to the relationship, if such exists, between the sodium, potassium, and calcium fluctuations in the blood serum and those of the estrual secretions, particularly of the mucus.

Veterinary medical investigators have directed their attention toward methods of detecting early pregnancy; methods of determining proper timing for breeding either naturally or artificially; and toward methods of diagnosing all types of interferences with reproduction. One of the most perplexing problems facing the veterinarian specializing in fertility problems in all species of domestic animals, but particularly in the domestic cow, is the female animal that clinically is normal, culturally and serologically shows no infection present in her genital tract and physiologically she demonstrates normal estrous cycles, still she fails to reproduce. It has been hypothesized that in such females the chemistry of the genital tract secretions may be incompatible with the sperm, the fertilized ovum or might even interfere with implantation and intrauterine growth and development of the embryo and fetus. It is further hypothesized that aberrant nutrition may influence the chemistry of the secretions and make them incompatible with spermatozoa in instances of herd infertility where no other etiology can be detected. It is not yet known what the normal variations of calcium, sodium, and potassium content of the mucus are; neither is it known what variations occur during the four stages of the estrous cycle in the cow that is capable of reproduction.

The normal functioning of the animal body is dependent upon many factors. Among these is the well balanced electrolyte level of the body fluids. It is known that certain inorganic substances, notably potassium, sodium, and calcium are necessary for the irritability and rhythmicity of smooth muscles and nerves. Potassium is the major ion of the intracellular fluid, and its concentration in the extracellular fluid is not great. Slight variations in potassium may impair many functions of the body. An excess of potassium in the extracellular fluid can dilate the heart and even stop it in diastole. A low value of potassium in the extracellular fluids may impair nerve impulses and muscular paralysis may result.

The cation present in the highest concentration in the extracellular fluids is sodium; its main function is the maintenance of membrane potentials. When a membrane becomes excited and an electrochemical impulse transmitted, the cell membrane becomes more permeable to sodium than to potassium. This change in permeability of the membrane will permit the electrochemical impulse to be transmitted along nerve fibers. Sodium also exerts an important influence on the osmotic pressure and chemical characteristics of the extracellular fluids.

Calcium normally decreases the permeability of the cell membrane to sodium, thus calcium plays an extremely important role in nervous excitability. A low calcium will permit an increase in the membrane permeability and the membrane excitability will be increased.

These three inorganic substances, potassium, sodium and calcium, were chosen for study during the normal estrous cycle of lactating cows. An attempt will be made to determine if variations in the blood have any effect on the existence of these elements in the estrual secretions.

It is known that the nervous system, endocrine system, and possibly the digestive system, through the nutrients supplied, play an important role in the normal physiology of the estrous cycle. The hormones, which are of great importance under ordinary physiologic conditions in regulating electrolyte metabolism and water balance, do so principally through their actions on renal function. Aldosterone, the sodium-retaining hormone of the adrenal cortex and the posterior pituitary gland secretion of antidiuretic hormone (ADH) by its antidiuretic action, plays an important role in an animal's electrolyte balance. The extracellular calcium ion concentration is regulated mainly by the parathyroid hormone. This hormone increases calcium absorption from the body, especially the skeleton, and possibly enhances the absorption of calcium from the intestine and from the renal tubules.

The role of nutrition as a cause of sterility has been suspected for quite some time now, but is has been difficult to prove that nutrition alone is a primary factor in reproductive failures. Smythe (1931) observed that when Indian corn is utilized as a staple food, a higher incidence of functional sterility exists in cattle.

It is admitted that estrogenic hormones have an important effect upon bone and mineral metabolism by their stimulating effect on the osteoblasts, thus a greater deposition of calcium and phosphorus occurs in bones. Estrogen and progesterone are thought to cause calcium and sodium retention by the kidney tubules. It will be seen in reviewing the literature that various authors have different opinions regarding the importance of estrogens in maintaining the body's electrolyte balance. The control mechanism of these inorganic electrolyte substances in body fluids is very complex and is dependent upon many factors, including the animals' nutrition.

It is known that demonstrable variations exist in the blood supply to the female genitalia during the estrous cycle, pregnancy, and the postparturient period. These variations suggest possible changes in the chemistry of the estrual secretions, the secretions associated with pregnancy and the secretions in the involutionary period. It is known that all three elements, sodium, potassium and calcium, are present in the blood supplying the female genitalia and in the genital secretions. The normal chemistry of these secretions must be determined in proestrum, estrum, postestrum, and diestrum before clinical tests in these sterility problem patients can be interpreted. A possible explanation of infertility in the otherwise clinically sound cow is the incompatibility of the chemical composition of the tubular genital tract secretions to spermatozoa and/or the conceptus. (Early embryonic death; fetal resorption).

The cyclic changes in the quality and quantity of the estrual secretions of the genital tract of the cow have been known for many years. Even the general chemistry of the secretions is known but the correlation of the sodium, potassium and calcium content of the blood serum and its influence on these same substances in the estrual secretions during the four recognized stages of the estrous cycle is as yet unknown. Likewise, the effect of various sex hormones on the estrual secretions has been known for many years.

Human gynecologists attach great significance to the hydrogen ion concentration of the vaginal mucus in human infertility. In the bovine species, vaginal acidity is considered a very minor factor in causing sterility. Furthermore, parenteral estrogens or vaginal infections have failed to alter the pH of the vaginal secretions of the cow sufficiently to cause death of spermatozoa as has been reported for the human female. Even if the pH of the vaginal secretions of the cow were altered sufficiently by the aforementioned means, a most economical and practical method of bypassing this form of sterility exists in the rather extensive and widespread usage of artificial insemination in cattle.

While the most complete knowledge of the biochemistry of the genital tract secretions is that of the human female, it is inadvisable to attempt to apply that information *in toto* to the females of our domestic animals. As will be shown later, both the pH and total water content of the bovine cervical mucus differs significantly from that of the human female.

The objectives of this study were threefold:

1. To determine whether or not there is a significant variation in the calcium, sodium, and potassium of the blood at various stages of the estrous cycle of the domestic cow known to be capable of reproduction;
2. To determine the normal values and to find whether or not there is a significant variation in the calcium, sodium, and potassium in the collectable estrual secretions at the various stages of the estrous cycle of the domestic cow known to be capable of reproduction;
3. To correlate the relationship, if any, of these

three elements in the blood with their appearance in the genital secretions during the various stages of the estrous cycle in the domestic cow (Bos taurus) known to be capable of reproduction.

It has been pointed out by Santamarina (1961) that one of the factors which disrupts the continuity of efficient reproduction in domestic animals may be a natural imposition of a period of rest to conserve the body economy of the female animal. It is most difficult to differentiate the above from so called metabolic infertility, fetal resorption and early embryonic death. The human race is seeking chemical and other ways of controlling the world population explosion. The veterinary profession is seeking ways and means of increasing the proficiency of reproduction in food producing animals and birds to provide food for the ever increasing human population. It is believed that basic knowledge of the chemistry of the genital system, through studying the genital secretions, will not only lead to ways and means of controlling the "population explosion" but also to ways and means of increasing the reproductive efficiency of our food producing livestock.

REVIEW OF LITERATURE

Blood Serum Sodium, Potassium, and Calcium

The role of sodium, potassium, and calcium has been discussed in the INTRODUCTION. It would be proper now to find the normal values and variations of those three elements in the blood stream, particularly in the bovine species. It will also be interesting to investigate the opinion of researchers as to the significance of those variations related to the estrual cycle or gonadal hormones.

In 1930 Palmer and Eckles stated that significant fluctuations of plasma calcium occur from day to day not at that time explainable, even when the samples are taken under presumably identical physiological conditions. The maximum variation encountered was 4.05 mg. of calcium per ml. of blood plasma. The majority of the normal samples showed a range of 9.0 to 12.0 mg. per cent. Dukes (1937) gives the same range of 9.0 to 12.0 mg. per cent for blood plasma calcium. In 1946 Rusoff and Piercy made determinations over a period of two years on pooled citrated blood. They give variations of the plasma calcium in three different dairy cow herds; in the first one the variations were from 9.00 to 15.28 mg. of calcium per cent ml. of plasma, with a mean of 10.89 mg. per cent; the second, from 9.35 to 13.87 mg. of calcium per cent ml. of plasma with an average of 11.36 mg. per cent; and the third one from 7.22 to 12.73 mg. of calcium per cent with an average of 9.95 mg. per cent. They concluded that significant differences existed between animals in a herd; and significant differences existed in the same animal in different months of the year.

Charton et al. (1962) give values of 145.7 milliequivalents per liter (114.8-160.8) for serum sodium, and 4.8 milliequivalents (3.6-5.9) for serum potassium in lactating ewes. Benjamin (1961) gives the following normal values; 142.0 milliequivalents per liter (132.0-152.0) for serum sodium and 4.8 milliequivalents per liter (3.9-5.8) for serum potassium in the bovine species. Cornelius and Kaneko (1963) give the same average for serum sodium but state that the average of serum potassium in cattle is 4.8 with 3.9 as a minimum and 9.5 milliequivalents per liter as the maximum value.

As early as 1934 Zwarenstein stated that the endocrine relationship between the gonads and calcium metabolism was by no means well established in mammals. There is some evidence that a decrease in total serum calcium and an increase in dialysable calcium occurs during the later months of pregnancy, but this may be due to an indirect effect of pituitary activity at this time. He stated that in amphibia the results suggest that the ovary, directly or indirectly, controls the calcium content of the serum. Riddle and Reinhart (1926) found that the serum calcium of female pigeons rose during the preovulation phase to a value double that of the resting period; this high level is maintained during the greater part of the ovulation period. The level falls during the post-ovulation period.

Emmerson (1930) studied the serum calcium levels of cattle showing various stages of the estrous cycle and other ovarian involvements. Of 285 animals studied, blood samples were taken from cows in the Veterinary Hospital and from the Zürich abattoirs. He found a normal range of 8 to 13 mg. per cent of serum calcium and showed that variations occurred in the normally cycling cow. He states that the serum calcium falls during late diestrum, rises to its highest level prior to ovulation, drops markedly following ovulation and then rises and falls with the development and regression of the corpus luteum. He further states that the serum calcium levels are maintained at a rather high level in cystic ovary nymphomania, actually higher than during the follicular phase of the normally cycling cow, and then gradually fall off to a level commensurate with that observed in both male and female castrates as the cystic ovary nymphomania condition persisted.

Levin and Smith (1938) ask the question: "Is the blood calcium level of mammals influenced by estrogenic substances"? Their results indicate very little if any effect of estrin administration or ovariectomy on the serum calcium level. The slight elevations they observed were considered to be only transitory.

Thorn _et al._ (1938) the same year stated that pregnandiol, estrone, progesterone and testosterone induced the retention of Na, Cl, and H_2O in the normal dog. Thorn and Engel (1938) found that subcutaneous injections of progesterone, estrone, α-estradiol, or testosterone proprionate were followed by a decreased renal excretion of NaCl. On the day of injection a slight increase in the renal excretion of potassium frequently followed administration of progesterone and testosterone proprionate. Ruffo (1940) concluded that in adult female and male rats, repeated injections of progesterone caused a slight decrease in serum calcium. Eveleth _et al._ (1941), the next year, revealed that the changes in the serum calcium as a result of spaying are slight but may be significant. On the other hand their findings have not shown a constant hypercalcemia at estrum. They also felt that if the controlling factors were influenced by either the central nervous system or the sexual glands, it would appear that the actual act of copulation would produce changes in the blood chemistry that might be absent under ordinary conditions. In 1951 Sellers and Roepke found that the most noticeable changes were a fall in total serum calcium and serum inorganic phosphate at the time of parturition. In some individuals, a slight fall in total serum calcium was noted following estrogen administration. No appreciable or consistent changes were noted in blood levels of sodium, potassium, magnesium, or chlorides following either estrogen administration or parturition. It must be remembered that each time a hormone is injected into an animal, the resultant situation is no longer normal, therefore the experimental results cannot be interpreted as being physiological.

Phillips et al. (1952) investigated serum electrolytes in the menstrual cycle in the human and results showed that the serum electrolytes, sodium and potassium, reached a peak at the time of ovulation. Serum calcium increased from a premenstrual low to an ovulatory high. LaTorretta and Paladini (1960) determined the daily sodium and potassium value by flame photometry during the menstrual cycle in the serum and saliva of six women. Only slight variations were observed; the blood levels of sodium and potassium reached their highest values during the ovulation period; in saliva, potassium decreased at the same time and sodium did not change. Also in the human Bianchi et al. (1961) reported that progesterone had no direct action on reabsorption of sodium and potassium ions by the proximal convoluted tubules, but had an irregular effect. In all five cases examined the hormone decreased the excretion of potassium by the distal convoluted tubules. Arthur Guyton (1961) revealed that estrogens cause sodium, chloride, and water retention by the kidney tubules. However this effect of estrogens is very slight and rarely of significance except in pregnancy. He also states that when massive doses of estrogens are administered, the plasma calcium level may rise to values considerably above normal in the same manner that administration of parathyroid hormone and extreme quantities of vitamin D produce this effect. According to Williams (1963) gonadal hormones such as estradiol and testosterone have a slight sodium retaining activity, presumably through promoting sodium reabsorption by the renal tubules. This effect is slight and inconstant. There is no good evidence to indicate that gonadal hormones are responsible for premenstrual edema. The same author stated that in most clinical conditions, the administration of androgenic or estrogenic hormones alone or in combination does not induce an alteration in the concentration of calcium in the plasma. From Turkey, Ersoy and Şentürk (1963) give their results of the research of blood serum sodium and potassium of 53 normal Angora goats, by the flame photometric method. Sodium variation was between 150.9 and 169.6 mEq. per liter, potassium between 4.74 and 5.77 mEq. per liter. The average amount of sodium was 160 mEq. per liter and 5.41 mEq. per liter for potassium. In the goats used, the differences in blood serum sodium and potassium between the males and the females or between those of two years age difference were not significant. Soliman _et al._ (1964) reported that thyroid activity varies during the estrous cycle, with maximal activity during estrus. This increased thyroid activity at estrus and in animals with ovarian cysts is probably due to an increase of estrogen in the blood. Nocenti and Cizek (1964) studied the influence of estrogens on electrolyte and water exchanges in the ovariectomized rat. They state that the estrogens did not alter glomerular filtration rates or serum electrolyte concentrations. The same year Schröter <u>et al.</u> (1964) reported their findings on the values of sodium, potassium, and serum calcium in 130 pigs. Here are the values of these three parameters in this investigation: sodium, 136.1 to 158.7 mEq. per liter, potassium 3.95 to 6.28 mEq. per liter, and calcium 8.1 to 13.8 mg. per cent. They attributed these variations to the influence of numerous factors such as: the individual, the age, and the daily changes in stress, weather, or nutrition.

Estrual Secretion Sodium, Potassium, and Calcium

For many years researchers have been interested in the cyclic changes occurring in the female during the estrous cycle. As early as 1855 Smith recognized the periodic nature of mucus secretions during the human menstrual cycle. In the animal kingdom Benjamin Zupp (1924) has been one of the leaders in this field by his study of the secretory phenomena of estrum in the ox and pig, and the cyclic anatomical changes at the cellular level. He stated that the amount and nature of the estrual flow varies considerably, being most abundant at the time of estrum when it is clear and has a watery appearance. After ovulation it decreases rather rapidly in amount and becomes more viscous. McNutt (1924) has contributed to a better understanding of the normal physiology of the estrous cycle in the cow by his study of the corpus luteum in this species. Cole (1930) studied the cyclic changes of the genital tract mucosa of the cow. Herrick in 1950, in addition to the cytological changes in the cervical mucosa of the cow throughout the estrous cycle, gives a range of time in duration of each period of the estrous cycle. The diestrus period ranges from twelve to seventeen days, the proestrus from eighteen to thirty-six hours, the estrus from six to thirty-six hours and the metestrus from three to seven days. Like Asdell (1955) he feels that the mucus is constantly secreted by the columnar epithelium of the cervix with maximum secretion at the time of estrus. Pommerenke and Breckenridge (1952) give a good summary of what was known at that time regarding the biochemical studies of the human female genitalia. Von Kaulla et al. (1957) studied the secretory function of the human cervix with I^{131} which was administered intravenously to 34 women. Radioactivity was detected within two minutes in the cervical mucus. They concluded that the cervix has an ability to concentrate this isotope and that that ability was impaired in some patients with infertility problems. In addition they also noticed that the cervical glands appeared to concentrate this isotope in a manner grossly similar to that of the salivary glands. Parisier et al. (1964) stated that the I^{131}-uptake by the human cervical mucus is significantly higher during the second phase of the menstrual cycle than during the first.

The reaction of the vagina and its secretions has long been under discussion as a possible cause of sterility. But McNutt et al. (1939) investigated the hydrogen ion concentration of vaginal secretions of cows and their data tend to indicate that the pH of the cow's vagina is not of much significance for diagnostic purposes in breeding diseases. Roark and Herman (1950) made studies of the hydrogen ion concentration of the exocervical and vaginal mucosa of bovine genitalia, and their results indicate a tendency for the pH values to be grouped near neutrality. They stated that the vaginal pH of difficult breeding cows tended to be more acid than that of normal and pregnant cows. They also found that the pH of the bovine reproductive tract and its secretions decline the day before, the day of, or the day after estrus. According to them, the bovine cervix is more acid than the vagina. On the other hand, it is known that the cervix of the human female is more alkaline than the vagina, and it has been suggested that this pH differential aids spermatozoa in their passage through the female genitalia. It seems to be the opposite in the bovine species, since the cervix in this latter species is more acid than the vagina. The same authors related that studies in human nutrition have shown that the pH of some body fluids can be altered by the diet. Can bovine mucus be made acid by feeding a diet that has an acid residue? Schockaert and

Delrue (1938) studied the chemical composition of the vaginal mucus and uterine secretions, emphasizing that in the human female sterility is related to the higher acidity of the exo-cervical mucus. Rakoff et al. (1944) indicated that the pH of the vaginal mucosa of woman gradually falls to reach its lowest point at the midcycle and then gradually rises to its highest value at the premenstrual stage. Breckenridge et al. (1950) are in agreement and postulated that the cervical mucus in the woman is alkaline in all phases of the intermenstrual cycle.
A simple and inexpensive instrument, the oestroscope, has been designed for the measurement of the flow elasticity of bovine cervical mucus by Scott-Blair et al. (1941). They found that when the flow elasticity values were five mm. or more, the animal was in estrus or within a few hours of its onset or end. They also noted that the flow elasticity values began to rise about twenty-four hours preceding estrus and did not return to normal until about sixty hours after estrus.
Papanicolaou (1945) reports the crystallization phenomenon of dried cervical mucus from the human female as being a characteristic of the time of ovulation. He was the first to observe this phenomenon in the human species and thought that it was dependent upon salt concentration in the mucus and to the mucin content. One year later Viergiver and Pommerenke (1946) revealed that in the woman the "ovulatory process" is characterized by a period of increase in the secretion, decrease in

the viscosity of the cervical mucus with a shift in the basal temperature, and the penetrability being maximum when the viscosity is lowest. Pommerenke (1946) concluded his study by stating that the cervical mucus of woman has a water content of 92 to 94% during the preovulatory and postovulatory periods, and 97 to 98% during the ovulatory period. De Vuyst et al. (1960) placed emphasis on the study of the fern pattern during the follicular phase of the cycle in the bovine species. They found that this characteristic of the mucus was due to three factors; mucoprotein, sodium-potassium chloride, and an increase in the quantity of water in the mucus. They also stated that the water content of the cervical mucus varies from 97.71% on the fifth day to 98.83% on the day of estrus. Roark and Herman (1950) found by drying 413 specimens of bovine genital mucus that the water content varied from 95.07% to 99.12% during the first day after heat began. The cyclic change in the physical quality and quantity of the secretions from the genital tract has been reported in various species. In the bovine female these changes in the genital secretions have been thoroughly studied. Pommerenke (1946) made a good review of what is known in the human, and Roark and Herman (1950) reviewed the result of the first half of the 20th century research in this field in the bovine species. Bergman and Lund (1951) stated that the human cervical mucus is isotonic at the ovulatory phase. Glover and Scott-Blair (1953) worked on the flow properties of cervical secretions in the cow and concluded that these properties are governed by the ovarian hormones, and that the changes represent the resultant effect of the level of estrogen and progesterone in the body. Cohen _et al._ (1952) talk about "spinnbarkeit" as a characteristic of the cervical mucus, and concluded that artificial insemination is more successfully done when genital secretions are profuse, thin, acellular and at maximum "spinnbarkeit". Also in the human, Campos da Paz (1953) uses the crystallization test as a guide to the treatment of cervical hostility, and he states that when the crystallization phenomenon is absent, the mucus as a rule is hostile to spermatozoa. In the bovine species Bone (1954) contends that the crystallization patterns in vaginal and cervical mucus smears are related to bovine ovarian activity, and states that this phenomenon is present from three days before estrum to seven to nine days after estrum. The same year Igarashi (1954) studied the same phenomenon in the human female and reported that of the elements, sodium, potassium, calcium, magnesium, and copper, sodium showed the most marked cyclic changes in the cervical mucus. In this report, the amount of sodium in the dry matter paralleled the degree of arborization of the dried cervical mucus. Furthermore, he concluded that the increased sodium in the mucus was a result of an active secretion of the cervical cells and not that of transudation from the blood because it has been verified that the sample correlation coefficient is 0.18 and that the correlation in population can be regarded as zero ($\alpha = .01$).

All the physical changes that bovine mucus undergoes is of great interest. These changes could be perhaps related to conditions allowing bacterial invasion of the uterus through the cervix. Also the physiology of the ascent of the sperm as well as its capacitation are probably related to these physical changes. Bergman (1953) stated that the cervical mucus characteristics before and after the watery phase are unfavorable to, and possibly completely inhibit, sperm migration through the cervix into the uterine cavity. VanDemark and Hays (1954) found that it takes two to four minutes after artificial insemination into the cervix or after naturally mating for the spermatozoa to be transported to the oviducts. Sujan et al. (1963) present proof that the quality of the cervical mucus is of prime importance to sperm migration. Perloff and Steinberger (1963) described the in vitro penetration of ovulatory cervical mucus by spermatozoa and seminal fluids. They related the phalanx formation to the penetration process. Moghissi et al. (1964) described also the mechanism of sperm migration but they stated that phalanx formation is found to be a nonspecific physical phenomenon.

In 1958 VanDemark said that information concerning the environment afforded the spermatozoa by the human female genitalia is meager. More complete data on the composition and changes that occur in the luminal fluids should lead to a clearer understanding of the factors that affect sperm survival in the female reproductive organs. Yet they had in the human at that time much more information than we have today concerning the bovine species. In fact, Pederson and Pommerenke (1950) had identified seventeen amino-acids in the human cervical mucus; Breckenridge and Pommerenke (1951b) one year later, analyzed the lipid constituents of cervical mucus in the human, and the same authors (1951a) the same year analyzed the carbohydrates in human cervical mucus. Also the same year Shettles (1951) studied the polysaccharide composition of human cervical mucus.

In 1957 Olds and VanDemark concluded that more than 80% of the osmotic pressure of the luminal fluid in 58 bovine female genitalia appeared to be attributable to sodium and potassium compounds. In general the sodium values were lower than for normal blood plasma, while potassium values were considerably higher. They also stated that sodium levels were much higher in uterine and follicular fluids during the luteal stages of the cycle while potassium concentrations increased in vaginal mucus at that time. Calcium values were somewhat higher in vaginal mucus and uterine fluids near estrus. One year later Kurzrok and Birnberg (1958) showed that the human cervical mucus increases in amount and becomes thinnest at midcycle; and explained that this physical change represents the hydrolytic cleavage. The end products of hydrolysis are glucosamine and glucuronic acid. Glucosamine appears at mid-cycle only and corresponds to the time of ovulation. Odeblad et al. (1958) determined the sodium and potassium in human cervical mucus with the radioactivation technique and found by means of a Geiger-Mueller counter the Na^{34} and P^{32} in the mucus. Their results support the view that the NaCl concentration remains at about a constant level throughout the menstrual cycle. Moghissi et al. (1960) stated that albumin and gamma-globulin are normally present in cervical secretions of the woman. Heap (1962) studied some constituents of uterine washings. A technique is described for the recovery of uterine washings from four species, the rat, rabbit, sheep, and cow. In the latter species, sodium was found to be 86 mg. at estrus and 249 mg. during the luteal phase per uterine horn; potassium has been measured as being 29 mg. at estrus and 110 mg. during the luteal phase per uterine horn. Only traces of calcium are mentioned to exist in the uterine washings. In 1963 Gáspár and Cseh from Budapest found an unknown compound related to, or even identical with, hippuric acid which is found in exceedingly large quantities in herbivora as a final product of detoxification of benzoic acid and its derivatives. Estrous secreta from 84 living dairy cows and 60 slaughtered cows were used. Material from living cows was collected directly from the cervix by aspiration. With greater quantities of this compound in the secreta of the genital epithelium, the less the chances of successful insemination. This hippuric acid-like compound is the result of some yet unknown effect or condition. Gamčik and Hájovský (1964) studied the chloride levels in the cervical mucus of the cow in relation to the arborization phenomenon. They concluded that the phenomenon of the cervical mucus depended on the concentration of proteins in the mucus and on the effect of ovarian hormones. The same year Herzberg et al. (1964) from Israel studied the cyclic variations of sodium chloride content in the mucus of the cervix uteri in the human female. They concluded that the sodium chloride content of fresh mucus from the human cervix uteri does not show any cyclic variation and is approximately isotonic. On the other hand the percentage of sodium chloride in the dry mucus has a cyclic variation. The authors feel that the remarkable increase in the cervical water excreted during the normal midcycle is clearly due to hormonal regulation of the female cycle. A conventional explanation of the increased discharge attributes the phenomenon to the action of estrogens. During periods characterized by increased estrogen content, such as pregnancy, there is no significant rise in the sodium chloride percentage of the dry mucus. Furthermore, in women with hormonal disturbances (non-fertile) injected with large doses of estrogens, there was no significant rise in sodium chloride percentage of the dried mucus. They concluded that the maximum observed is related directly to the ovulation process itself. They advance a tentative hypothesis that the luteinizing hormone activity would be responsible for the phenomena described above. They also stated that sodium chloride is the major salt component and has a determining effect on the ionic strength of the mucus. It might therefore be assumed that the rheological periodicity is due to a cyclic variation in the sodium chloride concentration. Moreover since sodium chloride determines the osmotic pressure of the mucus, any appreciable variation in its concentration would cause osmotic irritation of the cervix. Finally they stated that there is little chance of interpreting the variations in the rheological properties through periodic variations in sodium chloride concentration.

MATERIALS AND METHODS

General Comments

The cows selected for this study had all previously demonstrated their ability to reproduce. This will be evident on reading the breeding history of each animal.

All cows were housed in the Department of Obstetrics stable, cared for by the same caretakers and fed the following 12% grain ration:

Ground corn	800 lbs.
Ground oats	800 lbs.
Soybean oil meal	200 lgs.
Bran	200 lbs.
Molasses	50 lbs.
Salt	10 lbs.
Dicalcium phosphate	20 lbs.

The grain was fed at the rate of approximately 3/4 lbs. per 100 lbs. of live weight per day.

U. S. Grade #1 (occasionally U. S. Grade #2) alfalfa hay was fed <u>ad libitum</u>. Water was available at all times. As long as the animals were giving milk, they were milked twice daily.

A complete history has been obtained for each subject, and a thorough physical examination was done, so it was determined before starting the experimentation that each animal was clinically normal. The estrous cycle has been timed by rectal examination, vaginal smear examination, exogenous and endogenous symptoms, and by teasing with the male animal if needed. The cows were stabled.

Blood samples as well as estrual secretion samples were taken each day of the cycle, and at the same time of the day. All the glassware used was cleaned in an acid preparation[1] and rinsed four times in deionized water. The pipettes were dried in an oven, and the other glassware was dried at room temperature.

Experimental Animals Used for This Investigation

1. Suneslope E. Elipse, Registration No. 1871809. Herd No. 194. This Guernsey was born October 28, 1957, she first calved January 20, 1960. Following this first parturition, she had some difficulty in conceiving. She finally had her second calf July, 1961. She then calved during the month of August in 1962 and again in August, 1963. After one breeding on October 11, 1963 she conceived and delivered on July 28, 1964. She was in heat in September and has been cycling every 20-21 days since. We obtained this cow November 13, 1964. She was used first to develop skill in the detection of the endogenous signs of estrus, and to learn and adapt methods for serum and exocervical mucus collection and analysis. The daily collections were begun on January 2, 1965. We detected a heat

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[1]Concentrated sulfuric acid (35 pints) and saturated aqueous potassium dichromate (600 ml.).

period January 9. She was removed from the experiment on January 20, because she developed a severe staphylococcal mastitis. We followed her 17 days including one heat period.

2. Suneslope E. E. Beauty, Registration No. 2223527. Herd No. 262. A Guernsey cow born August 17, 1961, and calved for the first time August 16, 1963. She was bred artificially October 15 the same year and had her calf from this insemination. We received her November 13, 1964 and the daily samplings were begun by January 2, 1965. She was in estrus January 10, and ovulated on the 11th and 14th. She was back in heat January 29, 1965. We collected samples from her until the 12th of February. Forty-one consecutive samples were taken from that cow. After completing the investigation we heard that this cow showed signs of heat the 16th of February, 1965, was bred and conceived. Pregnancy was confirmed by manual pregnancy examination on April 1, 1965.

3. Ames A. Mamie, Registration No. 5667368. Herd No. 5275 L. This Holstein was born July 24, 1962. She was bred one time the second of January 1964, conceived and calved October 10, 1964. She was bred artificially January 11, 1965 but did not conceive. We purchased her January 20, she had a silent heat January 23, and she was in heat the 31st of the same month, February 20, and March 15, 1965. She was used for this study until March 22, 1965. She then had given 62 daily samples of both cervical mucus and blood.

4. Ames A. Latty, Registration No. 5817101, Herd No. 5330 L. This first calf, open Holstein was born January 5, 1963; after one breeding on April 16, 1964, she conceived and calved January 19, 1965. When we received her February 15, she had endogenous signs of heat and she ovulated the 16th. She was in heat again March 9, March 31, April 22, May 14, and June 5. One hundred and seventeen samples were taken from this cow.

Technic of Collecting and Handling Blood Serum

The blood was drawn from the jugular vein by means of a bleeding needle into a test tube at nine o'clock in the morning. The blood was then centrifuged in an international clinical centrifuge[1] for thirty minutes at 3,000 rpm. The supernatant liquid was decanted and allowed to stand for thirty minutes. Then an applicator stick was used to rim and remove the fibrin. Serum was then withdrawn from the tubes, placed in sealed vials and frozen. The samples were stored in a frozen state at 9 or 10 F. until analyzed. All the parameters were run in duplicate, and one estrous cycle was analyzed at one time. The samples to be analyzed were thawed and brought to room temperature before being used. According to Miller (1961)

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[1]Model Cl No. 18583 H. International Equipment Co., Boston, Massachusetts.

there is no significant change in one freezing and thawing of the serum. The serum sodium and potassium determinations were made by means of the Flame Photometer,[1] and serum calcium determinations by means of a micro-quantitative method described by Harper (1959).

The development of the flame photometry marked great advances in the measurement of sodium and potassium. Contrary to conventional chemical methods, it is time saving and requires relatively small volumes. Only 1.5 ml. is required for an accurate determination of these two elements. The solution to be analyzed is discharged by suction through an atomizer and ingested in the flame. Light intensity ratios utilizing an internal standard are measured by a sensitive galvanometer after the filtered light, using an appropriate light absorption filter for each element, impinges on a barrier layer photocell.

Procedure for Determining Serum Sodium

A 1:500 dilution of the serum was prepared by placing 0.1 ml. of serum in a 50 ml. volumetric flask and diluted to volume with deionized water. A calibration curve for sodium was prepared using standard solutions, containing sodium chloride[2] in the following concentrations: 0.2, 0.4, 0.5, 0.6, 0.8, and

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[1]"EEl" Flame Photometer mark II. Gelman Instrument Co.

[2]Sodium Chloride crystals; "Baker Analyzed" reagent, J. T. Baker Chemical Co., Phillipsburg, New Jersey.

1.0 mg. of sodium per 100 ml. of solution. The serum dilutions were then read, and the values determined from the calibration curve. To obtain the number of Milliequivalents of Sodium per liter of serum, the following transformation was necessary:

$$\frac{\text{unknown from the standard curve x dilution factor x 10}}{23}$$

The dilution factor is 500.

Procedure for Determining Serum Potassium

A dilution of 1:50 was prepared by placing 0.5 ml. of serum in a 25 volumetric flask, and diluting to volume with deionized water. A calibration curve for Potassium was prepared using standard solutions containing potassium iodide[1] in the following concentrations: 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0 mg. of potassium per 100 ml. of solution. The serum dilutions were then read, and the values determined from the calibration curve. To obtain the number of Milliequivalents of Potassium per liter of serum, the following transformation was necessary:

$$\frac{\text{unknown from the standard curve x dilution factor x 10}}{39.1}$$

The dilution factor is 50.

An adaptation suggested by Newell and Duke (1962) has been used on the flame photometer. A six inch long capillary nylon

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[1]Potassium Iodide crystals analytical reagent; Mallinckrodt Chemical Works, St. Louis. New York. Montreal.

tube has been fixed to the capillary tube of the atomizer. The sample was taken directly from the volumetric flask, avoiding in this way one manipulation of the unknown solutions.

Procedure for Determining Serum Calcium

The serum calcium determinations were made by means of the method described by Harper (1959). The murexide-ammonium purpurate solution changes from purple to pink on addition of calcium, and the determination of the calcium murexide complex is made by means of a spectrophotometer. The method gives accuracy of ± 1%. It has been found that the Murexide techniques give lower results than oxalate precipitation methods, and the range of this latter method is the accepted one. In order to avoid confusion, the results obtained with Murexide techniques must be corrected in order to bring them into agreement with oxalate precipitation methods. The correction factor is 1.046.

Procedure:

1. Label three test tubes for blank, standard, and test.
2. Add 4.9 ml. of deionized water to the unknown and standard test tubes, and 5 ml. to the blank.
3. To the test tube add 0.1 ml. of serum, to the standard add 0.1 ml. of working standard calcium solution which contains 9 mg.% of calcium.

4. To all tubes add one drop of Piperidine and 0.2 ml. of Murexide.
5. Mix and read the optical density (OD) at 500μ wave length on a spectrophotometer which is set with the blank before.

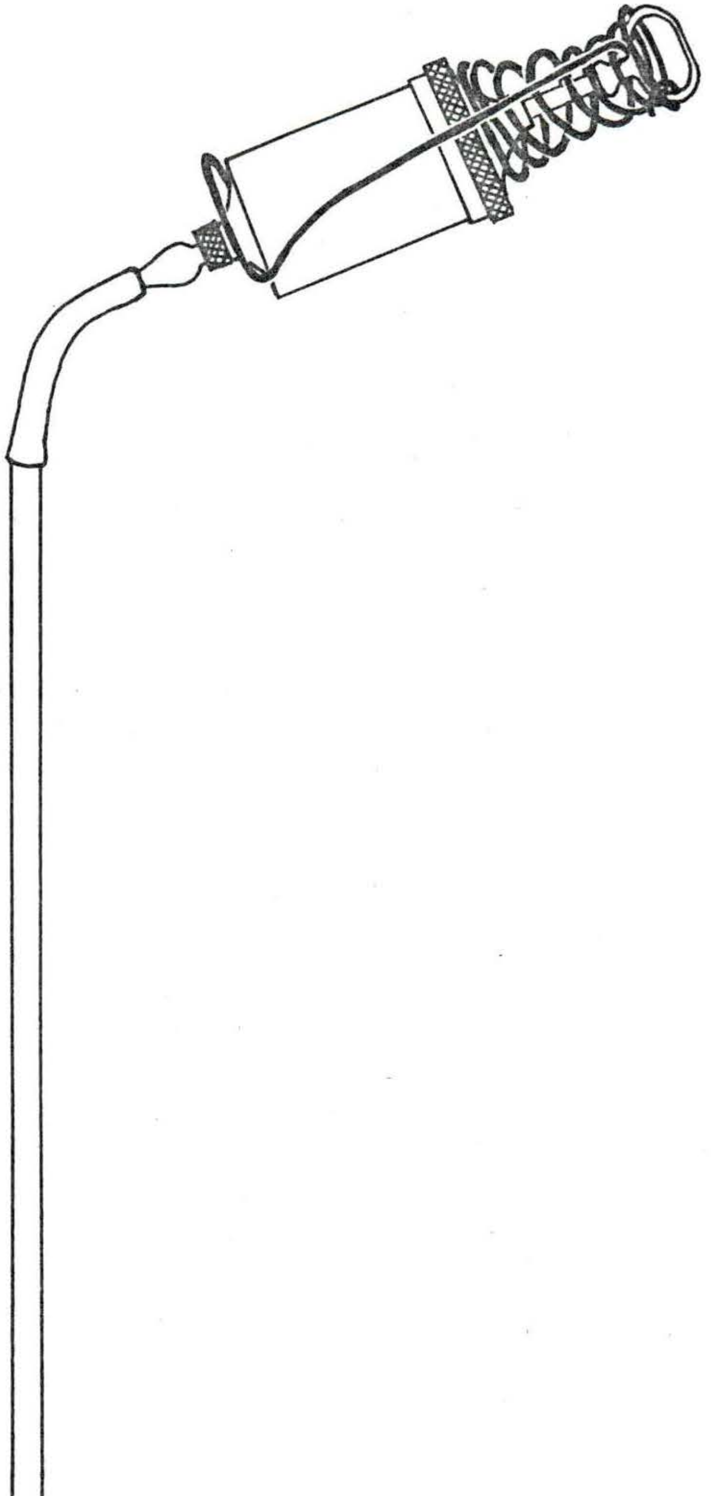
Calculations:

$$\frac{\text{OD test} \times 9 \text{ mg.\%} \times 1.046}{\text{OD standard}} = \frac{\text{Mg. of Calcium per}}{\text{100 ml. of serum}}$$

Technic of Collecting and Handling Genital Secretions

The cervical mucus was collected daily by means of a metal dose syringe (Figure 1) to which was attached a glass tube 16 inches long by 7 mm. inside diameter. One hand was introduced into the rectum and the cervix immobilized. The vulva and perineal region were washed with soap and water, and dried with a paper towel. The lips of the vulva were opened and the vestibulum exposed to permit the passage of the glass tube up to the external os of the cervix, avoiding contamination with the lips of the vulva. The mucus was then withdrawn by suction created by the release of the plunger which was forced outward by a spring located between the top of the barrel and the thumb ring of the syringe. The negative pressure of this apparatus is about 25 mm. of mercury. After collection the mucus was expelled into a test tube by means of a glass rod 7 mm. outside diameter, introduced into the glass tube. The mucus was then frozen in sealed tubes at 9 or 10°F. until the

Figure 1. Apparatus for mucus collection.



end of the estrous cycle. At that time, the mucus was thawed and brought to room temperature. Each sampling of mucus was weighed on a Mettler B5 balance,[1] and diluted with deionized water to obtain a concentration between 30 and 31 mg. of mucus per ml. of solution. The exact concentration of each sample was then recorded. Both mucus and water were blended in a Waring Blendor[2] for one minute.

Procedure for Determining Mucus Sodium

It was found necessary to use 3 ml. of the blended solution diluted to volume in a 50 ml. volumetric flask for sodium determinations. The same standard solutions of sodium were used for the calibration curve determination in this case, as were used for serum sodium determinations. Knowing the concentration of mucus per ml. of solution and the dilution factor which is 16 2/3 (3 ml.:50), it was then easy to convert the reading from the calibration curve to mg. of sodium per gram of mucus. Here are the calculations to obtain : mg. of Na per gram of mucus:

$$\frac{\text{unknown from the graph x dilution factor x 1000 mg.}}{\text{concentration of mucus per 100 ml. after blending}}$$

[1]No. B 1250-1, Balance, Analytical, Macro (Mettler B5), capacity 200 gm., accuracy ± 0.05 mg. Scientific Products, Evanston, Illinois.

[2]42110 A Plain Mills Emulsifying, Shearing, High speed, 12 oz., semi-micro, single speed, Monel container, 115 volts Waring Blendor. Type 700. Matheson Scientific, Chicago, Illinois.

Procedure for Determining Mucus Potassium

It was found necessary to use 3 ml. of the blended solution diluted to volume in a 25 ml. volumetric flask for potassium determinations. The same standard solutions of potassium were used for the calibration curve determination in this case, as were used for serum potassium determinations. Knowing the concentration of mucus per ml. of solution and the dilution factor which is 8 1/3 (3 ml:25), it was possible to convert the reading from the calibration curve to mg. of potassium per gram of mucus. The following mathematical formula was used to determine the number of mg. of potassium per gram of mucus:

$$\frac{\text{unknown from the graph} \times \text{dilution factor} \times 1000\ \text{mg.}}{\text{concentration of mucus per 100 ml. after blending}}$$

Procedure for Determining Mucus Calcium

The calcium content of the mucus was determined by means of Harper's (1959) technique. The only adaptation we found necessary was to use 1 ml. of the blended solution instead of 0.1 ml. as required for blood serum determinations. Consequently, instead of using 4.9 ml. of deionized water it was necessary to use 4 ml. The following formula was used in the calculations to obtain the number of mg. of calcium per gram of mucus:

$$\frac{\text{OD test} \times 9\ \text{mg.\%} \times 1.046 \times 1000\ \text{mg.}}{\text{OD standard} \times \text{mg. of mucus per 100 ml. of blended solution}}$$

Statistical Analysis

Concerning the statistical analysis, the orthogonal polynomial methods described by Anderson and Houseman (1942) were used. Within each animal the estrus cycles were of the same length of time. Cow 262 had 19-day cycles, cow 5275 L had 20-day cycles, and cow 5330L had 22-day cycles. All the statistical analyses have been computed on two cycles for the first cow, two cycles for the second, and five cycles for the third one. In other words the observations were equally spaced and the same number of observations was seen at each level. The first day computed was the estrus period, and the last day was the proestrus of the next estrus period.

Original Model:

$$(Y_i-\bar{Y}) = b_1x_{1i} + b_2x_{2i} + \cdots + b_4x_{4i} + b_5x_{5i} + b_6x_{5i}^2 + b_7x_{5i}^3 + b_8x_{5i}^4 + b_9(x_{1i}x_{5i}) + \cdots + b_{25}(x_{4i}x_{5i}^4) + \epsilon i$$

Now the transformed model with orthogonal powers of x_{5i} due to Yates ξ's method (Anderson and Houseman, 1942).

$$(Y_i-\bar{y}) = \beta_1x_{1i} + \cdots + \beta_4x_{4i} + \beta_5(\xi'_{1i}) + \cdots + \beta_8(\xi^1_{4i}) + \beta_9(x_{1i}\xi^1_{1i}) + \cdots + \beta_{25}(x_{4i}\xi^1_{4i}) + \epsilon i$$

Where variables x_1 through x_4 are classification variables for cycles; ξ_1 through ξ_4 are coded powers of x_{5i} from above, and subsequent variables are the interactions of cycles with the coded powers of x_5, i.e. the ξ's.

FINDINGS

Preliminary Observations

Our first concern has been to develop a proper technique for mucus collection. Then the right dilution, that could be used for flame photometry determinations of sodium and potassium and for chemical determinations of calcium, has been found.

Cow 194 has been used mostly for these purposes. This animal developed a vaginitis twice during the preliminary work, and after 17 days she had to be discarded because of a severe staphylococcal mastitis.

Our findings, on the exocervical mucus of that cow, are not from a normal animal, and they will not be used in this study.

Table 1. Arithmetic average of seventeen samples for each parameter, cow 194

	Serum, mEq./liter		mg%	Mucus, mg. per gram		
	Na	K	Ca	Na	K	Ca
	140.30	4.5	8.4	3.03	0.65	2.65
Range	129.4-156.5	3.5-5.5	6.9-11.7	2.12-4.46	.44-.86	1.62-4.72

The serum values are quite in agreement with the normals, even though they are somewhat lower.

Table 2. Serum value during the four stages of the estrous cycle, with mention of the values during the ovulation day, cow 194

Periods	Serum mEq./liter		mg%
	Na	K	Ca
Proestrus	150.9	5.17	8.2
Estrus	135.9	5.23	11.7
Ovulation	143.3	4.28	7.6
Postestrus	142.2	3.95	7.8
Diestrus[a]	139.1	4.59	8.3

[a]Only five days were available to form the diestrus.

Not enough observations were obtained from cow 194 to permit a good analysis of the variations during the estrous cycle.

Results

Experimental cow 262

First an arithmetic average of all the determinations of each parameter is given. Then follows a table of the average of the four stages of the estrous cycle and the value during the ovulation day. More emphasis is given to the ovulation day because it is a very important time, more so in the bovine female because it occurs during the postestrus.

The average of each parameter, using the period table, will not be the same as for the overall average of that animal because not all the observations were necessarily used to form a complete estrous cycle. In other words for this 19-day cycle cow, we had 41 samples total to determine the average, and only 38 days were used to form two complete estrous cycles. The same principles have been applied for each experimental animal depending on the number and length of the estrous cycles.

Table 3. Arithmetic average of cow 262, for 41 determinations

	Serum			Mucus		
	mEq./liter		mg%	mg. per gram of mucus		
	Na	K	Ca	Na	K	Ca
	138.9	5.27	7.63[a]	2.732	1.038	0.421[a]
Range	120.7- 159.6	3.7- 7.3	6.21- 8.78	2.034- 4.0	.419- 1.5	.102- .626
Standard error	8.23	0.89	0.67	0.387	0.1455	0.0659

[a]Note the correlation of the low serum calcium and low mucus calcium value of this cow. The average serum calcium value of 237 determinations is 8.93 mg.%; and the average of mucus calcium on 220 determinations is 1.213 mg. per gram of mucus.

Table 4. Arithmetic average of the four periods of two estrous cycles and ovulation, cow 262

	Serum mEq./liter		mg%	Mucus mg. per gram of mucus		
	Na	K	Ca	Na	K	Ca
Proestrus:	128.1	4.7	7.0	3.233	1.168	0.501
Estrus:	133.6	5.4	7.3	3.123	0.548	0.145
Ovulation:	138.8	5.2	8.1	3.140	0.516	0.107
Postestrus:	137.95	5.1	7.6	3.051	0.774	0.204
Diestrus:	139.4	5.4	7.8	2.464	1.135	0.496

On an individual cycle basis the serum sodium increases at estrum one time, and undergoes no change the other time. Serum potassium increases in one cycle at estrum and decreases in the other one. During ovulation serum potassium decreases in one estrous cycle and increases during the same period in the other one. Serum calcium had a tendency to increase on the day of ovulation for both estrous cycles.

The exocervical mucus sodium increases toward the estrus period being maximum the day following the ovulation on both estrous cycles. The mucus potassium undergoes a sharp drop the day of estrus with a minimum value during the ovulation day; and mucus calcium undergoes a drastic drop the day of estrus decreasing for four and five consecutive days later on.

The arithmetic average of the periods of the estrous cycle gives the trend of the values for the animal during that time, whereas the daily variations within each cycle is a better reflection of the fluctuations during the cycles.

Table 5. Analysis of variance of serum sodium, cow 262

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	138.3224	2.04
Days			
Linear	1	7.900	<1
Quadratic	1	312.5152	4.61[a]
Cubic	1	37.6191	<1
Quartic	1	110.3668	1.63
Cycles x days			
Linear	1	276.7608	4.08[b]
Quadratic	1	17.4816	<1
Cubic	1	80.7419	1.19
Quartic	1	33.9892	<1
Error	28	67.7655	
Total	37		

[a] P = 0.05.

[b] Close to (P = 0.05).

Table 6. Analysis of variance of serum potassium, cow 262

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	1.4411	1.80
Days			
Linear	1	0.6301	<1
Quadratic	1	0.4142	<1
Cubic	1	0.0877	<1
Quartic	1	0.0121	<1
Cycles x days			
Linear	1	0.0114	<1
Quadratic	1	0.3066	<1
Cubic	1	0.0031	<1
Quartic	1	1.0461	1.31
Error	28	0.7993	
Total	37		

Table 7. Analysis of variance of serum calcium, cow 262

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.8914	1.97
Days			
Linear	1	0.1538	<1
Quadratic	1	4.2803	9.46[a]
Cubic	1	2.4603	5.44[b]
Quartic	1	2.0437	4.52[b]
Cycles x days			
Linear	1	0.1802	<1
Quadratic	1	0.0282	<1
Cubic	1	0.0397	<1
Quartic	1	0.0355	<1
Error	28	0.4526	
Total	37		

[a](P = 0.01).

[b](P = 0.05).

Table 8. Analysis of variance of mucus sodium, cow 262

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.3703	2.47
Days			
Linear	1	1.3780	9.19[a]
Quadratic	1	2.0413	13.61[a]
Cubic	1	0.5156	3.44
Quartic	1	0.1295	<1
Cycles x days			
Linear	1	0.1461	<1
Quadratic	1	0.0465	<1
Cubic	1	0.4340	2.90
Quartic	1	0.0122	<1
Error	28	0.1500	
Total	37		

[a](P = 0.01).

Table 9. Analysis of variance of mucus potassium, cow 262

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.0791	2.73
Days			
Linear	1	0.4613	21.76[a]
Quadratic	1	0.6898	32.54[a]
Cubic	1	0.5647	26.64[a]
Quartic	1	0.0879	4.15
Cycles x days			
Linear	1	0.0013	<1
Quadratic	1	0.0077	<1
Cubic	1	0.0060	<1
Quartic	1	0.0107	<1
Error	28	0.0212	
Total	37		

[a](P = 0.01).

Table 10. Analysis of variance of mucus calcium, cow 262

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.0019	<1
Days			
Linear	1	0.37232	85.79[a]
Quadratic	1	0.25922	59.73[a]
Cubic	1	0.01966	4.53[b]
Quartic	1	0.07439	17.22[a]
Cycles x days			
Linear	1	0.00022	<1
Quadratic	1	0.00005	<1
Cubic	1	0.00730	1.68
Quartic	1	0.00000	<1
Error	28	0.00434	
Total	37		

[a](P = 0.01).

[b](P = 0.05).

The coefficient of partial correlation (r) is: +0.2573 for serum and mucus sodium, +0.034 for serum and mucus potassium, and +0.224 for serum and mucus calcium. The error degrees of freedom is 28 and either one of these correlations is significant. Since no significant difference was found between cycles and no significant interaction of cycles by days; the arithmetic average of those two cycles gives a good estimate of the average of each parameter during two estrous cycles (Table 4).

The quadratic fitting parabola was significant at .05 level for serum sodium daily variations. No significant fit was found for serum potassium and the quadratic model was highly significant for serum calcium. Concerning serum calcium, the cubic and quartic fitting models were also significant. Significant fitting parabolas were found for mucus sodium, potassium, and calcium daily variations. The linear and quadratic was significant for the three, sodium, potassium, and calcium. Moreover, the cubic fitting was significant for mucus potassium and the cubic and quartic models were also significant for mucus calcium.

Experimental cow 5275 L

Table 11. Arithmetic average of cow 5275 L, for 62 determinations

	Serum mEq./liter Na	Serum mEq./liter K	mg% Ca	Mucus mg. per gram of mucus Na	Mucus mg. per gram of mucus K	Mucus mg. per gram of mucus Ca
	139.5	5.10	10.3	2.94	0.6104	1.495
Range	110.0-163.0	3.5-6.8	8.4-11.3	2.52-3.93	0.29-0.963	.57-2.81
Standard error	8.44	0.614	0.52	0.198	0.088	0.432

Table 12. Arithmetic average of three proestrus, estrus and postestrus, and two diestrus periods; cow 5275 L

	Serum mEq./liter Na	Serum mEq./liter K	mg% Ca	Mucus mg. per gram of mucus Na	Mucus mg. per gram of mucus K	Mucus mg. per gram of mucus Ca
Proestrus:	136.1	5.1	10.4	3.01	0.553	2.31
Estrus:	127.4	4.4	10.6	3.06	0.468	1.14
Ovulation:	142.7	5.0	10.5	3.32	0.400	0.95
Postestrus:	144.7	4.9	10.5	2.94	0.485	0.93
Diestrus:	136.2	5.2	10.2	2.68	0.676	1.73

If we look at the individual cycle basis for the three proestrus, estrus, and postestrus periods, we notice that serum calcium increases the three times at estrus, drops two times during the ovulation day, and no change is noted during one ovulation. Serum sodium increases one time at estrus and drops two times; and during the ovulation day serum sodium increases on the three estrous cycles. Serum potassium decreases at estrum for the three estrous cycles, undergoes one ovulation without any change and shows an increase value two times during that same period.

This cow showed a silent heat six days after she was bought, she ovulated and the corpus luteum had a very short life and a smaller size than usual. It also regressed very rapidly. This silent heat happened during the diestrus, and corresponded with the endogenous symptoms of estrus. Drastic changes in the composition of the exocervical mucus were noted. During the day the first signs of heat were noted and the day of ovulation, mucus sodium increased to a value as high as 3.35 milligrams per gram of mucus; the average of that cow is 2.94 mg. A drop in mucus potassium was also noted at the same time. Mucus calcium showed an increased value the day before we detected the endogenous signs of heat, and underwent a gradual decrease for three consecutive days following the estrus. Mucus calcium reached then a value as low as 0.57 mg. per gram of mucus. Also during this irregular estrus the mucus was less cohesive, and easier to collect. Six days later the cow was back with the regular estrus period and she had then regular 20-day cycles.

The cyclic variations of mucus sodium of this cow have been constant, showing an increase during proestrus with a maximum on the day of ovulation, except during one cycle when the maximum was reached during the estrus period. Mucus potassium had a tendency to decrease at estrus, being minimum three times at ovulation or just before it. Mucus calcium starts to decrease at estrum for four or five days. One time the drop was found during the proestrus.

Table 13. Analysis of variance of serum sodium, cow 5275 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	328.329	4.61[a]
Days			
Linear	1	328.3133	4.61[a]
Quadratic	1	91.6212	1.286
Cubic	1	1334.9491	18.75[b]
Quartic	1	117.9633	1.657
Cycles x days			
Linear	1	712.4117	10.007[b]
Quadratic	1	324.4468	4.557[a]
Cubic	1	5.7175	<1

[a](P = 0.05).
[b](P = 0.01)

Table 13 (Continued)

Source of variation	Degrees of freedom	Mean square	F test
Cycles x days			
Quartic	1	3.7958	<1
Error	30	71.1921	
Total	39		

Table 14. Analysis of variance of serum potassium, cow 5275 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	1.521	4.03
Days			
Linear	1	1.563429	4.15[a]
Quadratic	1	1.355982	3.58
Cubic	1	0.413715	1.82
Quartic	1	0.061792	<1
Cycles x days			
Linear	1	0.145271	<1
Quadratic	1	0.762274	2.02
Cubic	1	0.005172	<1
Quartic	1	1.29237	3.43
Error	30	0.376766	
Total	39		

[a]Close to 0.05 level significance.

Table 15. Analysis of variance of serum calcium, cow 5275 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	6.16225	2.28
Days			
Linear	1	0.02937	<1
Quadratic	1	0.361737	1.33
Cubic	1	0.00276	<1
Quartic	1	0.004879	<1
Cycles x days			
Linear	1	0.64328	2.38
Quadratic	1	0.596323	2.20
Cubic	1	0.000292	<1
Quartic	1	0.842183	3.11
Error	30	0.270689	
Total	39		

Table 16. Analysis of variance of mucus sodium, cow 5275 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.0839973	2.14
Days			
Linear	1	0.3165172	8.07[a]
Quadratic	1	0.2254556	5.75[b]
Cubic	1	0.0377954	<1
Quartic	1	0.2528629	6.48[b]
Cycles x days			
Linear	1	0.0194837	<1
Quadratic	1	0.0305264	<1
Cubic	1	0.0267395	<1
Quartic	1	0.020763	<1
Error	30	0.0392128	
Total	39		

[a](P = 0.01).

[b](P = 0.05).

Table 17. Analysis of variance of mucus potassium, cow 5275 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.0885481	11.37[a]
Days			
Linear	1	0.05538939	7.11[b]
Quadratic	1	0.24702578	31.74[a]
Cubic	1	0.01381098	1.77
Quartic	1	0.01490512	1.91
Cycles x days			
Linear	1	0.00428045	<1
Quadratic	1	0.00436078	<1
Cubic	1	0.00409977	<1
Quartic	1	0.00015921	<1
Error	30	0.00778246	
Total	39		

[a](P = 0.01).

[b](P = 0.05).

Table 18. Analysis of variance of mucus calcium, cow 5275 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	1	0.25921	1.39
Days			
Linear	1	6.192152	33.14[a]
Quadratic	1	0.001577	<1
Cubic	1	1.214044	6.49[b]
Quartic	1	0.09687	<1
Cycles x days			
Linear	1	0.068861	<1
Quadratic	1	0.013436	<1
Cubic	1	0.000116	<1
Quartic	1	0.008412	<1
Error	30	0.186827	
Total	39		

[a](P = 0.01).

[b](P = 0.05).

The coefficient of partial correlation (r) is: +0.226 for serum and mucus sodium, +0.097 for serum and mucus potassium, and +0.337 for serum and mucus calcium. The error degrees of freedom is 30, and either one of these correlations is significant, but the last correlation is very close to the 0.05 level significance.

Significant difference of cycles by days have been found for serum sodium; thus it must be said that the arithmetic average concerning this parameter in the serum will not give the true picture of the variations.

The pooled average of the other determinations will give a good estimate of the variations during the estrous cycle.

The average of the two cycles was significantly different concerning serum sodium, and the linear and cubic fits (Table 13) were significant for the daily variations. It was not possible to find any significant parabola for serum potassium and serum calcium, but the fitting of polynomials of first degree was close to $P = 0.05$ significance (Table 14) for serum potassium. The linear, quadratic and quartic parabolas are significant for mucus sodium daily variations (Table 16), the linear and quadratic are significant for mucus potassium (Table 17) and the linear and cubic fits are significant for mucus calcium (Table 18).

Experimental cow 5330 L

Table 19. Arithmetic average of cow 5330 L, for 117 determinations

	Serum mEq./liter Na	Serum mEq./liter K	mg% Ca	Mucus mg. per gram of mucus Na	Mucus mg. per gram of mucus K	Mucus mg. per gram of mucus Ca
	141.38	4.88	8.85	2.648	0.728	1.723
Range	127.8-166.3	3.9-6.9	7.34-10.1	2.268-3.268	.291-1.07	.41-2.98
Standard error	6.56	0.51	0.51	0.1327	0.1058	0.315

Table 20. Arithmetic average of six proestrus, estrus, and postestrus, and five diestrus periods, cow 5330 L

	Serum mEq./liter Na	Serum mEq./liter K	mg% Ca	Mucus mg. per gram of mucus Na	Mucus mg. per gram of mucus K	Mucus mg. per gram of mucus Ca
Proestrus	142.9	4.84	9.23	2.692	0.728	2.61
Estrus	140.9	4.67	9.42	2.921	0.485	1.79
Ovulation	141.9	5.18	8.28	3.010	0.467	1.01
Postestrus	140.3	4.86	8.73	2.848	0.573	0.90
Diestrus	141.9	4.91	8.82	2.523	0.835	2.03

On the individual cycle basis, an increase of serum sodium was found during two estrus periods; a decrease during two estrus periods; and not much change was noticed in one estrus period. It was not possible to determine whether or not there was any change during one estrus period because the samplings were started on the day of estrum of the first cycle. Concerning serum potassium a decrease was noticed during four estrus periods; no change during one. Serum calcium showed three cycles with increased values at estrum; one without any change; and one with a decrease during that period. Always on the same parameter a sharp drop was noticed during four ovulation days and one was found without any change.

Exocervical mucus sodium concentration shows a constant increase starting usually during the proestrus and reaching its highest value on the ovulation day, or the day before; mucus sodium returns to normal at the end of the postestrus (Figure 5). Mucus potassium follows a constant decrease with the lowest values at, or just before, or after the ovulation day, returning to normal at the beginning of the diestrus (Figure 6). A sharp increase in mucus calcium is noticed during the proestrus on each cycle, and a constant decrease is noted, starting during the estrus for six or seven days until the end of the postestrus (Figure 7).

Table 21. Average of the daily variations during the estrous cycle of cow 5330 L; including six proestrus, estrus, and postestrus, and five diestrus periods (Figures 2 to 7)

Day of the cycle	Serum mEq./liter Na	Serum mEq./liter K	Serum mg% Ca	Mucus mg. per gram of mucus Na	Mucus mg. per gram of mucus K	Mucus mg. per gram of mucus Ca
1[a]	140.93	4.67	9.42	2.921	0.485	1.79
2[b]	141.38	4.97	8.65	3.030	0.465	1.30
3[c]	141.86	5.18	8.28	3.010	0.467	1.01
4	140.10	4.75	8.51	2.926	0.520	0.89
5	140.88	4.68	8.89	2.819	0.553	0.79
6	142.14	4.74	8.86	2.680	0.688	0.74
7	135.46	4.86	9.19	2.622	0.745	0.68
8[d]	142.78	4.82	8.78	2.556	0.805	1.45
9	139.08	5.30	8.70	2.466	0.808	2.40
10	143.10	5.28	8.24	2.523	0.770	2.89
11	150.82	4.98	8.92	2.457	0.878	2.66
12	139.68	5.04	8.50	2.581	0.913	2.54
13	141.22	4.76	8.51	2.554	0.927	2.19
14	147.56	4.76	8.88	2.538	0.810	2.25
15	141.60	4.66	8.87	2.564	0.790	1.92
16	143.22	5.26	8.18	2.544	0.824	1.76
17	139.40	4.64	8.65	2.305	0.897	1.73
18	137.82	5.16	8.91	2.532	0.840	1.58
19	142.22	4.66	9.29	2.549	0.842	1.53
20	137.56	4.76	9.63	2.568	0.777	1.69
21	140.02	4.72	9.36	2.586	0.810	1.86
22[e]	142.86	4.84	9.23	2.692	0.728	2.61

[a]Day 1 = estrus.

[b]Days 2 to 7 = postestrus.

[c]Day 3 = ovulation.

[d]Days 8 to 21 = diestrus.

[e]Day 22 = proestrus.

Table 22. Analysis of variance of serum sodium, cow 5330 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	4	34.7226	<1
Days			
Linear	1	1.1147	<1
Quadratic	1	23.57	<1
Cubic	1	4.5882	<1
Quartic	1	79.9907	1.86
Cycles x days			
Linear	4	46.019	1.07
Quadratic	4	24.6981	<1
Cubic	4	12.828	<1
Quartic	4	80.8798	1.881
Error	85	43.0094	
Total	109		

Table 23. Analysis of variance of serum potassium, cow 5330 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	4	4.144682	15.92[a]
Days			
Linear	1	0.243732	<1
Quadratic	1	0.157119	<1
Cubic	1	0.001321	<1
Quartic	1	0.120222	<1
Cycles x days			
Linear	4	0.38684	1.48
Quadratic	4	0.235082	<1
Cubic	4	0.221335	<1
Quartic	4	0.198987	<1
Error	85	0.260285	
Total	109		

[a](P = 0.01).

Table 24. Analysis of variance of serum calcium, cow 5330 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	4	0.427169	1.64
Days			
Linear	1	1.291048	4.95[a]
Quadratic	1	5.3325	20.47[b]
Cubic	1	0.088108	<1
Quartic	1	0.012221	<1
Cycles x days			
Linear	4	0.177653	<1
Quadratic	4	0.343869	1.32
Cubic	4	0.20167	<1
Quartic	4	0.084498	<1
Error	85	0.260546	
Total	109		

[a](P = 0.05).

[b](P = 0.01).

Table 25. Analysis of variance of mucus sodium, cow 5330 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	4	0.0659994	3.75[a]
Days			
Linear	1	1.3224349	75.15[a]
Quadratic	1	1.1077939	62.93[a]
Cubic	1	0.0033954	<1
Quartic	1	0.090021	5.11[b]
Cycles x days			
Linear	4	0.0692438	3.91[a]
Quadratic	4	0.0221349	1.26
Cubic	4	0.0106643	<1
Quartic	4	0.0286813	1.63
Error	85	0.0175962	
Total	109		

[a](P = 0.01).

[b](P = 0.05).

Table 26. Analysis of variance of mucus potassium, cow 5330 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	4	0.0051893	<1
Days			
Linear	1	0.9874915	88.18[a]
Quadratic	1	0.874723	78.11[a]
Cubic	1	0.0012512	<1
Quartic	1	0.0231567	2.06
Cycles x days			
Linear	4	0.1123377	10.03[a]
Quadratic	4	0.0080936	<1
Cubic	4	0.0420483	3.75[a]
Quartic	4	0.0343802	3.07[b]
Error	85	0.0111983	
Total	109		

[a](P = 0.01).

[b](P = 0.05).

Table 27. Analysis of variance of mucus calcium, cow 5330 L

Source of variation	Degrees of freedom	Mean square	F test
Cycles	4	0.031687	<1
Days			
Linear	1	9.307052	93.65[a]
Quadratic	1	3.107417	31.26[a]
Cubic	1	0.476629	4.79[b]
Quartic	1	25.509059	259.71[a]
Cycles x days			
Linear	4	0.055306	<1
Quadratic	4	0.025067	<1
Cubic	4	0.022818	<1
Quartic	4	0.006313	<1
Error	85	0.099375	
Total	109		

[a](P = 0.01).

[b](P = 0.05).

The coefficient of partial correlation is: +0.017 for serum and mucus sodium; +0.234 for serum and mucus potassium; and +0.235 for serum and mucus calcium. The error degrees of freedom is 85, and we found that serum and mucus potassium are correlated significantly (P = 0.05). Serum and mucus calcium are also correlated significantly (P = 0.05). That is to say that for a given cycle, there is a significant relationship between the concentrations of these two elements in the serum and in the mucus. It has not been possible to find any significant parabola for serum sodium during any cycle, nor has it been possible to find any for serum potassium. The statistical analysis has shown significant differences between the means of the cycles for serum potassium (Table 23). The linear and quadratic parabolas were significant for serum calcium daily variations (Table 24). Polynomials of first, second and fourth degree were significant for mucus sodium daily variations, with a higher significance for the straight line. There was a significant difference between the linear and quadratic fits when mucus sodium results were considered according to cycle days (Table 25), but the same general picture is observed when the best estimate of the parabola for each cycle is plotted (Figure 15). It is assumed that the arithmetic average of the different cycles concerning mucus sodium, will probably be a good estimate of the daily variations during the cycles (Figure 5). The first degree parabola was more significant for mucus potassium daily variations, and a significant difference was noticed between the linear, cubic, and quartic fits on the cycles by days (Figure 16). It must be noted that the significant difference in the variations of the parabolas does not seem to affect appreciably the reliability of the arithmetic average of mucus potassium. Figure 16 shows the best estimate of the parabolas to fit for each estrous cycle, and the difference mentioned by the statistical test is shown on this graph.

<u>Pooled results and averages from cows 262, 5275 L, and 5330 L</u>

Table 28. Average values of 237 serum determinations

	mEq. per liter		mg.%
	Na	K	Ca
	139.93	5.08	8.93
Range	110.0-166.3	3.5-7.3	6.21-11.3

Table 29. Average values of 220 exocervical mucus determinations

	Milligrams per gram of mucus		
	Na	K	Ca
	2.773	0.799	1.213
Range	2.019-4.00	0.290-1.500	0.102-2.98

Table 30. Arithmetic average of eleven pooled proestrus, estrus, and postestrus, and nine diestrus periods (Figures 8 to 13)

	Serum mEq./liter		mg%	Mucus mg. per gram of mucus		
	Na	K	Ca	Na	K	Ca
Proestrus	135.70	4.88	8.88	2.978	0.816	1.807
Estrus	133.97	4.82	9.11	3.035	0.500	1.025
Ovulation	141.13	5.13	8.96	3.157	0.461	0.689
Postestrus	140.98	4.95	8.94	2.946	0.611	0.678
Diestrus	139.17	5.17	8.94	2.556	0.882	1.419

The cervical mucus water content of cow 5330 L has been determined (Figure 14). The water content of the exocervical mucus during two estrous cycles, showed typical cyclic variations with a first peak at estrum and a second higher peak at the ovulation time. The smallest amount of water is found during the two days following ovulation, when blood is noticed in the mucus; the water content is then 92.9% and 90.5%. The presence of blood appears to be responsible for this important drop. The average percentage of water is 96.47% during the diestrus. During the estrus the average water content was 98.36, and at ovulation 98.76%. Eliminating the two days when the exocervical mucus contained blood, the range of the water content varies between 95.58 and 98.79% on this experimental animal.

Figure 2. Cow 5330 L. Daily variations. Arithmetic average of serum sodium during six proestrus, estrus, and postestrus, and five diestrus periods.

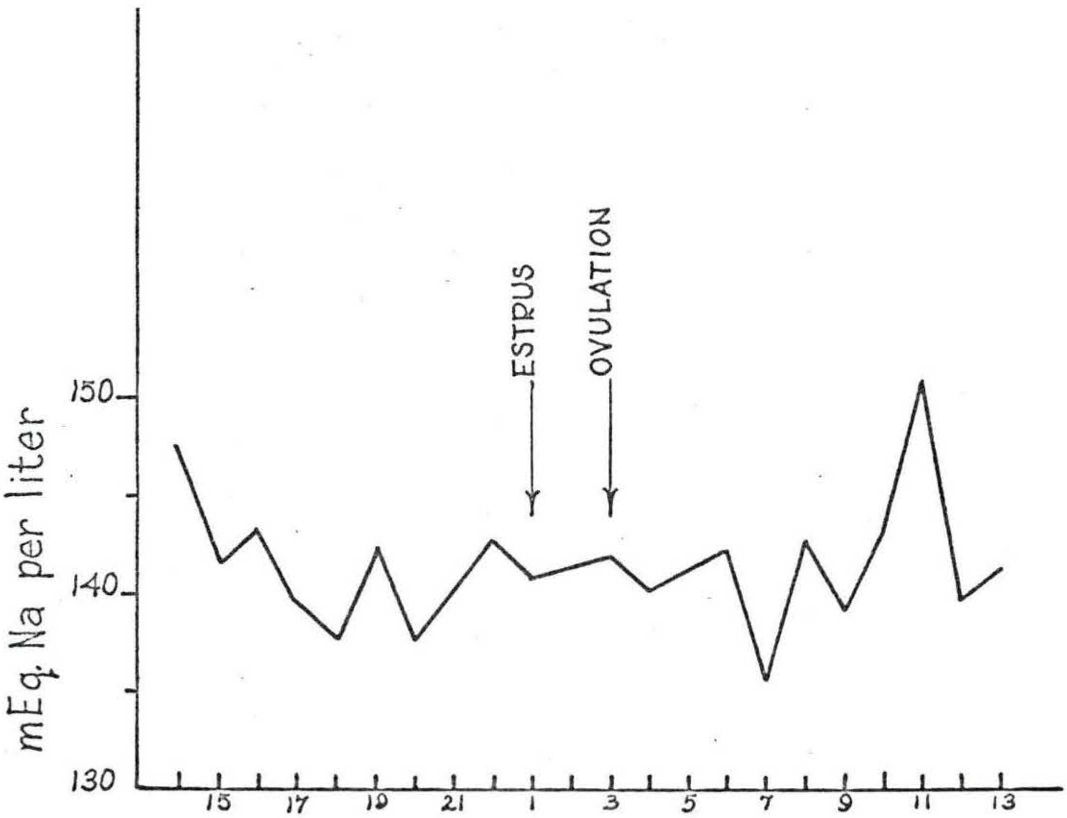

ESTRUS
OVULATION
mEq. Na per liter
150
140
130
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 3. Cow 5330 L. Daily variations. Arithmetic average of serum potassium during six proestrus, estrus, postestrus, and five diestrus periods.

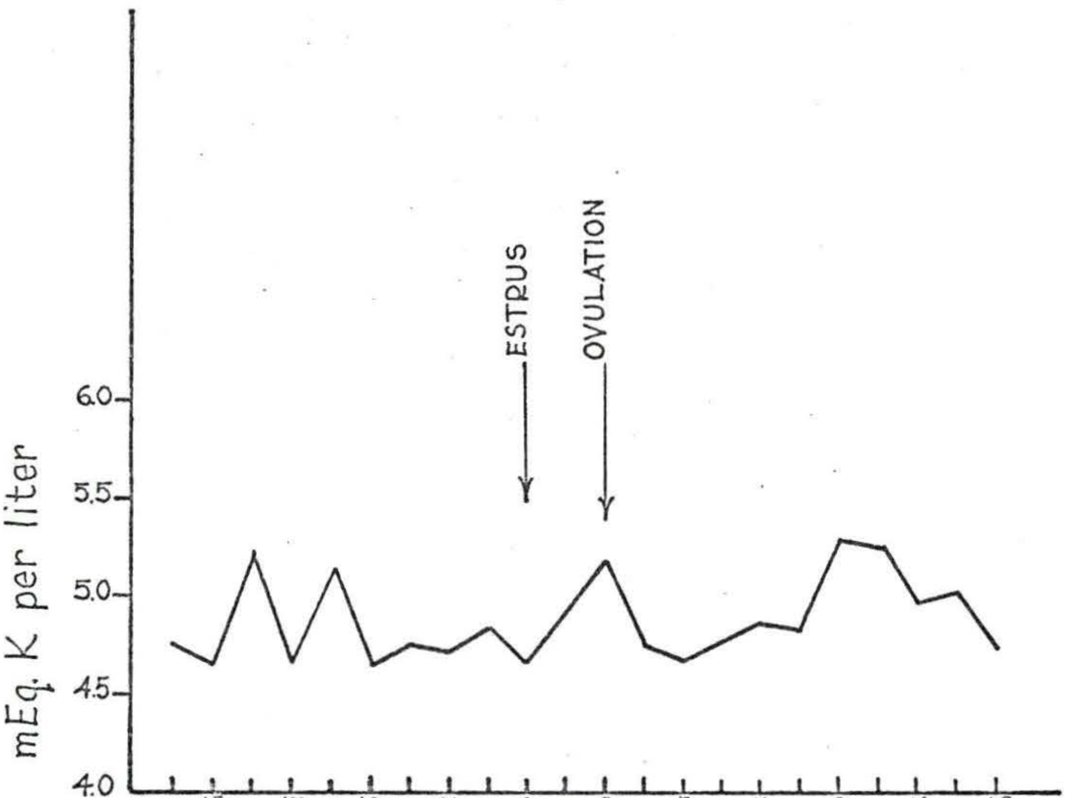

ESTRUS
OVULATION
6.0
5.5
5.0
4.5
4.0
mEq. K per liter
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 4. Cow 5330 L. Daily variations. Arithmetic average of serum calcium during six proestrus, estrus, postestrus, and five diestrus periods.

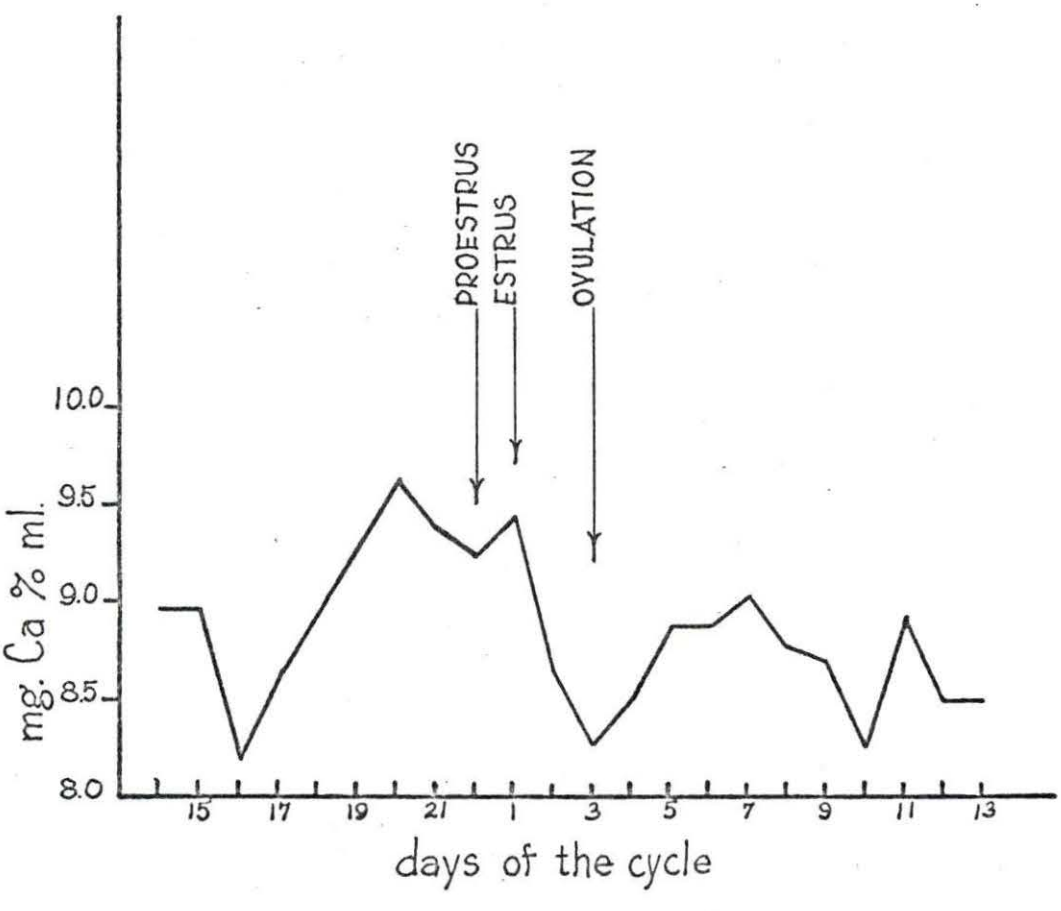

PROESTRUS
ESTRUS
OVULATION
10.0
9.5
9.0
8.5
8.0
mg. Ca % ml.
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 5. Cow 5330 L. Daily variations. Arithmetic average of exocervical mucus sodium during six proestrus, estrus, postestrus, and five diestrus periods.

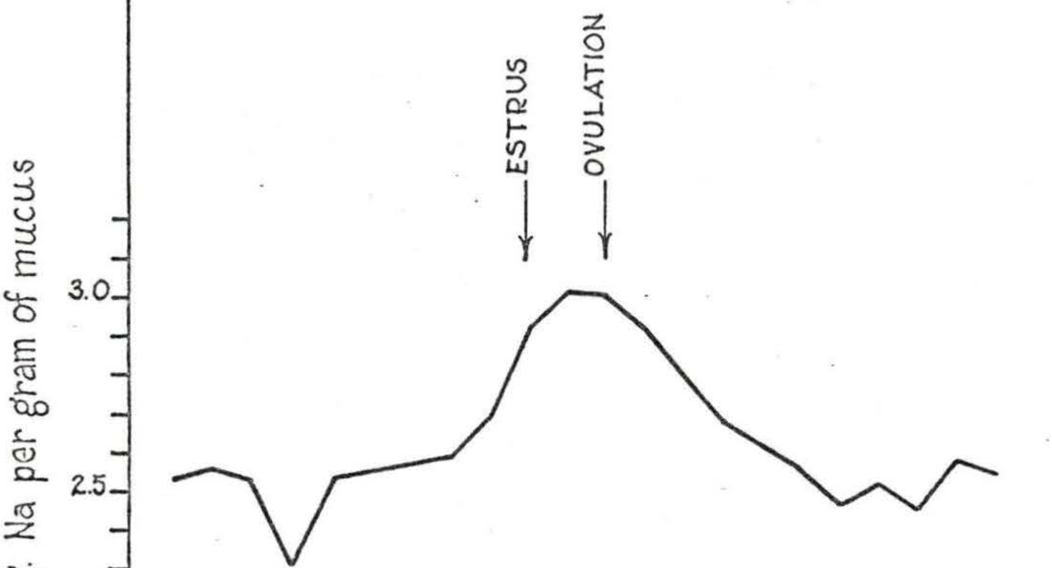
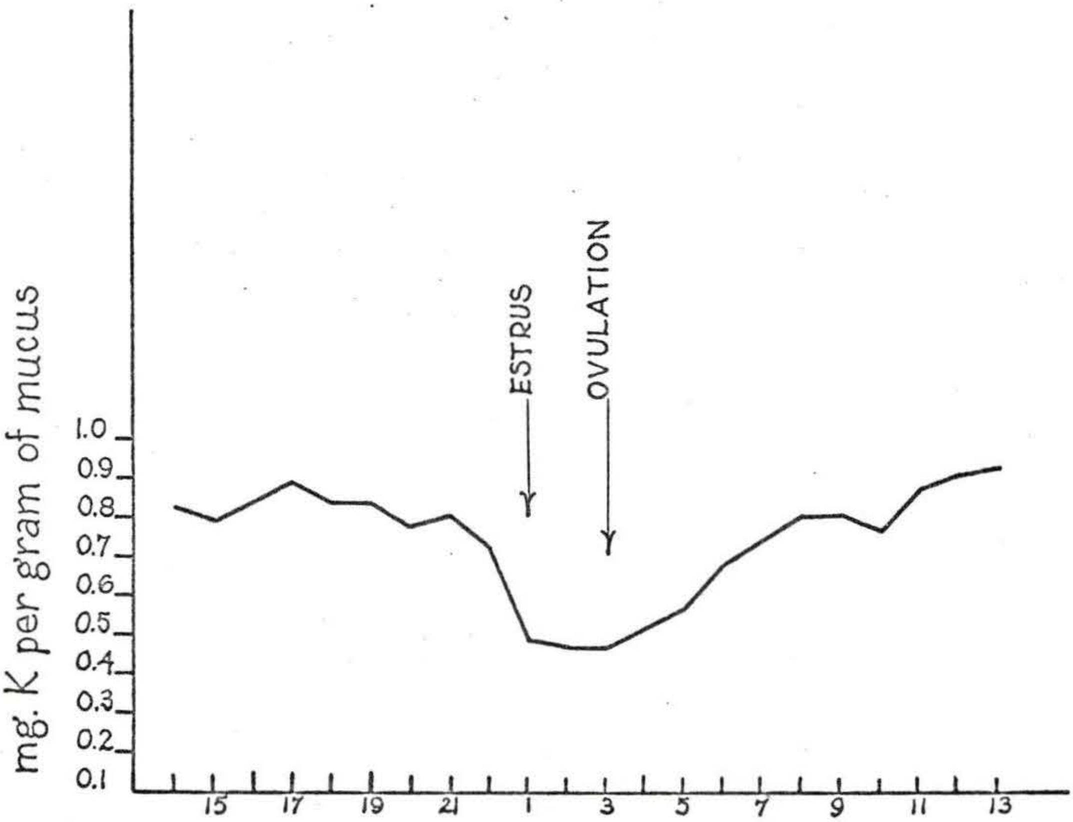

ESTRUS
OVULATION
mg. Na per gram of mucus
3.0
2.5
2.0
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 6. Cow 5330 L. Daily variations. Arithmetic average of exocervical mucus potassium during six proestrus, estrus, postestrus, and five diestrus periods.



mg. K per gram of mucus
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ESTRUS
OVULATION
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 7. Cow 5330 L. Daily variations. Arithmetic average of exocervical mucus calcium during six proestrus, estrus, postestrus and five diestrus periods.

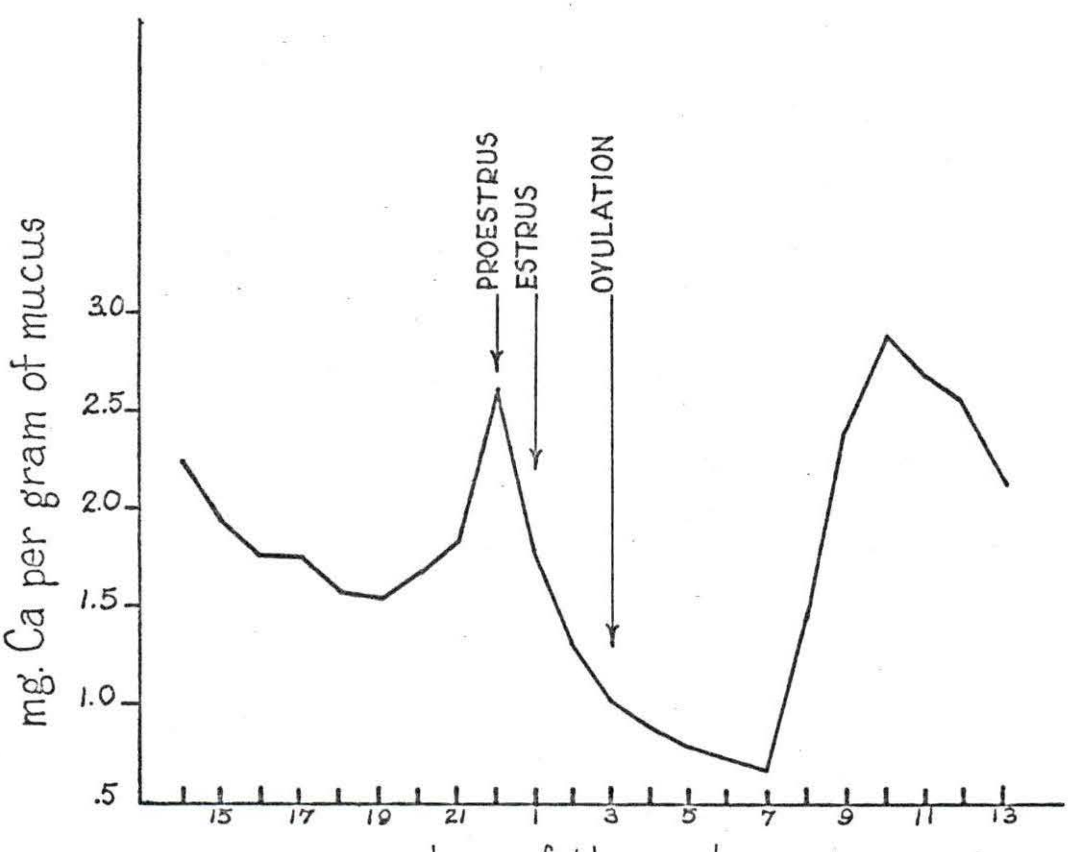

mg. Ca per gram of mucus
3.0
2.5
2.0
1.5
1.0
.5
PROESTRUS
ESTRUS
OVULATION
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 8. Cows no. 262, no. 5275 L, and no. 5330 L. Pooled values of serum sodium during eleven proestrus, estrus, postestrus periods, and nine diestrus periods.

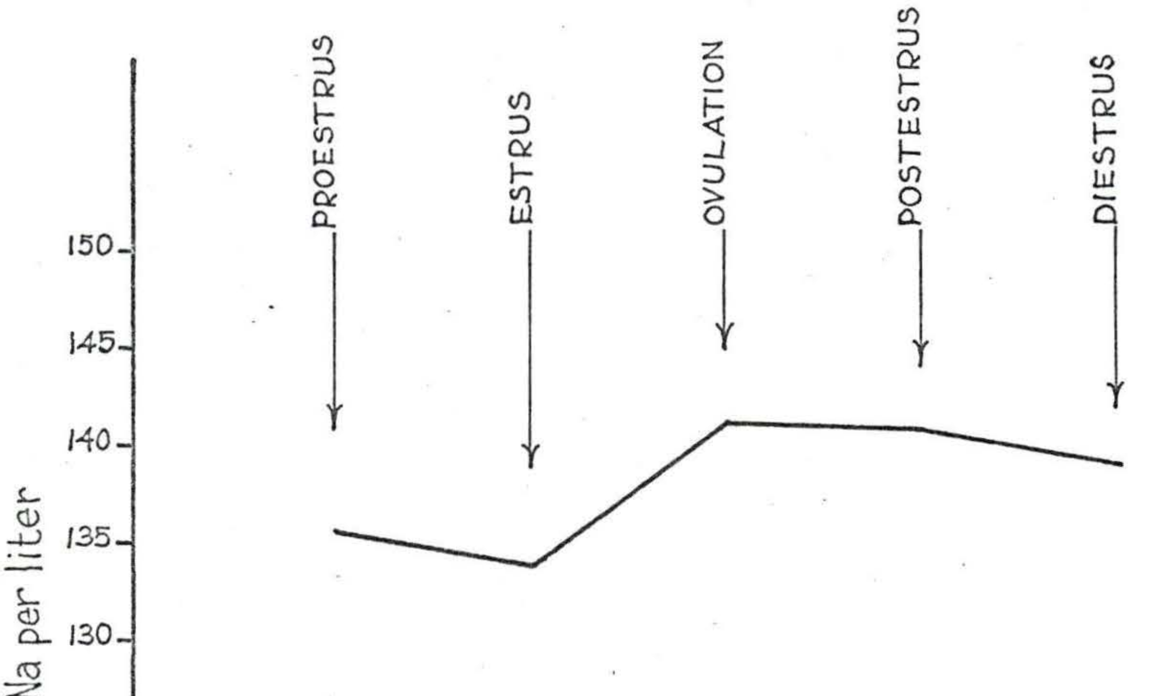

PROESTRUS
ESTRUS
OVULATION
POSTESTRUS
DIESTRUS
150
145
140
135
130
125
120
mEq. Na per liter

Figure 9. Cows no. 262, no. 5275 L, and no. 5330 L. Pooled values of serum potassium during eleven proestrus, estrus, postestrus periods, and nine diestrus periods.

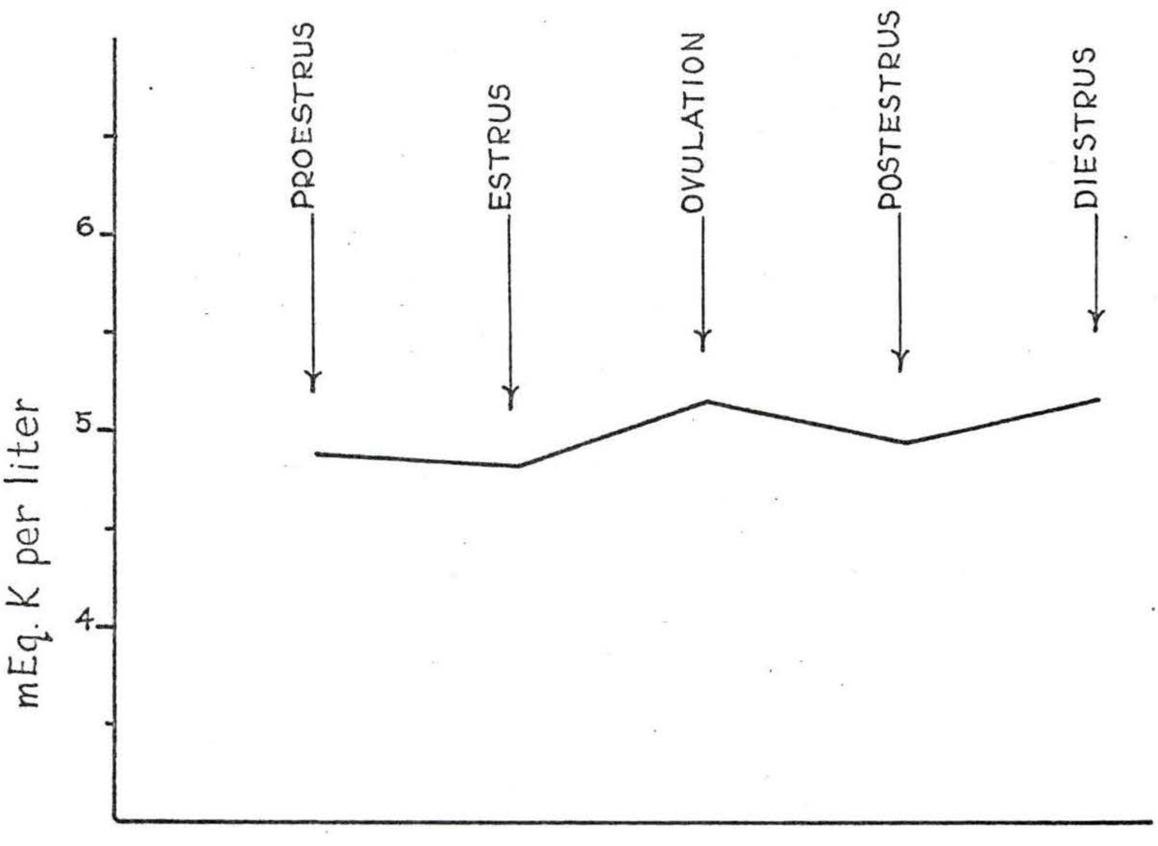

mEq. K per liter
6
5
4
PROESTRUS
ESTRUS
OVULATION
POSTESTRUS
DIESTRUS

Figure 10. Cows no. 262, no. 5275 L, and no. 5330 L. Pooled values of serum calcium during eleven proestrus, estrus, postestrus periods, and nine diestrus periods.

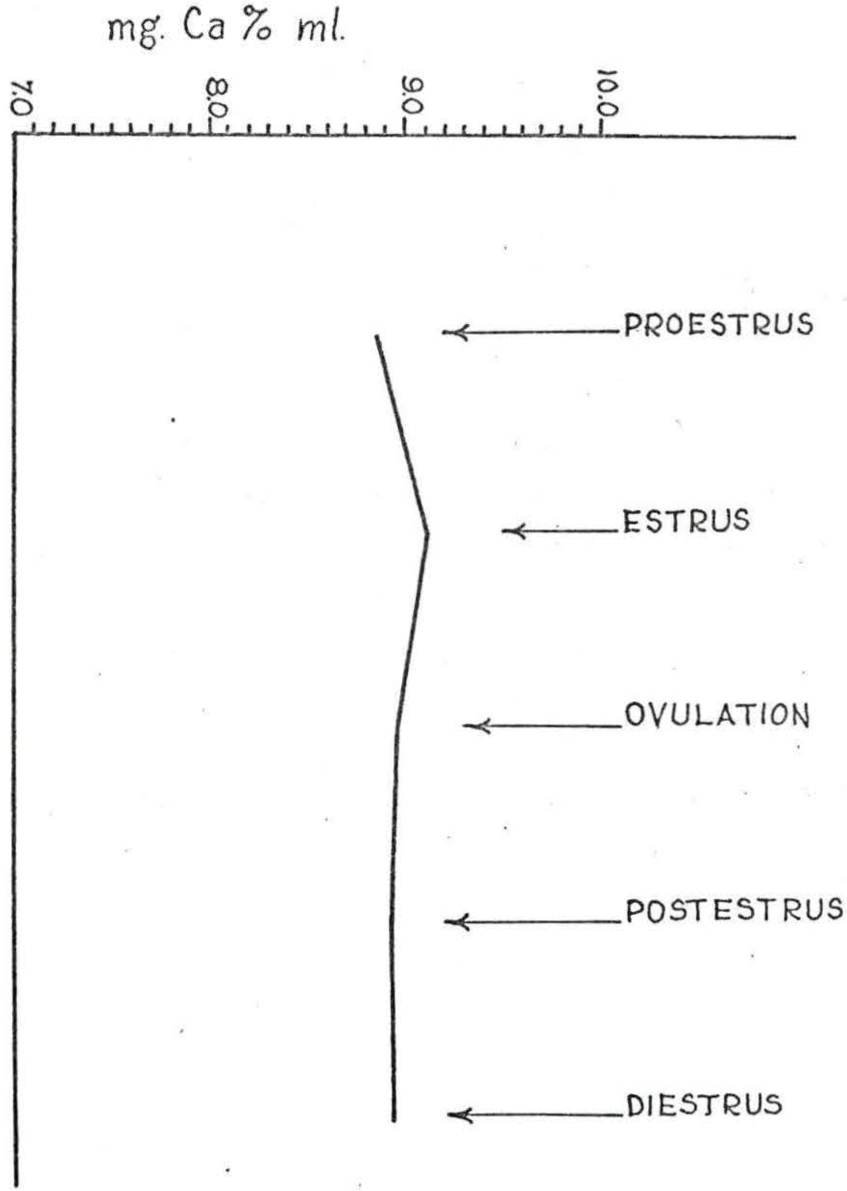

mg. Ca % ml.
7.0
8.0
9.0
10.0
PROESTRUS
ESTRUS
OVULATION
POSTESTRUS
DIESTRUS

Figure 11. Cows no. 262, no. 5275 L, and no. 5330 L. Pooled values of exocervical mucus sodium during eleven proestrus, estrus, and postestrus periods, and nine diestrus periods.

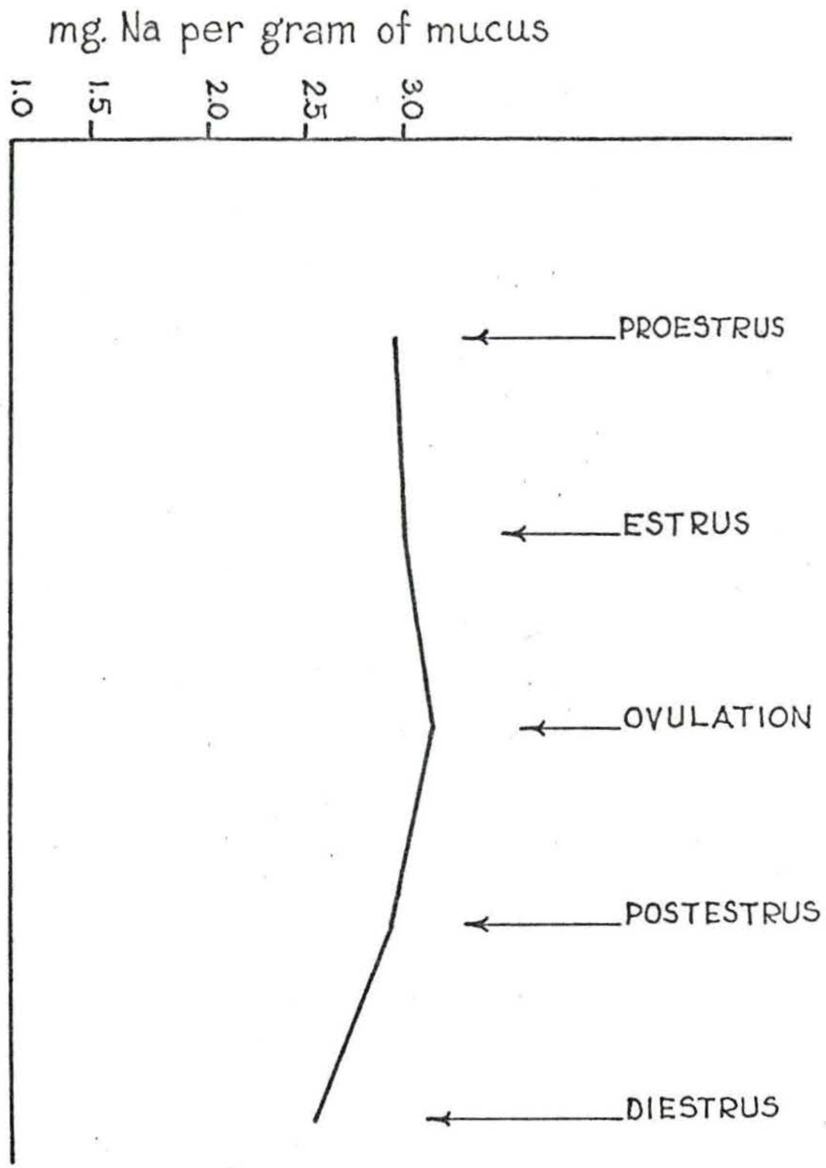

mg. Na per gram of mucus
1.0
1.5
2.0
2.5
3.0
PROESTRUS
ESTRUS
OVULATION
POSTESTRUS
DIESTRUS

Figure 12. Cows no. 262, no. 5275 L, and no. 5330 L. Pooled values of exocervical mucus potassium during eleven proestrus, estrus, and postestrus periods, and nine diestrus periods.

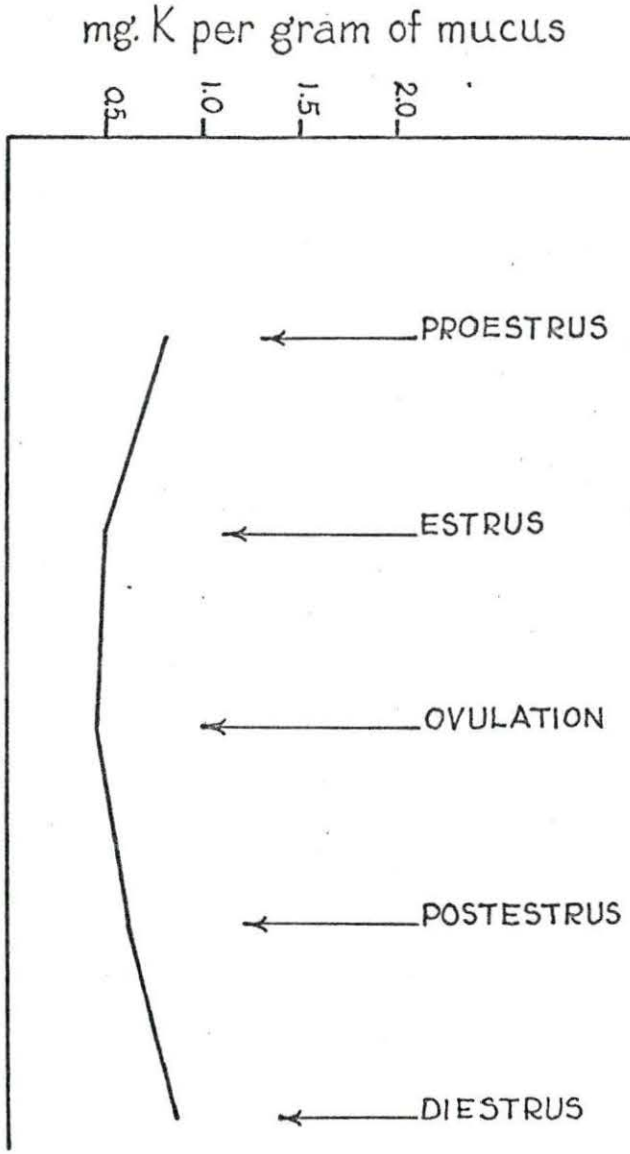

mg. K per gram of mucus
0.5
1.0
1.5
2.0
PROESTRUS
ESTRUS
OVULATION
POSTESTRUS
DIESTRUS

Figure 13. Cows no. 262, no. 5275 L, and no. 5330 L. Pooled values of exocervical mucus calcium during eleven proestrus, estrus, and diestrus periods, and nine diestrus periods.

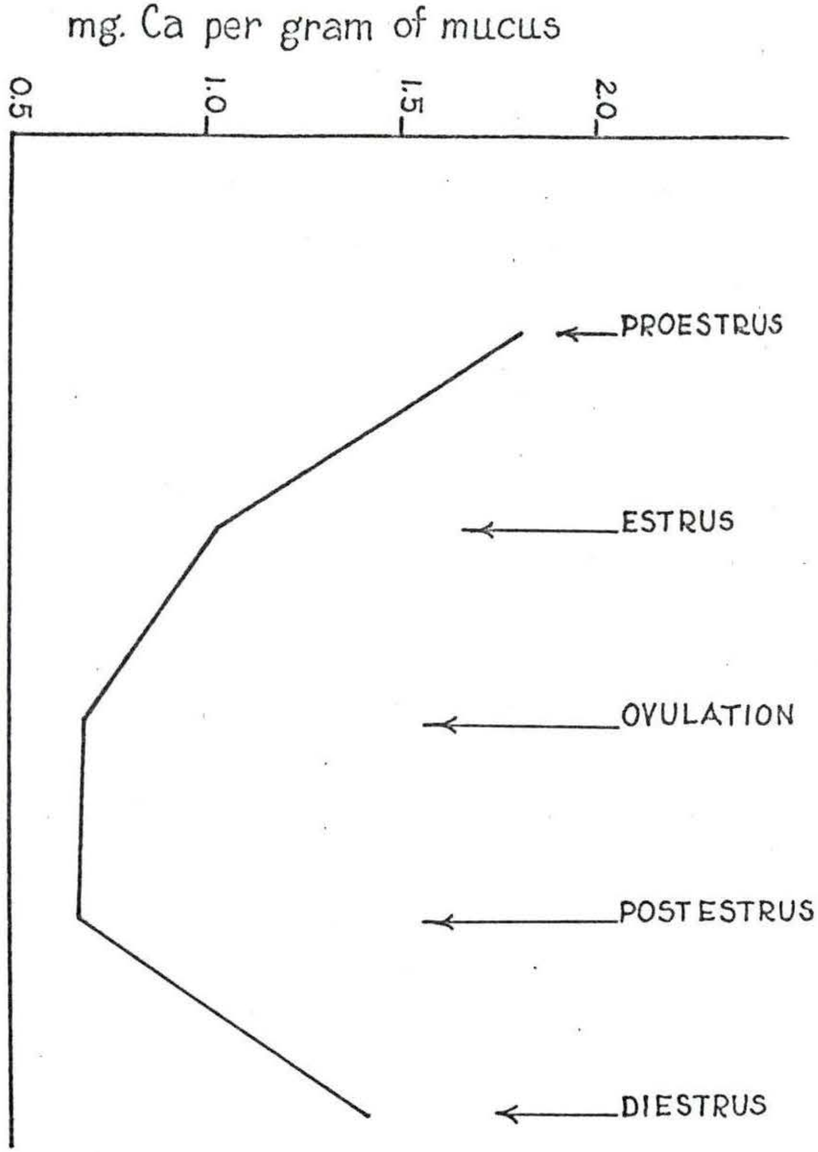
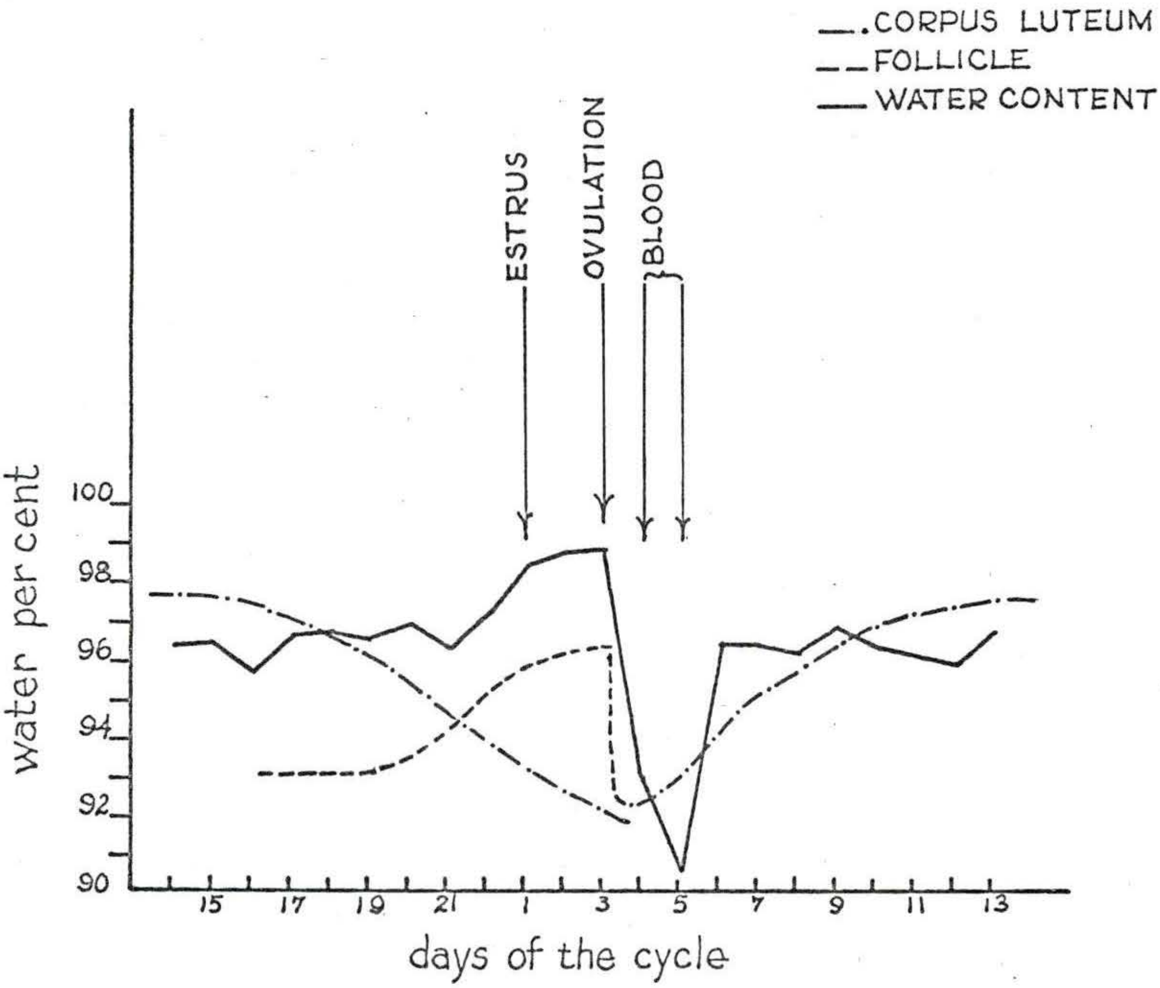

mg. Ca per gram of mucus
0.5
1.0
1.5
2.0
PROESTRUS
ESTRUS
OVULATION
POSTESTRUS
DIESTRUS

Figure 14. Cow 5330 L. Average of the daily variations of the exocervical mucus water content during two estrous cycles. The cut lines show the follicle development. The cut lines and dots show the corpus luteum development and regression.



.CORPUS LUTEUM
FOLLICLE
WATER CONTENT
ESTRUS
OVULATION
BLOOD
water per cent
100
98
96
94
92
90
15
17
19
21
1
3
5
7
9
11
13
days of the cycle

Figure 15. Cow 5330 L. Mucus sodium. Y-HAT values of five estrous cycles. Two cycles where the concentration tends to increase during the proestrus, but the same general curve is observed.

The solid line in this graph and in Figure 16 represents the same cycle. This is true of the other cyclic line designations.

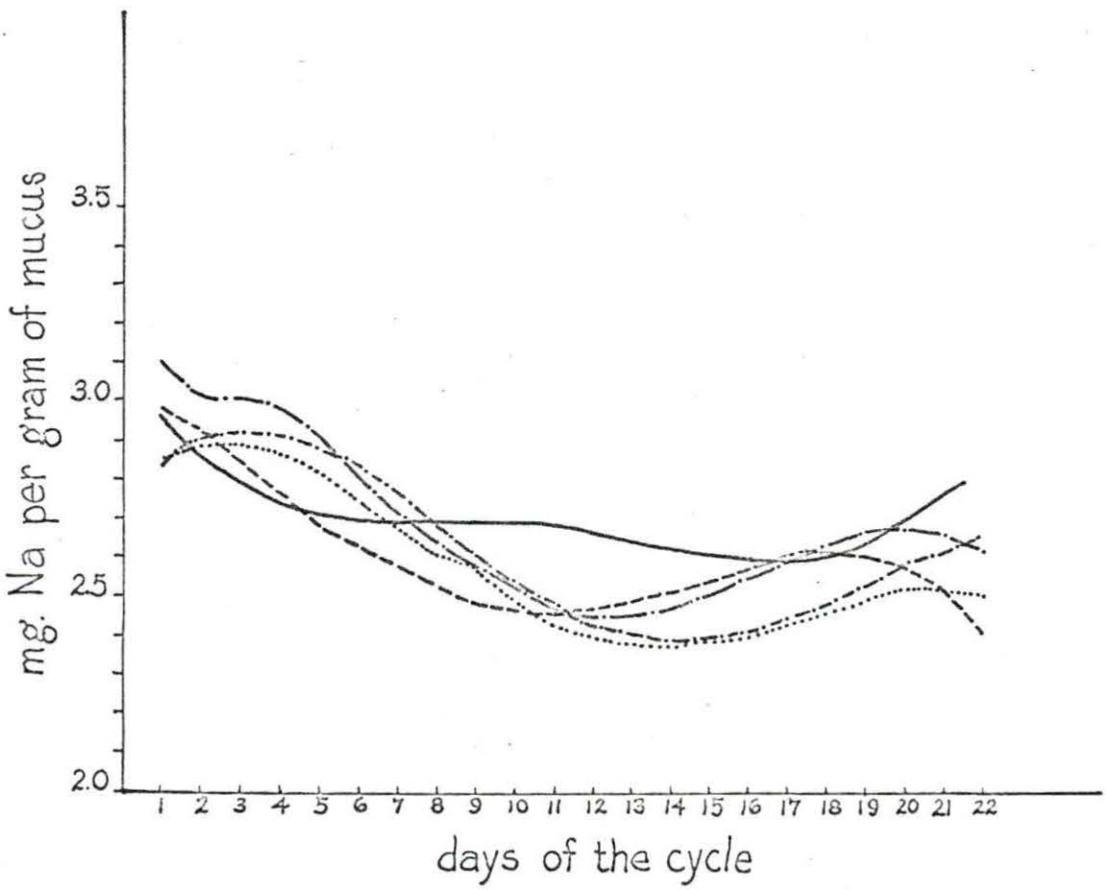
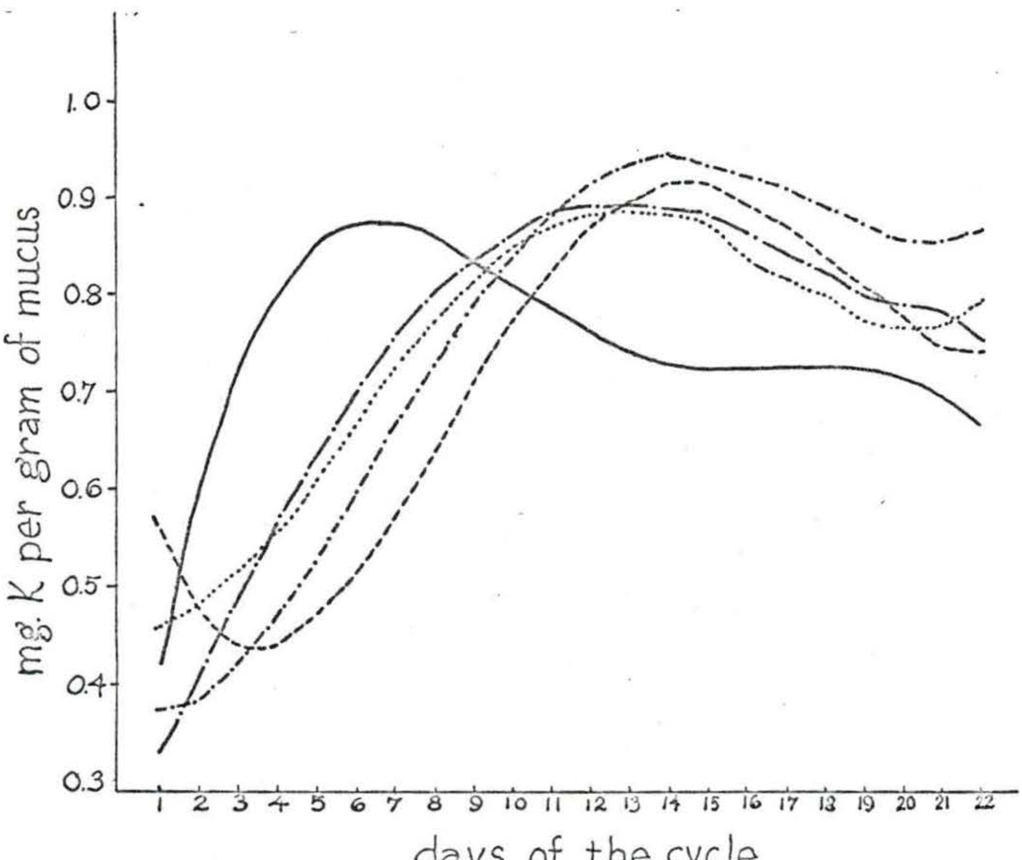

mg. Na per gram of mucus
3.5
3.0
2.5
2.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
days of the cycle

Figure 16. Cow 5330 L. Mucus potassium. Y-HAT values of five estrous cycles.

One cycle decreases for two days after the estrus; one other increases more rapidly during the dirst days of the cycle.


mg. K per gram of mucus
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
days of the cycle

DISCUSSION

The overall average of serum sodium is quite in agreement with the reports of the literature being 2 mEq. lower than the average given by Benjamin (1961). Serum potassium is somewhat higher than the average given by Benjamin (1961), and Cornelius and Kaneko (1963) by 0.28 mEq. per liter. But our range is larger than Benjamin's (1961) and smaller than Cornelius and Kaneko's (1963). Our average of serum calcium is 1.87 mg. % lower than the average given by Cornelius and Kaneko (1963). The cow number 262 has contributed largely to decrease the serum calcium average; we saw before (Table 3) that her average was 7.63 mg. %. It has been seen by reviewing the literature that important fluctuations have been found between animals in a herd and significant difference exists between months within animals (Rusoff and Piercy, 1946). Factors such as the age of the animals and/or environmental factors could be responsible for those fluctuations. The overall picture of serum sodium variations during the periods of the estrous cycle shows a decrease at estrum with an increase during the ovulation day (Figure 8). We must be careful in interpreting these changes because within each animal, four cycles are seen with a decrease at estrus, two cycles without any change and four cycles with an increase. Considering the ovulation period it was found that sodium decreased four times, increased five times and did not change one time. On cows 262 and 5275 L we noted significant polynomials of second degree and third degree as the best significant fit for the data. But by looking at the third cow no significant fit could be detected on any of the five estrous cycles. Because of these individual variations with apparently normal heat periods and because no significant parabola could be detected on this cow during five estrous cycles, it cannot be said that serum sodium variations are a determining factor in the estrus and ovulation processes. Serum potassium showed one increase during one estrus and a decrease during eight estrus, and not much change during another. The ovulation day showed a decrease in serum potassium three times, an increase six times, and no change one time. No fitting parabola was found considering the days of the cycles on either of the cows. Significant differences between the means of the cycles has been detected on cow 5330 L concerning serum potassium. It appears that within the normal range serum potassium changes are not a determining factor for the apparition of estrus or ovulation. Serum calcium has been found to increase eight times on the day of estrum; to decrease one time and to show no change one time. During the ovulation day serum calcium showed an increase twice, a decrease six times and no change during two ovulations. Fitting polynomials of second degree are noticed for cow 262 and cow 5330 L. It appears that serum calcium is more significant to the estrous cycle than serum sodium and potassium.

The results of the analysis of the exocervical mucus showed more constant results. On all the estrous cycles studied we have observed an increase in mucus sodium near the estrus starting usually during the proestrus, with the highest values ordinarily from the day of estrum to the day of ovulation (Figures 5 and 11). The linear fitting appears to be the more significant. When the Y-HATS of the five cycles of cow 5330 L are plotted the maximum is observed during the proestrus, estrus, or ovulation (Figure 15). The sodium contained in the mucus is definitely related to the estrous cycle process and particularly to the physical changes occurring in the cervical mucus. What causes the increase in mucus sodium secretion? We do not know.

Further study should determine if the increased secretion follows the estrogen level in the blood stream and/or the ovulation process itself.

Since a constant increase in mucus sodium has been observed, starting generally during the proestrus with maximal concentration around the ovulation while the water content is at its maximum (Figure 14), we must admit that there is an increased secretion of sodium in the exocervical mucus at that time. This observation is contradictory to the observations in the human by Herzberg _et al._ (1964) and Odeblad _et al._ (1958). We are in accord with the tentative explanation of Herzberg _et al._ (1964) regarding the osmotic pressure of the mucus; they say that sodium chloride is the major salt component and has a determining effect on the ionic strength of the mucus. It might therefore be assumed that the rheological periodicity is due to a cyclic variation in the sodium chloride concentration. Moreover since sodium chloride determines the osmotic pressure of the mucus, any appreciably variation in its concentration would cause osmotic irritation of the cervix. Finally the authors stated that there is little chance of interpreting the variations in the rheological properties through periodic variations in the sodium chloride concentration. According to the results obtained during this study, the chances of interpreting the physical variations of the cervical mucus through periodic variations in the sodium concentration are better because of the cyclic variations of mucus sodium. Generally mucus potassium undergoes a constant drop at estrus, being minimum at/or just before ovulation (Figures 6 and 12). The quadratic fit appears to be the most significant on our data; and significant differences between mean cycles have been detected in cow 5275 L, but the trend of the variations during the cycles is constant.

Mucus potassium is bound to the estrous cycle and the higher concentrations are observed during the luteal phase (Figure 6), the lowest values being seen at/or just before, or immediately after the ovulation. The fluctuations of mucus potassium seem to be related to the water content of the mucus;

the lowest values are observed when the dilution is the greatest, and the highest concentrations are found when the dilution is the smallest.

Mucus calcium showed a constant drop on the day of estrus and a decrease for four to seven consecutive days before returning to normal (Figures 7 and 13). The linear parabola is a constant figure for this element in the exocervical mucus. On the third cow (5330 L) the fourth degree is the most significant fitting, and it agrees with our graph (Figure 7).

Mucus calcium seems to be closely related to the stage of the estrous cycle. The highest levels occur during estrum and diestrum.

A partial correlation was found in cow 5330 L between the concentrations of serum and mucus calcium and serum and mucus potassium. That is to say, the concentrations found in the mucus for these two parameters are correlated with the concentrations noted in the blood stream ($P = 0.05$). Furthermore, corresponding low average values of serum and mucus calcium have been noted earlier (Table 3) on cow 262. The observation of the correlation of serum and mucus calcium and serum and mucus potassium could be an important one for those who believe in the significant role of nutrition on reproductive efficiency. In fact if there is a significant partial correlation between the levels of serum and exocervical mucus for the two elements above mentioned, the next step would be to correlate the feeding level of those elements with the levels and fluctuations in the serum and in the mucus. Thus, the direct effect of nutrition on the level of potassium and calcium in the genital secretions could be demonstrated.

Further study should be done to investigate if important changes in the three elements studied are found in serum and uterine secretions on cows that fail to reproduce when no clinical pathology or infectious etiology can be found.

SUMMARY

In this study, the serum sodium, serum potassium and serum calcium levels have been correlated with the mucus sodium, mucus potassium and mucus calcium levels, during each stage of the estrous cycle and at ovulation, in four cows that had previously produced living calves.

1. Serum sodium follows important fluctuations and no significant fitting parabola could be detected on cow number 5330 L, on which five estrous cycles were followed.

2. The partial correlation of serum and mucus sodium have been determined and no significant correlation could be detected between serum and mucus sodium during the normal estrous cycle. According to this it appears that within the normal range found serum sodium variations are not a determining factor for the normal estrual secretion variations; and the changes in the serum do not seem to affect the estrum or ovulation.

3. Concerning serum potassium no significant line or curve could be found to fit the data during the different cycles studied. Significant differences were found between cycles within cows. On the other hand a significant ($P = 0.05$) partial correlation was found between serum and mucus potassium on cow 5330 L for five cycles. It appears that serum potassium variations are not a determining factor for the apparition of estrus or ovulation process itself; but that serum potassium fluctuations could act directly on the concentration of this

element in the estrual secretions.

4. Serum calcium fluctuations seem to follow quite a constant course during the normal estrous cycle; fitting polynomials of second degree were observed during seven cycles; more, serum calcium is correlated at the 0.05 level significance with the estrual mucus calcium concentration. According to this we can say that serum calcium concentration appears to affect directly the exocervical mucus calcium level.

5. It has been found that the average concentration of exocervical mucus is: for sodium 2.773 mg. per gram of mucus, for potassium 0.779 mg. per gram of mucus, and for calcium 1.213 mg. per gram of mucus.

6. A constant increase in secretion of sodium has been observed in the estrual secretions around the estrus period and ovulation time.

7. Mucus potassium showed a constant drop at estrus, being minimum at/or just before, or after ovulation.

8. Mucus calcium has been found to decrease the day of estrus with a continuing drop during postestrum.

9. The water content of the exocervical mucus increases with the sodium concentration of the mucus and both are maximum around the ovulation day.

10. The limited number of animals used in this study is recognized, but it is pointed out that the methodology and recorded results provide a basis for continued and more extensive studies.

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