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Physiological and Histological Phenomena of the Bovine Estrual Cycle With Special Reference to Vaginal-Cervical Secretions

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ABSTRACT

A study of the physiological and histological phenomena during the bovine estrual cycle was made on more than 100 cows of Guernsey, Holstein, and Jersey breeds in the Missouri Station herd. Particular attention was given to the properties of vaginal-cervical mucus at varying stages of the estrual cycle.

Observations on the estrual behavior of 68 cows are described. The intensity of estrus tended to vary with age and season rather than with individuals. Data on 1503 estrual periods on 1182 cows showed that the majority of the periods began in the A. M. The average length of 504 estrual cycles was 21.41 days with a range of 11 to 35 days. Eighty per cent of the cycles were within the range of 18 to 24 days. Analysis of variance for length of estrual cycles showed significant differences between breeds, but there were no significant differences between seasons and age groups.

The mucosa of the vulva, vagina and cervix was most vascular at the approximate time of ovulation and least congested during 7 to 10 days postestrus. The cervix was usually relaxed during estrus and contracted during diestrus. Vaginal temperatures and heart rates were higher during estrus than during diestrus and pregnancy.

A study was made of the characteristics of mucus collected at various intervals during estrus. In general, the volume of mucus secreted, its flow elasticity, surface tension, and water content decreased as estrus progressed, but the number of leucocytes increased. *In vivo* vaginal pH was significantly lower than *in vitro* pH of withdrawn mucus. This difference did not appear due to carbon dioxide loss. The cervical mucus was more acid than vaginal mucus, the average difference being 1.1 pH units. Color reactions were obtained from mucus that were characteristic of glycogen, peptide linkage, and the amino acids tyrosine, cystine, phenylalanine, and tryptophane. The color tests were faintest during early heat and tended to become more intense as ovulation approached.

Cyclic changes in the histology of the bovine genital tract are described. The vagina reached its height of development during the follicular phase of the cycle. The mucoid epithelium of the upper vagina and cervix began to secrete mucus actively during late proestrus which probably serves to facilitate the ascent of spermatozoa. Uterine activity was maximum during midcycle in preparation for receipt of the zygote. The tubal cilia were longest and the epithelium highest and smoothest during early postestrus and coincides with the time the egg begins its journey down the tube. Cytoplasmic projections and nuclear extrusions were maximum at 9 to 10 days postestrus and probably represent a method of cellular regression.

A method of observing the penetration of bovine mucus by spermatozoa *in vitro* is described. The significance of the semen-mucus interface is discussed. The average penetration rate of sperm in estrual mucus was 2.81 mm. per minute with a range of 0 to 6 mm. per minute. Not all specimens

of semen penetrated a given mucus sample with equal facility. When incubated at 37-39°C. sperm motility was maintained an average of 3.30 hours longer in a semen-mucus mixture than in control semen samples. Sperm motility survival and maximal penetration were recorded in mucus collected during full and late heat.

A study of the relation of various characteristics of mucus to sperm penetrability and survival *in vitro* was made. Sperm penetrability and survival appeared to be correlated directly with the surface tension and flow elasticity and inversely to the concentration of leucocytes and pH of mucus.

The observations and results are discussed and interpreted with regard to their bearing on the explanation of the normal reproductive processes of the bovine and with regard to the practices of artificial insemination in the field.

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INTRODUCTION

Normal, efficient reproduction is dependent upon the production of viable sperm and ova and the maintenance of the genital tract of the female in an optimum condition for fertilization, implantation and fetal development. The failure of the cytogenic organs to produce viable germ cells may be a cause of lowered fertility or absolute sterility, but unfavorable conditions in the male or female genital tract may likewise make it impossible for perfectly normal germ cells to be fertilized or to continue development after fertilization.

Every animal in a breeding herd that temporarily or permanently loses its fertility becomes a financial burden upon its owner. It is difficult to obtain accurate figures on the extent of these losses. Temporary sterility, or delay in conception, causes losses by reducing the calf crop and by keeping the cow for a longer time on the declining part of the lactation curve, both factors difficult to assess. There is undoubtedly a small yearly loss in every herd in the United States due to breeding efficiency not reaching 100 per cent, whereas the total loss from this cause must be rather large.

In recent years, the use of artificial insemination has increased tremendously. On January 1, 1949, over 2,400,000 cows in the United States were enrolled in artificial breeding associations. In Missouri some 75,000 cows are inseminated annually by artificial breeding associations. Much research has been done to develop and improve techniques, yet many problems remain unsolved. One of the problems confronting artificial insemination technicians is why some apparently normal cows conceive and others do not when all are inseminated with semen of good quality. It is a common observation that many such cows have frequently conceived when bred following douching the vagina with a mild solution of sodium bicarbonate. None of the more usual methods of approach to the question of semen fecundity have, thus far, been able to give the answer.

These observations appear to be responsible for the opinion among cattlemen that the vaginal conditions are, in some unknown way, deleterious to normal spermatozoa. Prevalent also is the hypothesis that the hydrogen-ion concentration of the bovine reproductive tract and its secretions varies from the normal optimum for spermatozoa from time to time during the estrual cycle and this is, in the main, the reason why conception does not take place.

In order that adequate control of fertility and the correction of permanent and temporary sterility may be understood in any species, it is necessary that the physiological and histological events of the estrual cycle be clearly understood. This study was undertaken in an attempt to throw some light on the phenomena associated with the bovine estrual cycle; such as possible changes in the hydrogen-ion concentration of the reproductive tract, the character of the secretions, histological changes in the reproductive tract, and related phenomena Any contribution to the total present-day knowledge may aid in completing the picture of bovine reproduction, and thereby result in increased reproductive efficiency.

The experimental work includes 4 related phases which will be discussed in separate sections, namely:

I. THE ESTRUAL CYCLE
II. PHYSIOLOGICAL CHANGES OF THE GENITAL TRACT DURING THE ESTRUAL CYCLE AND VARIOUS STAGES OF PREGNANCY
III. HISTOLOGICAL CHANGES OF THE GENITAL TRACT DURING THE ESTRUAL CYCLE
IV. SPERMATOZOA PENETRABILITY AND SURVIVAL IN BOVINE MUCUS *IN VITRO*

SECTION I. THE ESTRUAL CYCLE

Review of Literature

The female reproductive system has in most species a well-marked functional rhythm called the estrus cycle. Although each species has its own peculiarities, there is similarity of pattern in all female mammals. Knowledge concerning the patterns of reproduction in mammals and the factors which determine these patterns have been reviewed by Asdell (1946).

Cattle differ from most animals in that estrus occurs regularly throughout the year. The psychological signs of estrus have been given little systematic study. Most workers have distinguished periods of receptivity and repulsion only and have not concerned themselves with the degree of these manifestations. Weber (1911) studied the symptoms of heat in cows and has reviewed the earlier literature on this subject. He noted considerable variation in estrual behavior. Cows in heat attempted to mount their companions and permitted other animals to mount them in mimic copulation. Animals with intense heat periods frequently went off-feed; however, no change in the frequency of micturition and/or defecation was observed. He found that reddening of the vaginal mucous membrane, swelling of the external genitals, and a flow of slime from the vulva always accompanied heat, but these symptoms varied with the intensity of heat exhibited by the cow. The signs of estrus were intensified by exercise and association with other animals, as well as by stimulation of the vulva or clitoris by friction.

Hammond (1927) observed a small amount of clear mucus hanging from the vulva an hour or so prior to heat. During estrus there was a large flow of mucus from the vulva and the character of this mucus flow changed as the duration of heat increased. At first the mucus was clear and extremely fluid, later it contained yellowish-cheesy lumps, and afterwards became whitish and thickened. He noted that congestion of the vulva was maximum about 1 to 2 days postestrus and frequently culminated, especially in heifers, in a flow of blood. He found the symptoms of heat to be more pronounced in summer than in winter.

Komarov (1938) reported that dilation of the cervix occurred irrespective of the degree of other manifestations of heat. Alba and Asdell (1946) divide the estrual behavior of cows into 3 strata: the lowest, a "coming in" stratum when the cow stands near the bull, but is not yet receptive; the middle, or receptive stratum, when the cow is receptive to the bull; and the higher, when the cow is an active partner displaying pursuing activities.

In some species there is a tendency for estrus to be initiated at some particular time of day. Hemmingsen and Kararup (1935) found in rats that the transition from proestrus to estrus commenced between noon and midnight. In the guinea pig about two-thirds come in heat between 6:00 P. M. and 6:00 A. M. (Young *et al.*, 1935.) Quinlan, Mare, and Roux (1932) tested ewes for estrus at hourly intervals from 6:00 A. M. to 6:00 P. M. In trials involving 563 animals, 48.1 per cent came into estrus between 6:00 A. M. and 6:00 P. M. McKenzie and Terrill (1937) found in ewes that a larger number of heat periods began between midnight and noon than between noon and midnight. Werner, Casida, and Rupel (1938) made observations on the heat periods of 35 dairy heifers on pasture during the season of 1938. Of a total of 113 periods observed, 73 began estrus in the A. M. and 40 in the P. M. Trimberger (1948) found that the onset of estrus in dairy females occurred at any time during the day. Of 132 animals studied, the onset of estrus in 94 was between 6:00 P. M. and noon, whereas only 38 began estrus between noon and 6:00 P. M.

In a general way, the duration of estrus in domestic animals is known, yet very few actual data have been collected. Weber (1911) tabulated the opinions of various authorities as to the average length of heat in cows. He reports that in cows with intense heat periods it varied from 12 to 36 hours, in cows with average heat periods from 6 to 36 hours, and in cows with feeble heat periods from 3 to 36 hours. Hammond (1927) reported that the length of estrus for dairy cattle varied from 6 to 30 hours with a mean of about 17 hours. He found that cows averaged 19.3 hours in heat as compared to 16.1 hours for heifers. No breed differences for the average length of heat period were noted, but seasonal variation was reported.

Asdell (1946) has compiled the available data on the duration of heat and reports a mean duration of 13.6 $\pm$ .16 hours, with a standard deviation of 3.9 hours. The modal length was about 14 hours, and 82 per cent fell between 10 and 18 hours. These figures are for cattle of all ages. Trimberger (1947, 1948) collected information on 132 dairy females. The duration of estrus averaged 15.3 hours for heifers and 17.8 hours for cows. In 90 per cent of these observations, the duration of estrus was from 10 to 24 hours. The cows first observed in estrus in the afternoon averaged 20.4 hours for the duration of estrus, as compared with 16 hours for cows that were in estrus in the morning.

Asdell (1946) postulated that the duration of estrus is dependent on the length of time that the central nervous system responds to estrogens. The basis for this view was the administration of threshold doses of estrogen to ovariectomized cows. The resultant estrus was not continuous, but ceased at the usual time in spite of continued daily injections. He reasoned that the central nervous system became refractory, since the usual change in the vagina and uterus continued. The average threshold dose of estrogens necessary to induce heat was very low, about 600 Rat Units. As this threshold was very low, he assumed that in the normal cow it was reached early in the development of the follicle. As refractoriness set in quickly, the cow went out of heat although the follicle was still growing. Hence, the fact that the cow, alone of all known animals except the bat, in which the cause may be different, is out of heat before ovulation.

The length of the estrual cycle in the bovine is usually stated as 3 weeks. Literature reports are in remarkable agreement. Schmidt (1902) observed that the majority of cows had 18 to 24 days between heat periods. He noted several cases over 24 days, which were probably due to unobserved heat periods as well as to delayed regression of the corpora lutea. Struve (1911) observed 38 cows during 249 cycles, and found a range of 16 to 28 days between cycles. Eighty per cent of the cases were between 17 and 23 days, 70 per cent between 18 and 22 days, and 53 per cent between 19 and 21 days. Weber (1911) observed 155 heat periods, 1 cow through 29 cycles and many through 5 or more cycles. He found the cycle lasted 3 weeks in cows with intense heat periods, 2.5 to 4 weeks in cows with average heat periods, and 3 to 4 weeks in cows with feeble heat periods. Wallace (1922) noted that estrus recurred every nineteenth day in summer and every twentieth to twenty-first day in winter. McNutt (1924) observed 19 animals and found the average cycle to be 21 days. Hammond (1927) found the estrual cycles to be 19.5 days apart with a variation of 17.5 to 24 days. Werner *et al.* (1938) made observations on the estrual cycles of 35 dairy heifers. In a total of 82 cycles, 54 ranged between 18 and 24 days. Alba (1944) gives a modal cycle length for heifers of 20 days, and for cows, 21 days. The cycle length was less variable in heifers, in which 85 per cent fell between 18 and 24 days. The mean for heifers was 20.23 ± .05 days, and the standard deviation was 2.33 days. For cows the mean was 21.28 ± .06 days with a standard deviation of 3.68 days. According to Lasley and Bogart (1943) the mean cycle length for beef cattle of all ages was 19.6 ± .12 days, the mode was 20 days, and 79 per cent fell between 17 and 23 days.

Materials and Methods

The estrual behavior of females in the Missouri Station dairy herd, which consists of Jersey, Guernsey and Holstein breeds, was studied over a 2-year period. Both heifers and cows were included in the observations. A heat-expectancy list was prepared on all females. Animals were checked for signs of heat 4 times daily, at 3:00 A. M., 7:00 A. M., 1:00 P. M., and 5:00 P. M. In addition to these periodic inspections, the herd attendants were instructed to report females observed in heat at times other than the

Artificial Insemination Association ....
Name of Inseminator ....
Herd Owner .... **Address** ....
Cow Identification .... **Breed** .... **Age Yrs.** ....
Heat 1st observed Hr. .... **Day** .... **Inseminated Hr.** .... **Day** ....
Number of Insemination (circle) 1 2 3 4 5 More
Volume of mucus—circle which

1. Large—flowing from cervix, no vaginal pool, drains through speculum.
2. Moderate—small stream flowing from cervix, small vaginal pool, does not drain through speculum.
3. Scanty—no mucus flowing from cervix, no vaginal pool, no draining through speculum.

Appearance of Mucus—circle which
1. Clear 2. Cloudy 3. Colored

Figure 1.—Survey card distributed to inseminators employed by artificial insemination associations throughout the State of Missouri.

regular intervals. Notations were made on the behavior of animals in and near estrus. The notations included such items as grazing and feeding habits, association with and attention to other animals in the herd, homosexual activities, frequency of urination and defecation, and milk production.

A record was made of all cows coming into heat, with particular attention to the time of day, from February 1, 1948, to November 1, 1949. Survey cards (Figure 1) were distributed to inseminators working for artificial insemination associations throughout the State of Missouri. These cards were completed and returned to the associations with the breeding reports of the inseminators. From the cards, the relation of the time of day and onset of estrus was determined.

Detailed reproductive records are kept on all cows in the University dairy herd. The occurrence of estrus has been recorded on all females for the past 20 years. The animals are observed from 2 to 4 times daily for estrus. When heat is noted the herd attendant makes this entry in the permanent breeding records. The length of the estrual cycle was determined on all normal cows during the period of January 1, 1946, to June 1, 1949. Excluded from the study were the cycles of cows that were classed as "shy" breeders, those that had aborted, and those used in research programs that interfered with normal herd management practices.

Results

Since the information obtained from the study of the symptoms of heat, time of day and onset of estrus, and length of the estrual cycle is of considerable importance to veterinarians, technicians, and farmers engaged in artificial insemination, it seems desirable to present the results in as much detail as possible.

Psychological behavior during estrus. The early diagnosis of heat for cows to be bred by artificial insemination is more important than for natural service. The dairyman must know early each morning and late each afternoon whether or not any females are in heat if he is to get artificial service at the proper time. The following description of the signs of heat is based on close observation of 112 periods in 68 different animals of the Jersey, Guernsey, and Holstein breeds.

Within 24 hours preceding the onset of active heat in 45 of the 68 animals observed, considerable amounts of milky-colored, gelatinous mucus were discharged. As active heat approached, the animals tended to become restless. They twitched their tails frequently and often raised them. Animals in this beginning stage of estrus, when approached by barn attendants, showed considerably more interest and affection than they did during the interestrual period. When on pasture they usually did not continue to graze but wandered about the field. Animals in this "coming-in" stage of heat sought the association of a larger or more aggressive cow and stood by her side passively. The intensity of this early estrual behavior appeared to be related, in part, to age as it was more marked in heifers and first-calf cows than in older animals. In the remaining 34 per cent of the observations the onset of estrus was abrupt and the "coming-in" behavior just described was completely absent.

The next two stages of heat, which are best referred to as mounting, male-like, or homosexual behavior, are indistinguishable in that both stages occur together. During this period the females in heat allow themselves to be mounted by other animals and do not run away as happens when an animal not in heat is mounted. This behavior was the most positive indication of active heat. Animals not in heat when mounted would arch the back and run away. When an animal in heat was mounted she braced herself, stood perfectly still, and in many instances tended to display a lordosis. In the majority of cases, the vulva of the animal in heat was sniffed by other cows in the herd, but the animal in heat was never observed to sniff the external genitals of other herd members. The homosexual behavior of the female in heat was always more intense than that of her active partner or partners. When mounting her active partner, the animal in heat frequently dribbled urine and often gave a short, low, "pre-

TABLE 1. RELATION OF TIME OF DAY TO THE ONSET OF ESTRUS IN DAIRY FEMALES ACCORDING TO AGE, BREED, AND SEASON

	Survey data of Missouri Artificial Breeding Associations from June to Sept. 1949		University of Missouri Dairy Herd from Feb. 1, 1948 to Nov. 1, 1949		Percent of Totals in Which Estrus Began	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
			A. Age in Years			
1-2	62	45	18	9	59.70	40.29
2-3	78	53	39	36	56.79	43.20
3-4	62	44	61	40	59.42	40.57
4-5	94	64	41	20	61.64	38.35
5-6	121	70	27	9	65.19	34.80
6-7	79	62	25	10	59.09	40.90
7-8	50	29	20	16	60.86	39.13
8-9	35	18	23	15	63.73	36.26
9-10	17	11	6	4	60.52	39.47
Over 10	15	11	16	8	62.00	38.00
			B. Breed			
Jersey	211	154	74	43	59.12	40.87
Guernsey	176	126	41	20	59.77	40.22
Holstein	157	96	161	104	61.38	38.61
Mixed	86	54	--	--	61.43	38.57
			C. Season			
Winter	--	--	38	23	62.29	37.70
Spring	--	--	106	29	78.51	21.48
Summer	--	--	85	75	53.12	46.87
Fall	--	--	47	40	54.02	45.97

mounting grunt" similar to the teasing action of a bull. The female in heat acting the part of the male made all movements of copulation including the thrust. As in the "coming-in" stage of heat, the intensity of homosexual behavior was greatest in the younger females.

The final stage of heat, which was a gradual cessation of estrual behavior, waned over a longer period than did the "coming-in" stage. Even though the symptoms of heat were more marked in animals in which estrus

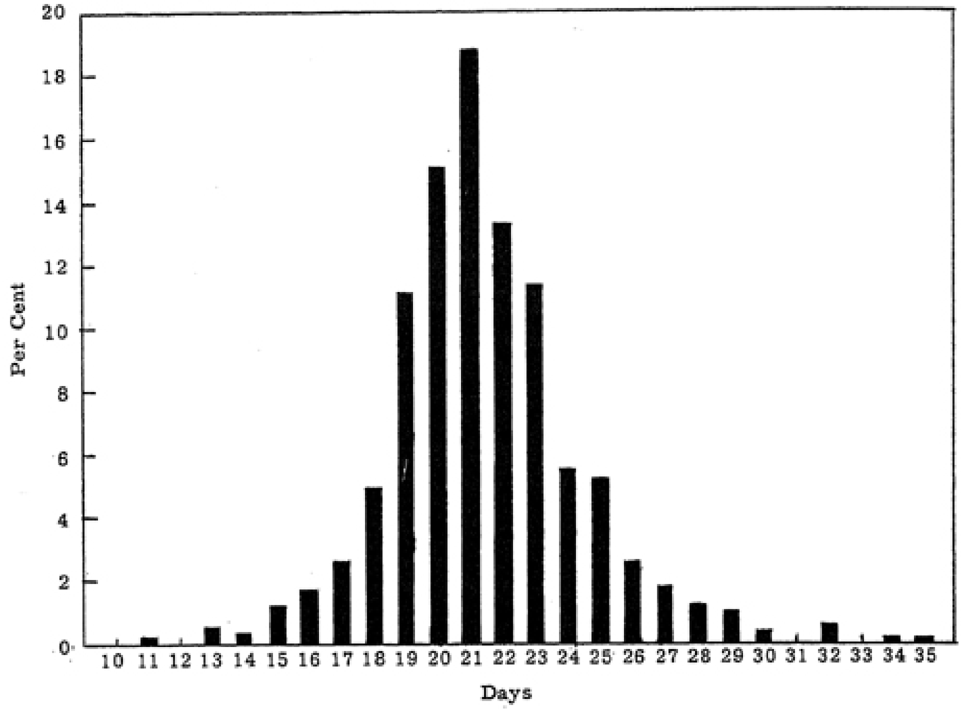

Figure 2.—The length of 504 estrual cycles of 110 dairy cows.

TABLE 2. LENGTH OF THE ESTRUAL CYCLE---
FREQUENCIES WITHIN BREEDS

Days between Cycles	Guernsey		Jersey		Holstein		All Breeds	
	No.	Percent Total	No.	Percent Total	No.	Percent Total	No.	Percent Total
11	1	1.449	--	--	--	--	1	.198
12	--	--	--	--	--	--	--	--
13	--	--	1	.826	2	.636	3	.595
14	--	--	2	1.652	--	--	2	.390
15	--	--	3	2.479	3	.955	6	1.190
16	2	2.898	2	1.652	5	1.592	9	1.785
17	1	1.449	4	3.305	8	2.547	13	2.579
18	4	5.797	6	4.958	14	4.458	25	4.960
19	8	11.594	5	4.132	44	14.012	56	11.111
20	15	21.739	11	9.090	50	15.923	76	15.079
21	16	23.188	26	21.487	53	16.878	95	18.849
22	8	11.594	14	11.570	44	14.012	67	13.293
23	4	5.797	16	13.223	38	12.101	57	11.309
24	5	7.247	9	7.438	14	4.458	28	5.555
25	5	7.247	7	5.785	14	4.458	26	5.159
26	--	--	6	4.958	7	2.229	13	2.579
27	--	--	3	2.479	6	1.910	9	1.785
28	--	--	2	1.652	4	1.273	6	1.190
29	--	--	2	1.652	3	.955	5	.992
30	--	--	1	.826	1	.318	2	.390
31	--	--	--	--	--	--	--	--
32	--	--	1	.826	2	.636	3	.595
33	--	--	--	--	--	--	--	--
34	--	--	--	--	1	.318	1	.198
35	--	--	--	--	1	.318	1	.198
Total	69		121		314		504	
	Cycles within Range of 18-24 Days							
	60	86.956	87	71.900	257	81.847	404	80.158
	Cycles outside Range of 18-24 Days							
	9	13.043	34	28.099	57	18.152	100	19.841

began in the A. M., the cessation of estrual behavior tended to be most abrupt in those animals that began estrus in the P. M.

Changes in the eagerness to eat and in milk production were noted. They were not reliable indications of estrus because of tremendous individual variations. For the most part, only those animals showing intense psychical symptoms of heat and a large flow of mucus were erratic in their eating habits and in milk production.

As a general rule, the symptoms of heat were more intense in the spring than at any other time of the year. Regardless of the season, the symptoms were more marked when the animals were on pasture than when they were housed. In 92 per cent of the observations, a clear, watery mucus discharge, individually variable in quantity, was observed during and immediately following the height of the psychical symptoms of heat.

Time of day and onset of estrus. Data are presented for 1,503 estrual periods on 1,182 animals of Jersey, Guernsey, Holstein, and mixed dairy breeding. Table 1 shows the relation of time of day and the onset of estrus in these animals. The majority of estrual periods began in the A. M. regardless of age, breed, or season. With advancing age, there was a slight tendency for the onset of estrus to shift even more toward the A. M. In the summer the percentage of estrual periods beginning in the A. M. was minimum at 53.12 and gradually increased to a maximum percentage of 78.51 in the spring. There were no significant breed differences with regard to time of day and onset of estrus.

Length of the estrual cycle. Data on the length of 504 estrual cycles of 110 cows of the Jersey, Guernsey, and Holstein breeds are presented in Table 2. A graphical representation of the frequency distribution of the total is shown in Figure 2. Eighty-six and ninety-five hundredths per cent of the Guernsey cycles, 71.90 per cent of the Jersey cycles, 81.84 per cent of the Holstein cycles, and 80.15 per cent of the cycles of all breeds were within the range of 18 to 24 days. The number of cycles outside the 18 to 24 days range was greatest in the Jerseys (28.09 per cent) and least in the Guernseys (13.04 per cent).

A comparison for length of the estrual cycle by analysis of variance between seasons and age groups within breeds showed no significant differences. When the average length of the estrual cycles for different breeds was compared by analysis of variance, it was found that the Guernsey and Jersey breeds were significantly different, but the average for the Holstein breed was not significantly different from the others. The average length of the estrual cycles was 21.61 days for Jerseys, 20.77 days for Guernseys, 21.47 days for Holsteins, and 21.41 days for all breeds (Table 3).

Discussion

The domestic cow has one of the most peculiar types of estrus behavior and estrus cycle of any mammal. In the majority of cases, the onset of estrus is gradual. The cow coming in heat becomes restless and seeks the association of a larger or more aggressive cow and stands passively by her side. This proestrus condition lasts about 24 hours during which time there is a discharge of milky-colored, gelatinous mucus. It is believed that considerable significance should be attached to this discharge as it precedes active heat. Close observation for this proestrus mucus discharge could increase the efficiency of early detection of heat which is of paramount importance to dairymen practicing artificial insemination.

The cow in heat displays a type of male-like behavior; she repeatedly mounts other cows and heifers. This reaction is not unique to the cow; it is shown by other species, but it is much more pronounced and may be regarded as part of the normal pattern of sexual behavior in this species. Young (1941) has pointed out that during heat females of various species mount others more frequently than has been believed. It is a common phenomenon in the cow, in which it is a manifestation of intense heat; however, the most positive indication of active heat was standing when mounted by animals not in heat. It should be pointed out that cows exhibit estrual behavior when they are not in heat, as such cows often mount

TABLE 3. AVERAGE LENGTH OF ESTRUAL CYCLES WITHIN BREEDS BY SEASON AND AGE*

	Guernsey av. days	Jersey av. days	Holstein av. days	All Breeds av. days
		A. Season		
Winter	20.71	21.94	21.65	21.60
Spring	21.04	21.65	21.20	21.32
Summer	20.77	23.04	21.45	21.39
Fall	20.36	22.36	21.23	21.26
		B. Age in Years		
Under 3	22.67	21.77	21.84	21.87
3-4	21.00	23.46	21.66	21.77
4-5	20.14	21.21	20.58	20.06
5-6	20.75	20.62	21.36	21.10
6-7	20.15	23.10	21.52	21.48
7-8	23.00	23.46	22.00	22.44
8-9	---	22.67	21.14	21.73
9-10	20.09	---	21.63	21.09
Over 10	21.80	21.00	21.39	21.32
		C. Breeds		
	20.77	21.61	21.47	21.41

* All averages are calculated from the original data.

those that are in heat. Experiments with ovariectomized cows injected with estrogens (Alba and Asdell, 1946) have shown that intense estrus behavior may be evoked at very low hormone levels, thus indicating that estrogens produce the psychical symptoms of heat.

The final "going-out" stage of heat and the "coming-in" stage could easily be confused since the psychical symptoms are so similar and the changes so gradual; however, the clear, watery mucus discharged during and immediately following the height of psychical symptoms provides sufficient distinction between those stages of estrual behavior.

The estrual behavior of heifers was more intense than that of cows and the behavior of females of all ages was most intense during the spring when pasture utilization was maximum. At present there is no satisfactory explanation as to why the intensity of estrual behavior varies with age and season. A seasonal trend in the intensity of estrual behavior could conceivably be related to the hormonal content of feedstuffs and/or to the changing gradient of light each day.

Cows seem to be consistent with other species (rat, guinea pigs, ewe) in showing a tendency for estrus to begin more frequently during a particular time of day. However, the period of the day varies with the species. The results of this study are in agreement with literature reports that the majority of bovine estrual periods begin in the A. M. The criticism offered by many that cows are observed more closely for heat in the A. M. than in the P. M. and that this accounts for the greater frequency of onset of estrus in A. M. is not substantiated by this study. Survey data collected under average farm conditions and data collected by periodic observations 4 times daily gave essentially the same results, namely, that the majority of cows began estrus in the A. M.

Data on the length between successive estrual periods reported in the literature are remarkably constant. This would indicate that environmental conditions have little effect upon the length of the estrual cycle. The percentage of cycles falling outside the normal range (about 18 to 24 days) was also fairly constant among the various reports and with the data reported here. The tendency for periods to be longer than the mean length was greater than for them to be shorter. Seven and ninety-two hundredths per cent of the periods were more than 24 days duration and only 6.73 per cent less than 18 days duration.

The number and percentage of abnormal cycles for the various breeds are shown in Table 2 (page 12). Observational error was a possible cause of abnormality but it was held at a minimum through frequent, individual checking of the cows for estrus and by ruling out all cycles where there was evidence of inaccuracy.

Cycles longer than the normal length may be the result of ovulation at the normal time unaccompanied by estrus. Trimberger (1948) observed 3 cows to have silent estrus periods, and examination of their ovaries showed that normal follicles developed and ruptured after they reached mature size. These cows were tested for estrus 3 times daily, but they exhibited no external signs of estrus other than discharge of the characteristic mucus. The occurrence of silent estrus periods could explain many of the abnormal cycles observed, but investigation into the factors responsible for the failure of estrus to accompany ovulation is necessary to explain this abnormality fully.

Contrary to the opinion of herdsmen, no significant age or seasonal differences were found for length of the estrual period among the 3 breeds of cattle represented in this study; however, significant breed differences were found.

SECTION II. PHYSIOLOGICAL CHANGES OF THE GENITAL TRACT DURING THE ESTRUAL CYCLE AND VARIOUS STAGES OF PREGNANCY

Review of Literature

The genital tract of the bovine undergoes rhythmic changes during the estrual cycle. Weber (1911) observed that the genitalia were always swollen and reddened by congestion during heat. Hammond (1927) further noted that this condition was at a maximum 1 to 2 days after the cessation of heat. Brown (1943) reported that vascularity of the vaginal and cervical mucosa was maximal during estrus and diminished rapidly during postestrus.

Cervical tone is considered a reliable indication of estrus. Immediately preceding and during heat there is a gradual relaxation of the cervix with a complete loss of rigidity in some cases. During diestrus the cervix is tightly closed (Weber, 1911; Albrechtsen, 1917; Hammond, 1927; Brown, 1943).

A cyclic change in the quantity and appearance of secretions from the genital tract has been reported in various animals. One of the main external characteristics of heat in the cow is the flow of large quantities of clear, stringy mucus from the vulva. Woodman and Hammond (1925) point out that in estrus the mucus in the cervix and vaginal vestibule is plentiful and fairly fluid, while in midcycle it is greatly diminished in amount and is more viscid.

Using an emptying tube viscosimeter, Scott-Blair *et al.* (1941a) found the viscosity of bovine cervical mucus to vary from 1 poise in estrus to 1,000 poises in midcycle.

A simple and inexpensive instrument (the oestroscope) has been designed for the measurement of flow elasticity of mucus by Scott-Blair *et al.* (1941a, 1941b). They found that a flow elasticity value of 5 mm. or more was indicative of estrus or within a few hours of its onset or end. It was further noted that flow elasticity values began to rise about 24 hours preceding estrus and did not return to normal until about 60 hours after estrus. They report the mean values on 41 samples of mucus from cows in, or near, heat to be 14.7 ± 9.6 mm. flow elasticity. The mean values of 143 midcycle samples were 2.4 ± 1.4 mm. flow elasticity.

The reaction of the vagina and its secretions as a possible cause of sterility has long been under discussion. The earliest interest in this field probably arose in human gynecology. As early as 1837, Alfred Donne directed attention to cervical mucus as a factor in human sterility. Meaker and Glasser (1929), in their review of the literature, cite Sims, who in 1866 stated: "The vagina and the canal of the cervix each secretes a mucus peculiar to itself. That of the vagina is acid; that of the cervix very slightly alkaline." Sims pointed out the possible harm of acidity, and soon hyperacidity was generally accepted as an important cause of sterility. That the human female genital tract is acid under average conditions has been shown by many, including Zweifel, 1908; Grafenberg, 1918; Heinlein, 1925; Kraul and Bodnar, 1925; Muschat, 1926; Kessler and Uhr, 1927; Oberst and Plass, 1936; Trussel and MacDougal, 1940; and Karnaky, 1941, 1945, 1946a, 1946b, 1947a, 1947b, and 1947c.

Work on cattle has shown a much different picture with respect to the pH of the vaginal contents, which in this case are secretions arising in part from the mucus-secreting goblet cells present in the vagina proper. In the human the vagina is lined not by a mucous membrane but by a squamous epithelium, and thus its contents are not vaginal secretions, but consist of the overflow of the cervix, cellular debris, bacteria and their products, and tissue fluids. In the bovine, vaginal acidity has been advanced as an important factor in sterility. So general is this contention that it is a common practice to douche difficult breeding cows with a mild sodium bicarbonate solution prior to breeding. The basis of this treatment is its supposed effect of creating a more favorable environment for spermatozoa by increasing the pH of the genital tract.

While many workers looked upon vaginal acidity as playing a major role in bovine sterility, some did not, as is shown by the statement of Reinhardt (1920): "The frequently held view that in disease the vaginal secretion is acid and that this condition has an especially deleterious effect on sperms, is not true, for in normal as well as in diseased vaginas the reaction for the most part is neutral or alkaline." Wester (1921) noted that normal bovine vaginal fluid was alkaline to litmus paper. He attributed favorable results to sodium bicarbonate douches in sterile animals to the mechanical removal of "spermatoxins" that arose from the existing infections, rather than to a neutralization of acidic materials. A survey of the vaginal reactions in 84 live and 39 slaughtered animals was made by Kaden (1921), using litmus paper or phenolphthalein to test the pH. Of 190 samples, litmus showed 189 to be alkaline and one acidic. In 23 diseased animals, the vaginal reactions of 19 were alkaline. Kaden cited Renkert, and also Find, who found alkaline reactions in cows that had aborted, had secondary placental retention, or endometritis. Kaden pointed out that the theory that much sterility in cattle can be traced to vaginal acidity is poorly supported.

Woodman and Hammond (1925) studied vaginal and cervical mucus from 34 slaughtered cows, and in no case was an acidic reaction obtained with litmus paper. The vaginal mucus in every case showed a much stronger alkaline reaction than did the cervical mucus. This finding is in direct contrast to the condition found in women. Woodman and Hammond postulate that the thin mucus of estrus results from the cervical mucus coming in contact with an extremely dilute alkaline medium; thus, the cervical canal is opened for the passage of spermatozoa.

Palaschini (1934) compared the pH of vaginal fluids from 30 healthy cows with those from 55 sterile cows suffering from such conditions as cystic ovaries, salpingitis, cervicitis, vaginitis, etc. Using colormetric methods on discharges collected with a sterile sponge and diluted with distilled water, he found neutral pH reactions in healthy cows and cows having ovarian lesions. The discharges from cows with uterine, cervical or vaginal lesions varied from pH 6.2 to 6.9; the more severe the lesion the more acid was the pH.

Lober (1938) working with 115 diseased and 55 healthy cows found the reaction of the vaginal secretions to be alkaline as determined by a quinhydrone electrode. He believed an alkaline reaction of the vagina was a fairly constant characteristic.

Lemke (1938) used an electrometric method in determining the pH of the vaginal secretions of 73 calves, 111 nonpregnant, and 16 pregnant cows. The average pH values for the groups were: nonpregnant, 8.2; pregnant, 7.6; and calves, 7.3. He found variations in pH during the sexual cycle, but all were alkaline.

McNutt, Schwarte, and Eveleth (1939) made pH determinations with a glass electrode on a 50 cc. physiological saline wash of the vaginal secretions of normal heifers and cows. They found the pH to fluctuate near neutrality except at estrus when it was distinctly alkaline. They also stated that "secretions of the cervical canal appear more alkaline than the vagina." These workers ovariectomized 2 heifers and found the ovaries to have no apparent effect upon the pH of the vagina.

Lardy, Pounden, and Phillips (1940) used a Coleman glass electrode apparatus in determining the pH of various fluids collected by pipette and speculum from the bovine genital tract. They indicate that sperm are first subjected to a feebly alkaline reaction, then pass through a stronger alkaline medium in the cervix, and arrive in the uterus which is acidic.

Fairly extensive investigations on the reactions of the vaginal secretions were made by Smith and Asdell (1941) and Asdell, Fincher, Smith, and Elliott (1942). The conclusion was drawn that vaginal acidity was a minor factor in the production of sterility. These workers found the vaginal pH to be alkaline (pH 7.0 to 8.9) at all times during the estrual cycle. The lowest values were observed during metestrus. They failed to produce an acid vaginal reaction, in the 4 cows studied, by injecting estrogens in amounts as high as 50,000 R. U. over a period of 2 weeks. Apparently then, cattle differ from the human and macaque monkey, in which Hall and Lewis (1936) reported that the vagina became highly acid following estrogenic treatment.

According to Sergin *et al.* (1941) in 30 freshly slaughtered cows the mean vaginal pH was 7.5, cervical pH 6.95, and uterine pH 7.1. There was little variation in the different stages of the sexual cycle, but mating caused a shift toward acidity in the vagina and cervix.

All of the foregoing results were obtained on samples either removed from the genital tract at slaughter or collected manually. Considering the various methods used the reports are in fairly close agreement. However, Dougherty (1941), Brown (1944), Lohmann and Ellenberger (1946), and Herman and Horton (1948), using specially constructed glass electrodes, measured the pH values *in vivo* and report different results from earlier workers in this field. Dougherty found that vaginal mucus is normally acid, for in only 21.5 per cent of the 400 cows examined was the pH above 7.0. He found no relation between breeding difficulties and vaginal pH. In a similar *in vivo* study by Brown the pH range was 6.2 to 8.3 with 86 per cent falling between 6.5 and 7.5. There was a slight tendency for the pH values to decline at the time of heat, but here again there was no apparent correlation between failure to conceive and the pH of the vagina. Lohmann and Ellenberger (1946) found in a total of 945 *in vivo* pH determinations that 615 were less than 7.0 and 330 were 7.0 or over. They found cows that conceived had higher vaginal pH values than those that failed to conceive.

In contrast to other workers who used *in vivo* methods of pH determination, Horton and Herman (1947) found the bovine vaginal pH to be alkaline throughout the estrual cycle, the lowest values being recorded on the day of heat. Data reported in the literature on the pH of the bovine genital tract secretions are summarized in Table 4.

Other animals whose vaginal pH has been studied are the mare (Dybing, 1937; Andrews, 1939), the chicken (Buckner and Martin, 1929), the ewe (Kardymovic, 1937), and the monkey (Hall and Lewis, 1936; Ransom

TABLE 4. DATA REPORTED IN THE LITERATURE ON THE pH OF THE BOVINE GENITAL TRACT SECRETIONS

Authority	Year	Country	Method of pH Determination	No. of Animals or Determinations	Region of Genital Tract		
					pH of Vagina	pH of Cervix	pH of Uterus
Reinhardt	1920	Germany			neutral or alkaline		
Wester	1921	Germany	litmus paper		alkaline		
Kaden	1921	Germany	litmus paper & phenolphthalein	123 animals 190 determinations	alkaline		
Woodman & Hammond	1925	England	litmus paper	34 animals	alkaline	less alkaline than vagina	
Falaschini	1934	Italy	colorimetric	85 animals	acid in disease neutral in health		
Lober	1938	Germany	quinhydrone electrode	170 animals	healthy cow average 7.36 diseased cow average 7.50		
Lemke	1938	Germany	electrometric	200 animals	predominantly alkaline		
McNutt, Schwarte & Eveleth	1939	United States	glass electrode in vitro	380 determinations	neutral to alkaline	more alkaline than vagina	
Lardy, Pounden & Phillips	1940	United States	glass electrode in vitro	33 determinations	average 6.4	average 8.33	average 6.8
Smith & Asdell;	1941						
Asdell, Fincher, Smith & Elliott	1942	United States	glass electrode in vitro	30 animals	range 7.0 to 8.9	more acid than vagina	
Sergin, Nesmejanova, Kuznecov & Kozlova	1941	Russia		30 animals	mean 7.5	mean 6.95	mean 7.1
Dougherty	1941	United States	glass electrode in vivo	400 animals	79.5% acid 20.5% alkaline		
Brown	1944	United States	glass electrode in vivo	38 animals 807 determinations	48.3% acid 51.6% alkaline		
Calisti;	1946						
Ellenberger & Lohmann	1946	Italy	glass electrode in vivo	1,170 determinations	average 4.0 mean 6.87		
Herman & Horton	1948	United States	glass electrode in vivo	18 animals	mean estrus 7.23 range nonestrus 7.25 to 7.61		

and Zuckerman, 1937; Ch'en Mai and Van Dyke, 1934; and Van Dyke and Ch'en Mai, 1936).

According to Woodman and Hammond (1925) there is a mucoprotein belonging to the class of mucin bodies containing mucoitin sulphuric acid in bovine mucus. They identified glucosamine in a specimen of cervical mucus. The chemical composition of mucus does not appear to have been investigated extensively since this observation was made, but the scanty evidence available indicates that it may contain a mucopolysaccharide-protein complex (Meyer, 1938).

Scott-Blair *et al.* (1941b) studied the variations in total nitrogen (micro-Kjeldahl) and total solids (by evaporation at 100°) of bovine cervical mucus during the estrual cycle. The total N of the secretion was minimal at estrus, the N content being about one-tenth of the midcycle value. Since the water content was highest at estrus, the question arose as to whether the fall in total N at estrus was due to an increased water content. Calculated values for the total N content of the solid matter of the secretion indicated that this was not the case. The N content of the dry matter was minimal at estrus with a value of 1 to 2 per cent, while in midcycle it rose to a value of 6 to 10 per cent. They concluded that nitrogenous substances probably arising from the breakdown of cellular debris from the epithelium of the reproductive tract, leucocytes, etc., entered the mucus secretion after estrus.

Boyland (1946) reported that bovine estrual cervical mucus appeared to be mainly carbohydrate, probably a mucopolysaccharide. Mucus from diestrus and pregnant cows contained both polysaccharides and proteins. The important differences between the chemical composition of estrus, diestrus, and pregnancy mucus were in the nitrogenous components.

Materials and Methods

The reproductive tracts of cows in the Missouri Station dairy herd were examined at varying intervals during the estrual cycle and gestation with special attention to color, vascularity, and muscular tone.

In making the examinations all cows were restrained in stanchions. The external genitals and surrounding areas were thoroughly washed with soap and water. All instruments were kept in a suitable disinfectant or else autoclaved prior to use. The examination included, in the sequence listed, the following: examination of vulva, speculum examination of the vagina and cervix for color, vascularity, and muscular tone, and collection of a mucus sample. Vaginal temperatures were secured with clinical thermometros following examination of the vulva and prior to insertion of the speculum. Heart rates were determined simultaneously with vaginal temperature measurements, using a stethoscope and stop watch. Initial high rates were discarded and the count was recorded when it was constant for 4 consecutive periods of 15 seconds each.

The degree of vascularity of the vaginal and cervical mucosa was estimated as *low*, *medium*, or *high*. The color of of the vulva, vagina, and cervix was rated as *red-yellow-red*, *red*, or *red-purple-red*. Cervical tone was estimated as *contracted*, *intermediate*, or *relaxed*.

In no case was an attempt made to measure the exact amount of mucus in the reproductive tract. Instead the volume was rated as *large*, *moderate*, or *scanty* depending upon the ease with which it could be aspirated and/or according to the method given in Figure 1 (page 9).

Mucus samples were collected at the first signs of estrus and at varying intervals for the duration of estrus or as long as samples could be obtained. An aspirator consisting of a glass tube one-fourth inch in diameter and approximately 15 inches long attached to a rubber suction bulb was used to collect mucus. After inserting the tube of the aspirator some 10 to 12 inches into the vagina, the rubber bulb was squeezed and the mucus drawn into the tube. The mucus was then transferred to sterile vials and sealed for laboratory study of surface tension, flow elasticity, pH, leucocytes, water content, color reactions, and sperm penetrability and longevity.

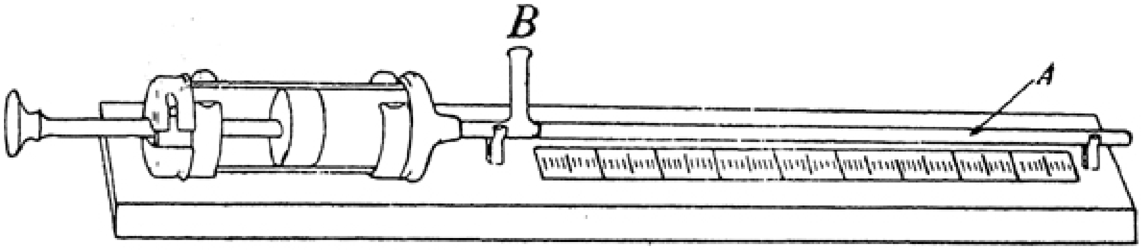

Figure 2a.—The oestroscope consists of a glass capillary tube of about 2 mm. bore and about 11 cm. long (A), graduated in millimeters. One end is cemented into a metal shank which will fit on to the nozzle of an ordinary hypodermic syringe. Between the shank and the 10 cm. mark there is an open side-tube B (from Scott-Blair *et al.*, 1941b).

Flow elasticity determinations on mucus were made by means of an oestroscope (Figure 2a); this instrument and the technic are described by Scott-Blair *et al.* (1941a, 1941b).

A smear was prepared from the mucus sample and the concentration of leucocytes rated microscopically. Using an average of 3 fields, the ratings were as follows: *small*, less than 5 leucocytes per field; *medium*, more than 5 but less than 25 leucocytes per field; and *large*, 25 or more leucocytes per field.

A Cenco du Nouy tensiometer was used to determine the surface tension of mucus. The instrument and its operation are described by Hawk, Oser, and Summerson (1947). A correction factor, which was computed for each reading, was applied to the reading because of variations in the torsion of the wire brought about by temperature and barometric pressure changes.

The pH determinations were made with a Beckman pH meter. For *in vivo* determinations specially constructed glass electrodes as described by Dougherty (1941) were used. For *in vivo* measurements the temperature compensator on the potentiometer was set at 38°C. The electrode and potentiometer were standardized against standard buffer solutions—pH 4.0 and 7.0, both at 38°C. and room temperature—each day before and after use. Between cows, the electrode was sterilized in alcohol and rinsed with distilled water. The electrode was inserted into the vagina until the bulb was in the immediate vicinity of the os uteri. After insertion and prior to taking readings the electrode was allowed to remain in the vagina for 1 to 2 minutes so as to reach body temperatures. Three readings were made; one ventral to the os in order to determine the pH of secretions that might have collected in the vagina; a second reading dorsal to the os to determine the pH of the wall of the vagina; and finally, in contact with the os uteri. These 3 readings were taken within 3 minutes and the arithmetic average taken as the *in vivo* pH at that time. *In vivo* pH determinations were made on animals throughout all phases of the estrual cycle and pregnancy.

In vitro pH measurements on collected mucus samples were made with the same potentiometer as the *in vivo* determinations. A standard glass electrode was used to measure pH except in those cases when the mucus sample was so small that a one-drop glass electrode was required. After all drifting had ceased, the arithmetic average of 3 successive readings was recorded. All *in vitro* pH determinations were made within 30 minutes following collection.

The water content of mucus was determined by drying specimens to constant weight in an oven at 55°C.

The following color reactions were tested on mucus: biuret, Millón's, xanthoproteic, sulfur, iodine, α-nitroso-β-naphthol, Hopkins-Cole, and ninhydrin (Hawk, Oser, and Summerson, 1947).

Results

Vaginal temperature. An attempt was made to study cyclic changes in the vaginal temperatures of cows during various phases of the sexual cycle. Thirteen hundred seven vaginal temperature readings were taken. There was marked individual variation, some of which was undoubtedly due to physiological causes and some of which was probably the result of such external factors as fright, movement prior to observation, and environmental temperature.

TABLE 5. VAGINAL TEMPERATURES DURING THE ESTRUAL CYCLE AND PREGNANCY

Period of Cycle	No. of Cases	Av. Temperature in °F.	Range of Temperature in °F.
Hours in estrus			
0- 4	267	101.57	99.0 to 104.2
4- 7	173	101.60	100.2 to 103.6
7-10	152	101.61	99.8 to 103.2
10-13	67	101.68	99.2 to 103.5
13-19	62	101.26	100.4 to 102.6
19-25	21	101.31	100.2 to 102.4
Diestrus	290	101.28	100.2 to 104.4
Following parturition but prior to heat	55	101.35	100.4 to 101.9
Pregnant	220	101.06	100.2 to 102.6

The lowest vaginal readings (Table 5) were made during pregnancy and the highest during estrus. The average vaginal temperature increased gradually from the start of estrus to a maximum at about 12 hours and then subsided to the diestrus level.

Heart rates. Heart rates were determined daily on 25 cows. Determinations were made at the same time each day. The average heart rate was approximately 5 beats per minute faster during estrus than during diestrus (Table 6). The wide range in heart rates during estrus was probably the result of observation at varying intervals after estrus began. In pregnant animals, the heart rate average 59.62 beats per minute and ranged from 48 to 80 beats per minute.

TABLE 6. HEART RATES
DURING THE ESTRUAL CYCLE AND PREGNANCY

Period of Cycle	No. Cases	Average Rate per Minute	Range in Rate per Minute
6-12 days before estrus	146	63.13	52 to 80
1-5 days before estrus	107	62.01	51 to 76
Day of estrus	18	67.46	52 to 82
1-5 days after estrus	88	64.38	56 to 72
6-12 days after estrus	104	61.17	52 to 80
Pregnant	132	59.62	48 to 80

Vascularity of the vaginal and cervical mucosa. Over 500 examinations, per speculum, were made of the reproductive tracts of cows in various phases of the estrual cycle and immediately after parturition to study variations in the vascularity, color, and muscular tone.

With rare exception rhythmic changes in the vascularity of the vaginal and cervical mucosa were observed throughout the estrual cycle. The development of hyperemia was rapid, developing from normal (low) to medium about 24 to 48 hours prior to the onset of estrus and reaching the highest rating about 12 to 24 hours after the cessation of heat. There was a definite tendency for the maximal degrees of vascularity in the vagina to coincide with the time of ovulation. Following ovulation there was a gradual decrease in vascular congestion with minimal values being observed 7 to 10 days interestrus.

The cervical mucosa underwent changes in vascularity similar to those of the vaginal mucosa. However, during estrus the os uteri was frequently studded with small red spots or "blood points" apparently caused by an extravasation of blood.

Some individual cows showed little vascular change in the vaginal and cervical mucosa during any stage of the cycle and some showed a higher degree of vascularity during interestrus than did others during estrus. Cows with intense heat periods tended to exhibit more marked vascular changes than did cows with average heat periods.

Vascularity was marked in both the vaginal and cervical mucosa following parturition, but had usually receded to the interestrual condition within 20 to 30 days after calving.

Color of vulva, vaginal mucosa, and cervical mucosa. Changes in the color of the vulva were not as marked as those in the vaginal and cervical mucosa. In most cows, the vulva showed more color during estrus and 1 day postestrus, usually a bright cherry red, than during the interestrual period, when the color was whitish-pink. Some cows exhibited little change during the estrual cycle.

The color of the vaginal and cervical mucosa ranged from a pale red to a red-yellow-red at the start of estrus and became red-purple-red about the time of ovulation. After ovulation the color intensity gradually decreased to minimal intensity at 7 to 12 days interestrus. As was found in examination of the vulva, color was most intense at the first estrual period after parturition.

Although there was a tendency for the color intensity of the vulva and the vaginal and cervical mucosa to be greatest during estrus and up to about the time of ovulation and least during diestrus, the high degree of individual variation prevents the phenomena from being of any diagnostic value.

Cervical muscle tone. The changes in the muscular tone of the cervix during the estrual cycle were as marked if not more marked than any of the other vaginal and cervical physiological phenomena studied. In the majority of cows, there was a gradual decrease in cervical muscle tone 2 to 3 days before the onset of estrus, with maximal relaxation occurring near the time of ovulation. After ovulation cervical muscle tone gradually increased to reach the highest degree of contraction about midcycle.

After parturition the cervix remained completely relaxed until the membranes had been expelled. Approximately 3 days later, cervical tone began to increase and usually reached the diestrus state of contraction within 4 to 5 weeks after calving.

TABLE 7. AMOUNT OF MUCUS AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	Total No. of Cases	Amount					
		Large		Moderate		Scanty	
		No. Cases	%	No. Cases	%	No. Cases	%
Hours in estrus							
0- 4	254	135	52.36	59	23.22	60	23.62
4- 7	161	60	37.26	65	40.37	36	22.36
7-10	150	12	8.00	60	40.00	78	52.00
10-13	64	8	12.50	20	31.25	36	56.25
13-19	59	3	5.08	15	25.42	41	69.49
19-25	24	3	12.50	3	12.50	18	75.00

Amount and appearance of mucus. The amount of mucus secreted during estrus varied considerably with individuals. The largest volume was usually noted during the first 3 hours of estrus. The amount of mucus gradually decreased as the interval from the start of estrus increased (Table 7).

In 92 per cent of the cases rated, the mucus was relatively thin and serous in nature. As the quantity of mucus decreased, the secretions tended to become thicker and slightly mucilaginous, with a yellowish to brownish or blood-tinged color near the time of ovulation and for one day thereafter.

Leucocytic concentration of mucus. In normal mucus (water clear) leucocytes appeared single. In abnormal (colored) mucus leucocytes were nearly always aggregated. Despite marked individual variation, the number of leucocytes usually increased with prolongation of estrus. Table 8 shows the variation in the number of leucocytes present in 435 samples of bovine mucus. In the majority of cases, the concentration of leucocytes was small during the first 18 hours of heat and increased sharply thereafter.

TABLE 8. CONCENTRATION OF LEUCOCYTES IN MUCUS AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	Total No. of Cases	Concentration					
		Large		Medium		Small	
		No. Cases	%	No. Cases	%	No. Cases	%
Hours in estrus							
0- 4	99	12	12.12	18	18.18	69	69.69
4- 7	116	7	6.03	24	20.68	85	73.27
7-10	112	12	10.71	34	30.35	66	58.92
10-13	40	11	27.50	14	35.00	15	37.50
13-19	51	9	17.64	13	25.49	29	56.86
19-25	17	7	41.17	7	41.17	3	17.64

Flow elasticity of mucus. The results of flow elasticity determinations on 668 samples of mucus are presented in Table 9. The flow elasticity of mucus varied rather regularly during estrus and early postestrus. It averaged 17.64 mm. during the first 6 hours of heat and then gradually decreased as the interval from the beginning of estrus increased. The lowest values for flow elasticity (average 16.00 mm.) were recorded from 19 to 25 hours after the start of estrus.

TABLE 9. FLOW ELASTICITY OF MUCUS AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	No. Cases	Average Flow Elasticity in mm.	Range of Flow Elasticity in mm.
Hours in estrus			
0- 4	240	17.64	7 to 26
4- 7	147	17.64	9 to 25
7-10	144	16.77	9 to 26
10-13	57	16.70	10 to 21
13-19	57	16.31	5 to 22
19-25	23	16.00	9 to 21

Surface tension of mucus. Surface tension determinations were made on 650 mucus samples collected at varying intervals during the estrual cycle. The averages and ranges in surface tension expressed in dynes/cm. are presented in Table 10. Surface tension values of mucus from an individual cow showed little variation during and immediately following estrus, but considerable variation between samples from different cows in comparable stages of heat was noted. In general, the surface tension tended to decrease as the duration of estrus was prolonged.

pH of mucus. Hydrogen-ion concentration will be expressed in the conventional manner, as pH. A total of 676 *in vitro* pH determinations was made on bovine mucus collected during the estrual cycle. The highest

TABLE 10. SURFACE TENSION OF MUCUS AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	No. of Cases	Average Surface Tension in dynes /cm.	Range in Surface Tension in dynes /cm.
Hours in estrus			
0- 4	232	55.57	41.13 to 65.36
4- 7	146	55.74	40.09 to 62.92
7-10	140	54.48	43.18 to 62.93
10-13	55	54.99	45.50 to 61.14
13-19	56	54.27	42.21 to 61.62
19-25	21	53.28	46.19 to 58.84

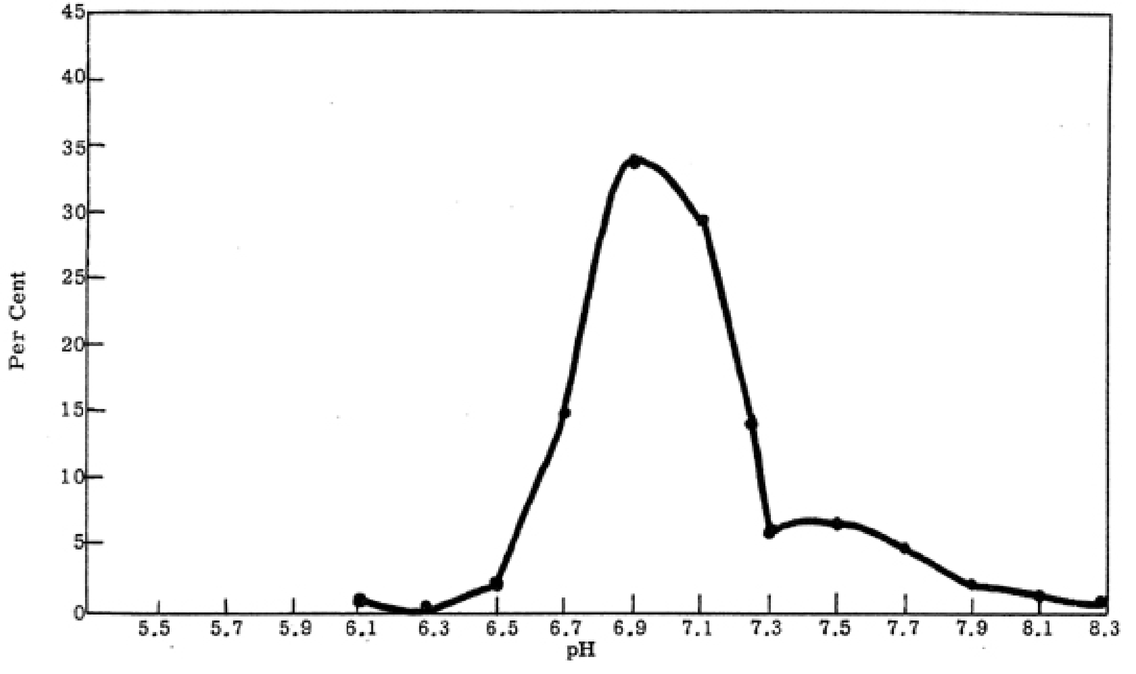

Figure 3.—Distribution of 676 *in vitro* pH values on bovine mucus collected during estrus.

pH reading obtained was 8.30 and the lowest 6.18. It will be noted from the frequency distribution curve in Figure 3 that there appears to be a tendency for the pH values to be grouped near neutrality. Whether this distribution represents a significant physiological difference has not been determined. Two and twenty-one hundredths per cent of the pH values varied between 6.10 and 6.69, 14.64 per cent between 6.70 and 6.89, 33.87 per cent between 6.90 and 7.09, 29.14 per cent between 7.10 and 7.29, 11.97 per cent between 7.30 and 7.69, and 8.12 per cent between 7.70 and 8.30.

The *in vivo* vaginal pH of difficult breeding cows was compared with that of normal and pregnant cows. The results are presented in Figure 4. Cows having 4 or more services without conception were classed as difficult breeders. Vaginal pH values of cows so classified varied from 5.5 to 7.0 and tended to be grouped around a pH of 6.70. The vaginal pH of difficult breeding cows tended to be more acid than that of normal and pregnant cows. The vaginal pH of pregnant cows, without regard to stage of preg-

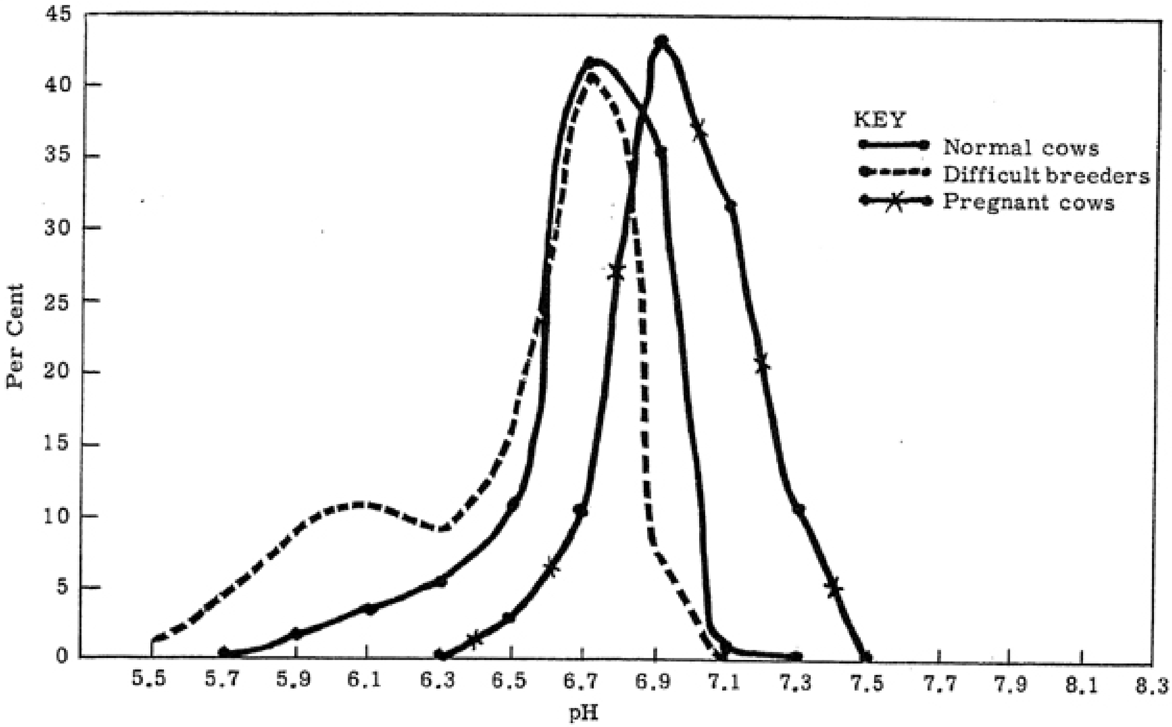

Figure 4.—Comparison of *in vivo* vaginal pH of normal, difficult breeding, and pregnant cows.

nancy, was more alkaline than that of normal and difficult breeding cows. Seventy-five per cent of the vaginal pH values of pregnant cows fell within the pH limits of 6.90 and 7.29.

A graphic illustration of the variation of *in vivo* vaginal pH in all phases of the estrual cycle and gestation is shown in Figure 5. Recordings of pH were made every fourth day for a period of 41 days. The pH values of 2 normal cows, 2 pregnant cows, and 2 difficult breeding cows were selected at random as representative of the picture presented by each group. It will be noted that the pH fluctuated less in the pregnant cows than in the normal and difficult breeding cows. In cows which were in estrus during the course of the measurements, there was a distinct drop in pH just prior to, during, or immediately after heat. Such a drop in pH appeared to be eliminated by pregnancy.

The average *in vitro* pH of mucus at various intervals during estrus is presented in Table 11. It is interesting to note that the lowest average pH was 7.09, at from 4 to 7 hours after estrus began, and that the pH tended to become more alkaline as estrus progressed.

In vivo pH readings of mucus were considerably lower than *in vitro* measurements. Table 12 shows a comparison of vaginal pH determinations made *in vivo* and vaginal-cervical mucus pH determinations made *in vitro*. A total of 30 such paired observations was made on 10 cows during estrus. While the *in vivo* pH was invariably lower than the *in vitro*, the extent of the differences was not uniform. The *in vitro* pH values averaged 7.45, or 0.88 above the *in vivo* average. They ranged from 0.40 to 1.33 pH units higher than *in vivo* values. These differences between *in vivo* and *in vitro* pH values were significant beyond the 1% level.

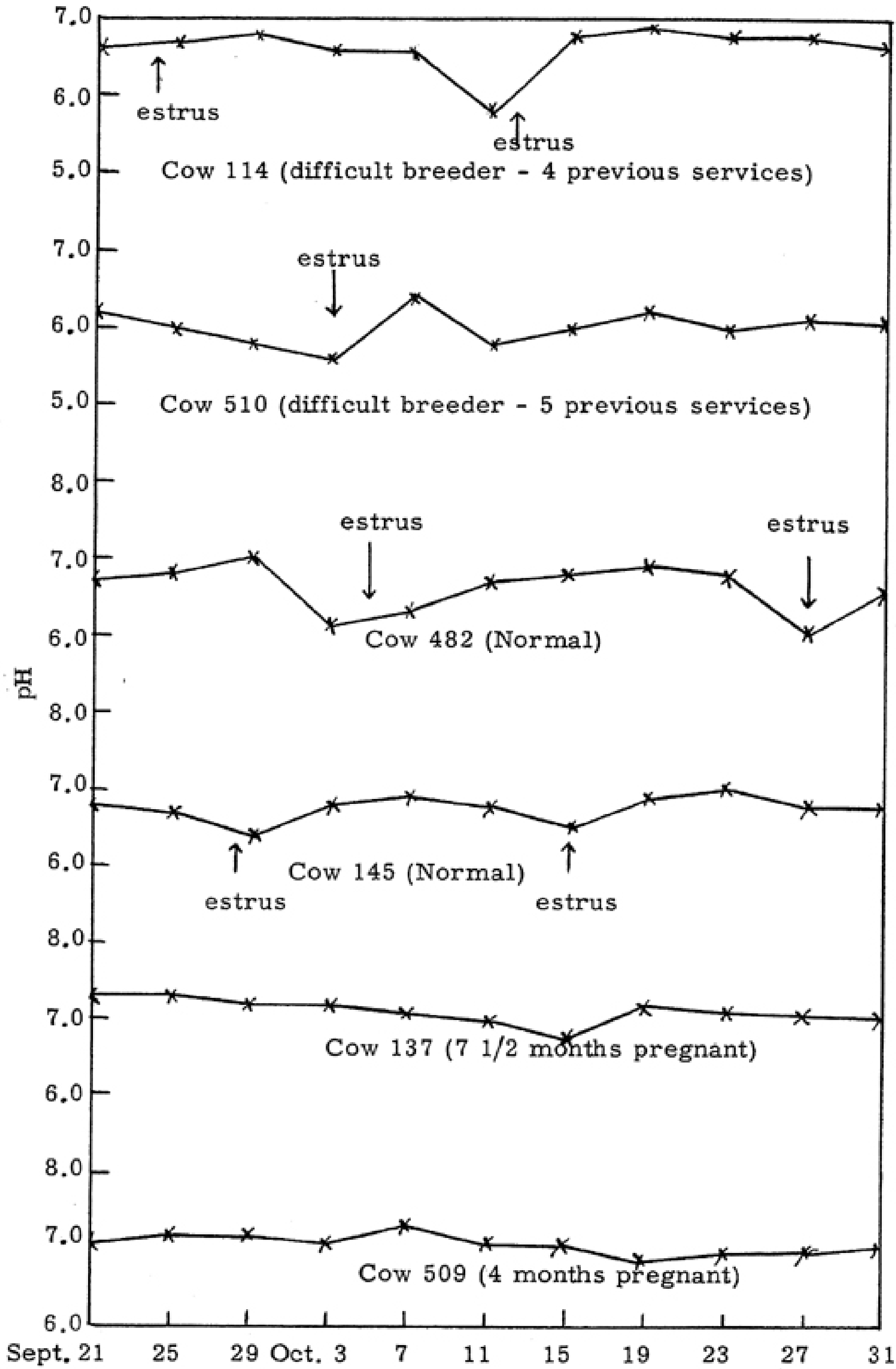

Figure 5.—Changes in the *in vivo* vaginal pH of pregnant, normal, and difficult breeding cows over a period of 41 days.

TABLE 11. IN VITRO pH OF MUCUS AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	No. Cases	Average pH	Range in pH
Hours in estrus			
0- 4	242	7.13	6.52 to 8.25
4- 7	151	7.09	6.18 to 8.30
7-10	143	7.11	6.70 to 8.22
10-13	60	7.15	6.20 to 8.00
13-19	57	7.17	6.70 to 7.80
19-25	23	7.32	6.82 to 8.00

TABLE 12. COMPARISON OF pH VALUES OF 30 IN VIVO AND IN VITRO DETERMINATIONS ON THE VAGINAL-CERVICAL MUCUS OF 10 COWS DURING ESTRUS

(Range of 0.40 to 1.33. P beyond 1% level)

Cow No.	In Vivo pH	In Vitro pH	Difference
109	6.90	7.75	0.85
	7.00	8.00	1.00
508	6.70	7.57	0.87
	6.60	7.42	0.82
134	6.90	7.70	0.80
	7.10	7.75	0.65
490	6.80	7.35	0.55
	6.90	7.55	0.65
	7.00	7.62	0.62
	7.00	7.69	0.69
502	6.60	7.80	1.20
	6.30	7.63	1.33
149	6.40	7.65	1.25
	6.60	7.92	1.32
	6.80	7.95	1.15
507	6.10	7.23	1.13
	6.50	7.51	1.01
	6.30	7.45	1.15
	6.70	7.90	1.20
156	6.20	7.02	0.82
	6.00	6.85	0.85
	6.40	7.41	1.01
	6.50	7.62	1.12
957	6.70	7.12	0.42
	6.50	7.00	0.50
	6.80	7.20	0.40
81	6.30	6.87	0.57
	6.20	7.01	0.81
	6.00	6.75	0.75
	6.40	7.28	0.88
Totals	197.20	223.57	26.37
Average	6.57	7.45	0.88

That this difference was not due to escape of carbon dioxide from the *in vitro* samples was indicated by another experiment in which the pH of 10 paired samples was determined within 1 minute following the *in vivo* determinations (Tables 13 and 14). In these cases the mucus was not taken to the laboratory before *in vitro* determinations were made. Instead, the potentiometer was standardized after the *in vivo* measurement, and the mucus samples, collected simultaneously by drainage and aspiration, were transferred as rapidly as possible from the cow to the potentiometer sample cup. The same difference between the readings from the 2 methods still existed despite sample transfer within 1 minute. The *in vitro* pH of both the draining and aspirated samples was always more alkaline than the *in*

TABLE 13. TYPICAL CHANGES IN THE pH VALUES OF DRAINING AND ASPIRATED MUCUS UPON EXPOSURE TO AIR FOR VARYING LENGTHS OF TIME

(Cow 81, in vivo pH 6.30)

Time between Air Exposure and pH Determination in Minutes	pH of Draining Sample	pH of Aspirated Sample
1	6.97	6.78
2	6.97	6.70
3	6.97	6.65
4	6.92	6.64
5	6.90	6.64
6	6.87	6.60
7	6.86	6.60
8	6.85	6.60
9	6.84	6.60
10	6.82	6.55
15	6.82	6.55
20	6.80	6.55
25	6.80	6.55
30	6.80	6.55

TABLE 14. DIFFERENCES BETWEEN INITIAL IN VITRO pH AND IN VITRO pH AFTER 20 MINUTES EXPOSURE TO AIR OF MUCUS OBTAINED BY 2 METHODS

Cow Number	Difference between the Initial and 20 Minutes in Vitro pH of Draining Samples	Difference between the Initial and 20 Minutes in Vitro pH of Aspirated Samples
134	0.19	0.17
490	0.12	0.19
109	0.13	0.21
149	0.15	0.18
502	0.13	0.21
156	0.17	0.21
957	0.22	0.20
508	0.18	0.17
507	0.13	0.16
81	0.17	0.23
Totals	1.59	1.93
Average	0.16	0.19
Range	0.12 to 0.22	0.16 to 0.23

vivo pH. Table 13 shows the changes in the pH values of mucus collected by draining through the speculum and aspirating with a pipette when exposed to air for varying lengths of time. In every case, the pH of the draining sample was slightly more alkaline than the aspirated sample. A slow drop in pH occurred during the first 20 minutes after the samples were obtained. Table 14 shows the difference between the pH values 1 minute after collection and after 20 minutes exposure to air on mucus samples secured by 2 methods and involves simultaneous samples from 10 cows. The drop in pH after 20 minutes exposure to air averaged 0.16 for draining samples and 0.19 for aspirated samples and was not statistically significant.

The pH of vaginal mucus and cervical mucus was compared in 10 cows. The genital tracts were secured from the animals immediately after slaughter. The vaginas and cervices were cut open and a quantity of mucus sufficient for pH determination taken from each. The results of these studies are presented in Table 15. In all the cervical mucus was more acid than the vaginal mucus, the average difference being 1.1 pH units.

TABLE 15. COMPARISON OF THE pH
OF VAGINAL AND CERVICAL MUCUS FROM THE SAME COW

Cow No.	Reason for Slaughter	Stage in Cycle	pH of mucus from		pH difference
			Vagina	Cervix	
513	Difficult breeder	8 days proestrus	8.3	6.8	1.5
969	Difficult breeder	2 days proestrus	8.1	7.0	1.1
759	Difficult breeder	2 days proestrus	7.9	7.2	0.7
509	Poor udder	6 hours in estrus	7.8	6.7	1.1
14	Poor producer	2 days postestrus	7.7	6.8	0.9
5	Difficult breeder	9 days postestrus	7.8	6.9	0.9
83	Aborted	1 day postpartum	8.5	7.3	1.2
165	Traumatic pericarditis	40 days postpartum	8.2	6.9	1.3
114	Aborted	5 days postpartum	8.3	6.7	1.6
499	Difficult breeder	3 days postestrus	7.5	6.6	0.9

Water content of mucus. By drying 413 specimens of mucus to constant weight in an oven at 55°C., it was found that the water content varied from 95.07 per cent to 99.12 per cent during the first day after heat began. Variations in the percentage of water in mucus at varying intervals during estrus are shown in Table 16. The highest average percentage of water recorded was 98.23 during the first 3 hours of heat and the lowest was 97.32 at 19 to 25 hours after the beginning of estrus.

TABLE 16. WATER CONTENT OF MUCUS
AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	No. Cases	Av. % Water	Range % Water
Hours in estrus			
0- 4	157	98.23	96.28 to 99.12
4- 7	92	98.01	96.81 to 98.87
7-10	89	97.84	95.07 to 98.97
10-13	33	97.83	96.87 to 98.99
13-19	32	97.67	95.09 to 98.97
19-25	10	97.32	95.50 to 98.73

Color reactions of mucus. Color tests were made on 61 mucus samples gathered at various periods during the estrual cycle. The results are summarized in Table 17. Color reactions were obtained that are characteristic of glycogen, the peptide linkage, the phenyl group, as in phenylalanine, tyrosine, and tryptophane, the hydroxyphenyl group, as in tyrosine, the condensation of tryptophane with an aldehyde group, and unoxidized sulfur, as in cystine. The ninhydrin color reaction gave a negative test. The color reactions obtained were fainter during the first part of heat than during the latter part and were most intense about 1 day after heat started.

TABLE 17. COLOR REACTIONS TESTED ON MUCUS

Name of Test	Reaction presumably Due to Presence of	Remarks
Iodine	Glycogen	Positive test
Biuret	Peptide linkage	Feebly positive test
Ninhydrin	α-amino acid groups	Negative test
Xanthoproteic	Phenyl group as in tyrosine, tryptophane, and phenylalanine	Positive test
Millon's	Hydroxyphenyl group as in tyrosine	Positive test
Hopkins-Cole	Condensation of tryptophane with an aldehyde	Positive test
α-nitroso-β naphthol	Tyrosine	Positive test
Sulfur	Unoxidized sulfur as in cystine	Positive test

Discussion

In these studies the highest vaginal temperatures for cows were observed during estrus, the follicular phase of the cycle. In humans it has been observed that the highest temperatures occur during the luteal phase of the menstrual cycle.

The increased heart rate during estrus probably results from increased physical activity. The heart rate increased even in cows that did not feed normally, thus indicating that feed intake was not a contributing factor.

In the bovine reproductive tract there was observed a tendency for the maximal degrees of color, vascularity, and cervical relaxation to coincide with the time of ovulation, but wide individual variation prevents the phenomena from being of any diagnostic value in predicting heat or ovulaton. These phenomena may offer possibilities in the detection of silent heat in cattle.

Bovine mucus is of interest because of the cyclic changes it undergoes. It may play an important role in connection with the physiology of the ascent of sperm in the female tract and could conceivably be related to conditions which allow bacterial invasion of the uterus through the cervix with consequent sterility.

Edema which begins with congestion of the blood vessels and migration of leucocytes into the subepithelial tissues is probably responsible for gradual relaxation of the cervix during heat. Edema is followed eventually by breakdown of blood capillaries to produce the small red spots or "blood points" frequently observed on the external os during estrus. The presence of brownish or blood-tinged mucus in early postestrus is probably due to decomposing blood.

In this study significant differences were noted between *in vivo* and *in vitro* pH determinations on the reproductive tract secretions. Dougherty (1941) suggested that *in vivo* pH might be more accurate than *in vitro* because of the rapid change in pH that some body fluids undergo when exposed to air. In this study the differences between the *in vitro* readings were well within the range of experimental error, but a significant difference between *in vivo* and *in vitro* pH existed. If this difference were due to exposure to air, the more alkaline *in vitro* pH would suggest a loss of carbon dioxide. This appears highly improbable since the first *in vitro* readings were always more alkaline than those taken at varying time intervals after exposure to air. The method of collection should also make a difference between *in vitro* pH if the escape of carbon dioxide were involved. Aspiration should remove more carbon dioxide to make the reading more alkaline; however, in every case the aspirated sample was more acid than the draining sample.

Some workers (Taylor and Whitaker, 1927; Dubuisson, 1937 and 1940) have reported that when an electrode was inserted into a mass of living or moist tissue, a film was formed about it. Across such a film there would tend to be differences of electrical potential which would seriously interfere with electrical pH measurements. Since the glass surface of the *in vivo* pH electrode was considerably greater than that of the *in vitro* electrode, it would appear reasonable to expect the *in vivo* pH to be more acid than *in vitro* pH, because surface glass catalysis tends to produce a more acid pH. Perhaps these were contributing factors to the significant differences between *in vivo* and *in vitro* pH values.

The results of this study agree with literature reports that the pH of bovine reproductive tracts and its secretions decline the day before, the day of, or the day after estrus. The cause for this decrease in pH is not known. Woodman and Hammond (1925) suggested that it might be due to a flowing of the more acid cervical mucus into the vagina. If it were the cervical mucus alone causing the shift in pH, the shift should occur during proestrus and early estrus since it is largely during this period that greatest amounts of cervical mucus drain into the vagina. Perhaps this lower pH is an estrogenic effect resulting from increased follicular activity. The pH of the vagina of humans and monkeys (Hall and Lewis, 1936) becomes highly acid after estrogenic treatment. The mode of action of estrogenic hormones in lowering vaginal pH is not known.

The fact that the bovine cervix is more acid than the vagina raises some interesting questions regarding the ascent of spermatozoa in the genital tract. The cervix of the human has been reported (Kessler and Uhr, 1927; Meaker and Glasser, 1929; Miller and Kurzrok, 1932) to be more alkaline than the vagina, and it has been suggested that this pH differential aids sperm in their passage through the female genital tract. If sperm are attracted by the more alkaline cervix in humans, would they be repulsed by the relatively acid cervix in cattle? If a general mechanism guides the ascent of sperm in mammalian genital tracts, it would seem to be other than a pH differential, since the differences between cattle and man are in opposition.

If, as has been stated, alkaline douching of the vaginas of animals leads to the production of a preponderance of male offspring and acid douching leads to the production of a preponderance of female offspring, should we not expect that those species having an acid vagina, as man and monkey, should have a greater number of female offspring, or on the other hand, that animals having alkaline vaginas as cows and mares, should have a preponderance of male offspring? The secondary sex ratio of the above species is near equality.

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? Lohmann (1943) found the *in vivo* pH to vary with the season. Is it possible that feeding practices may influence the pH of the bovine reproductive tract?

Since weakly alkaline solutions are known to be mucus solvents, it is conceivable that douching with such materials and the resultant liquefaction of the mucus would materially aid sperm to ascend the tract. Wester (1921) attributed success of use of sodium bicarbonate douches in "shy" breeding cattle to a mechanical removal of spermatoxins of an unknown nature. This explanation is sufficiently interesting to warrant further study. Zwaenpole and Roger (1943) douched vaginas of mares with warm water and found an exhibition of the symptoms of estrus. Douching in cattle, if it is successful as is the popular conception, probably exerts its effect partly by increasing the normal symptoms of heat.

Study at intervals during estrus showed the flow elasticity and water content of bovine mucus to be maximal during early estrus. Since more determinations were made on early estrus samples than on later samples, this time relationship may account for the average flow elasticity of 17.21 mm. in contrast to 14.7 mm. flow elasticity reported by Scott-Blair *et al.* (1941b) during estrus.

The fact that the color reactions given by bovine mucus were usually fainter during the first part of heat than at succeeding stages is probably due to the acellularity and water content. The positive color reaction to the iodine test which is presumably due to the presence of glycogen is of interest. Because of its molecular size glycogen cannot escape by diffusion through the cellular membrane. Traces of glycogen or glycogen-like polysaccharides have been detected in blood by a number of investigators (Best, 1919; MacLeod, 1917). Unshelm (1935) found from 5 to 6 milligrams per cent in normal whole blood, confined almost entirely to the cellular elements. Its concentration varied with the number of leucocytes. The increase in leucocytic concentration of mucus toward the end of heat may explain the more intense iodine test at this time. On the other hand, the mechanism of mucus secretion is obscure. Heilbrunn (1947) states: "solid materials may be definitely extruded; this is true in the case of mucus-secreting goblet cells found in both invertebrates and vertebrates." Within the cell, the mucus may be concentrated in a plug on that side of the cell near the free surface, and when rapid extrusion of mucus is required, the entire plug may be cast out. It is therefore possible that glycogen, as such, is a normal constituent of mucus.

The significance of the amino acids suggested by the color reactions is not established. One can offer the presumption that they share with carbohydrates the function of nutrition of the gametes, and perhaps the fertilized ova.

SECTION III.—HISTOLOGICAL CHANGES OF THE GENITAL TRACT DURING THE ESTRUAL CYCLE

Review of Literature

In histological studies of bovine reproductive tracts, Hammond (1927) distinguished 3 areas in the vagina: (1) next to the os uteri, (2) above the urethra, and (3) next to the vulva. While there was no sharp demarcation between these areas, the character of the epithelium gradually changed from the vulva to the external os. Near the vulva stratified squamous epithelium was thick. It decreased in thickness as the os was approached. Near the external os the epithelium was thin and the superficial layers consisted of tall columnar mucus-secreting cells with many goblet cells interspersed between them.

Murphey (1924) found that the vaginal epithelium was only 2 to 4 cells thick at midcycle (10½ days) and remained this way until the sixteenth day. Then it thickened until the eighteenth day. On the eighteenth day desquamation began and leucocytes were noted occasionally in the epithelium and frequently in the mucosa. Desquamation was completed within 24 to 36 hours following the onset of heat. On the twentieth day lymph flowed from the mucosa through spaces in the epithelium. From the nineteenth to the third day leucocytic migration and infiltration was heavy and then passed rapidly into a quiescent state.

Cole (1930) agrees with Hammond on the fact that cyclic changes occurred throughout all areas of the vagina. The entire epithelium was thickened during estrus and postestrus and decreased in thickness during early proestrus. The most marked changes occurred in the area adjacent to the cervix. Instead of cornification of the epithelium during estrus, as in other species, the superficial epithelium was composed of tall columnar mucus-secreting cells. Brown (1943) pointed out that the epithelium was reduced to a minimal number of layers during estrus. In spite of this, he found that it attained maximum thickness because of an increase in height of the individual cells of the superficial layer. This condition persisted through estrus. During the first day postestrus the number of layers in the epithelium increased, but the superficial layer began to recede in height. The second day postestrus the number of layers in the epithelium was even greater than 1 day postestrus and the superficial cells had decreased to a polyhedral form. From this time on the epithelium tended to decrease in thickness and the superficial layer varied from flattened to low columnar. Leucocytes were in greatest concentration 1 to 2 days postestrus.

Sergin *et al.* (1940) reported that smears from the vaginal vestibule showed increased numbers of cornified cells during proestrus and estrus, but leucocytes were never absent. There was a sudden, marked increase in leucocyte content 3 hours after mating, which was probably due to the presence of semen in the tract and progressing estrus.

The cervix is covered with a single layer of mucoid epithelium which is much folded. Murphey (1924) has described the typical loaded and secreting cell as high columnar and granular with a circular or laterally compressed granular nucleus. He noted goblet cells frequently which were nearly always flanked by pyknotic cells. The maximum height of the loaded state occurred about the seventeenth or eighteenth day. Active secretion of mucus began at the tips of the primary and secondary folds of the cervix on the nineteenth or twentieth day of the cycle. An exhaustion stage gradually proceeded down the sides of the folds and reached the bottom of the crypts by the fourteenth day. Hammond (1927) found the histological changes of the cervix to be similar to those occurring in the upper vagina: congestion and edema during and just after heat; cubical cells in diestrus, becoming columnar and full of mucus during heat, discharging and becoming ragged and cubical about 72 hours after heat.

The uterus contains numerous cotyledons which function in fetal attachment. Hammond (1927) observed that the cotyledonary area had a dense stroma, was highly vascularized, and had no gland openings. The intercotyledonary area was richly supplied with glands. At the end of diestrus the uterine epithelium was tall columnar and pseudostratified and the stroma was congested and edematous. Cole (1930) further noted that the intensity of these changes increased during, and 1 day after, heat. By the second day postestrus the congestion and edema had diminished and many of the congested blood vessels broke down, but at no time did the epithelium break. The glands were relatively quiescent and their lumina straight during proestrus and estrus. At 2 days postestrus they were coiled and their lumina filled with secretion. These glands began to hypertrophy, as the corpus luteum grew, to reach their greatest growth about 12 days postestrus. Glandular atrophy began about the fifteenth day. Murphey (1924) found that vacuolar changes in the epithelium of the uterus gradually increased from the twentieth to the fourth day, when this change was maximum. He further noted that leucocytic infiltration began about the nineteenth day and reached its maximum on the fifth day and gradually subsided until the fourteenth day. Leucocytes were mostly polymorphonuclear, but many mononuclears were also present. Cupps and Asdell (1944) found the muscle fibers of the uterus to be longest during and for 2 days following heat. Afterwards the fibers diminished in size to become shortest about the seventeenth day.

Asdell (1946) has summarized the literature dealing with the cyclic changes in the tubal epithelium of the bovine. Mucus and leucocytes were most abundant in the lumen about 2 days postestrus. The epithelium decreased in height about 8 days postestrus and at this time numerous globules of cytoplasm were found. During proestrus the cilia were most prominent and the cells began to enlarge to reach their greatest height during heat. Epithelical height was lowest at 8 to 12 days postestrus, which Asdell believed was due to the extrusion of protein.

Materials and Methods

Tissue sections of the vagina, cervix, uterus, oviducts, and ovaries were taken from the organs of 13 cows sold for slaughter from the Missouri Station dairy herd. The animals were slaughtered for such reasons as poor production, pendulous udders, traumatic pericarditis, and difficult breeding, even though the estrual cycles were fairly regular and examinations by veterinarians revealed no pathological abnormalities. It was assumed that these conditions which elicited slaughter would not interfere with the normal histology of the reproductive tract. The breed, age, and reproductive history of each animal are presented in Table 18. The majority of the animals were observed through 2 or more complete estrual cycles prior to slaughter.

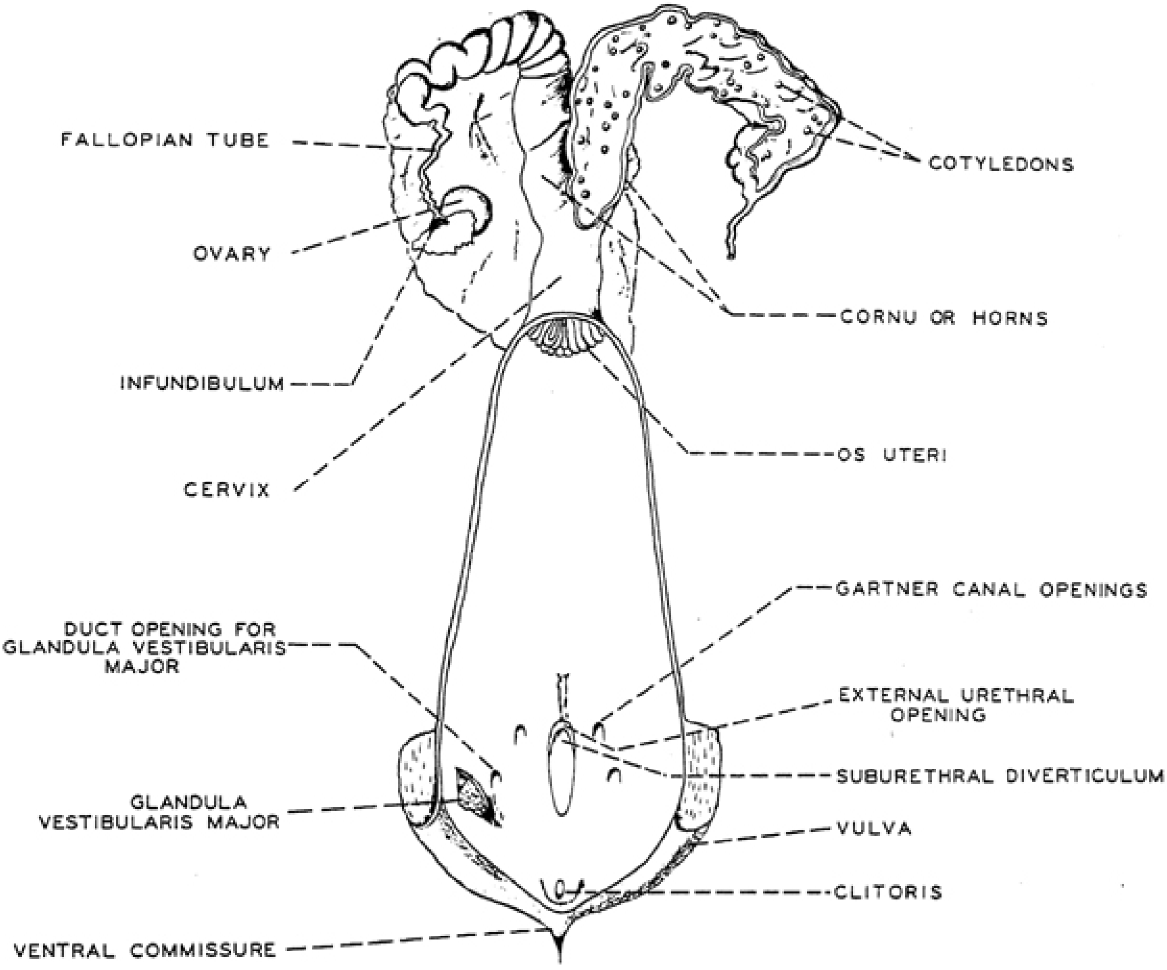

Figure 5a.—Diagrammatic view of the genital organs of the cow (from Herman and Madden, 1949).

Immediately after the animals were slaughtered the entire genital tract was removed (Figure 5a). Within 15 to 30 minutes following removal, the genital organs were examined thoroughly and notations on the gross anatomy recorded. The slaughter data and gross anatomy notations are presented in Table 19. The genital organs secured represent various successive stages of the estrual cycle and constitute the material upon which this study is based.

The tissues were placed in Bouin's fixative, subsequently dehydrated in alcohol, embedded in paraffin, and sectioned at 8 to 10 micra. Delafield's hematoxylin, Mayer's hemalum, eosin, and erythrosin were used for routine stains. Special stains used were Mayer's mucicarmine to demonstrate mucus and Wilder's silver impregnation technique for reticulum and collagenous fibers. Measurements of the thickness of the epithelium and stroma were made with an eyepiece micrometer.

TABLE 18. BREED, AGE, AND REPRODUCTIVE HISTORY OF COWS FROM WHICH TISSUE SECTIONS WERE TAKEN FOR HISTOLOGICAL STUDY

Cow No.	Breed	Age Years	Age Months	Previous Calves	Length of Previous Cycles in Days
104	Holstein	5	4	3	18, 20, 21, 18, 20, 29, 11, 19, 18, 23, 19, 15
507	Jersey	4	1	2	24, 22, 24, 24, 24, 23, 22, 23
90	Holstein	5	11	1	20, 23, 23, 22, 22, 22, 22, 24, 21, 22, 21, 24, 25
513	Jersey	3	9	1	22, 21, 20, 23, 23, 22, 23
969	Jersey	6	10	4	29, 27, 21, 30, 24, 24
759	Holstein	11	10	9	19, 23, 20, 20, 18, 20, 21, 19, 19, 21, 19, 20, 18, 21
509	Jersey	4	3	2	22, 23, 23, 22
14	Holstein	9	4	4	Incomplete records 23, 23, 23
5	Holstein	9	9	6	22, 22, 18, 20, 20, 21, 21
83	Holstein	6	5	4	20, 20, 19, 20, 21, 23, 26, 19
165	Holstein	3	0	1	21, 22, 21
114	Holstein	5	8	3	16, 18, 18, 19, 18
499	Guernsey	3	1	0	21, 21, 18, 18, 22, 23 30

Results

Cyclic changes in the vagina. For descriptive purposes the stratified squamous epithelium of the bovine vaginal mucosa may be divided into 3 layers: a basal layer of germinal epithelium, a middle layer of irregular polyhedral cells, and a superficial layer of flattened squamous cells or columnar mucus-secreting cells.

The epithelium of the vagina near the urethra was irregular in depth throughout the cycle due to varying degrees of penetration by low, broad, stromal papilla. The epithelial cell layers varied from 4 to 33 during the cycle. Blood vessels were present in all levels of the stroma and increased in size as the depth of the stroma increased. Folds in this region of the mucosa were rare and when present were merely small pockets in the wall.

During proestrus the epithelium consisted of small, compact, deeply staining cells. Small cysts or epithelial follicles, usually filled with lymphocytes, were frequent. The epithelium was not true stratified squamous since the superficial cells were polyhedral rather than squamous in character. Lymphocytes formed a nearly continuous layer subjacent to the basement membrane. Blood vessels of the superficial stroma were congested.

TABLE 19. SLAUGHTER AND GROSS ANATOMY DATA ON COWS FROM WHICH TISSUE SECTIONS WERE TAKEN FOR HISTOLOGICAL STUDY

Cow No.	Gross Anatomy of:					Stage of Cycle at Slaughter
	Ovaries	Oviducts	Uterus	Cervix	Vagina	
104	Large follicle on right ovary. Large, deep corpus luteum with fluid on the left ovary.	Normal. Irrigated.	Edematous. Right horn larger and more vascular than left.	Filled with clear, watery mucus.	Bright red in color. Edematous.	1 day proestrus.
507	One large mature follicle on right ovary. Large corpus luteum and one deep, small corpus luteum left ovary.	Right oviduct normal. Slight adhesions in fimbriated portion of left oviduct.	Large amounts of watery fluid in left horn which was more vascular than right.	Gelatinous plug in external os. Good tone throughout. Opaque mucus in cervix proper.	Normal, no visible mucus.	6 days postestrus.
90	About 20 developing follicles right ovary. Small corpus luteum and 10 developing follicles left ovary.	Normal. Irrigated.	Mucosa hard and thick.	Edematous, large amounts of transparent mucus.	Vestibule coated with thin layer of glistening mucus.	7 days postestrus.
513	Thick walled follicle and one corpus luteum on right ovary. Two small, tough, thickwalled follicles on left ovary.	Normal. Irrigated.	Edematous. Left larger and more highly vascularized than right horn.	Small amounts of clear, watery mucus.	Normal in appearance.	8 days postestrus.
969	One large follicle and corpus luteum on right ovary. One large follicle, thickwalled and filled with bloody fluid on left ovary.	Free of obstructions. Irrigated.	Right horn more highly vascularized than the left. Some clear watery fluid.	Small mucus plug in external os.	Good tone. Slightly edematous near vulva.	2 days proestrus or 18 days postestrus.
759	Three, large, deep follicles on right ovary. One large, apparently double corpus luteum.	Normal. Irrigated.	Highly vascularized, walls of left horn about 1/2 inch thick.	Slightly relaxed. Mucus plug in external os.	Normal. Slightly edematous.	2 days proestrus or 18 days postestrus.
509	One large follicle, one fluid filled corpus luteum on right ovary. Extremely large corpus luteum on anterior end of left ovary.	Normal. Irrigated.	Small, poorly vascularized. Large amounts watery fluid.	Could insert index finger in external os. Between rings filled with clear mucus.	Engorged with blood. Some clear mucus in the vestibule.	6 hours in estrus.
14	Large follicle with heavy wall on right ovary. Deep golden colored corpus luteum on left ovary.	Normal. Open. Irrigated.	Small, poorly vascularized. Small amount of watery fluid.	Double at external os. 4 rings present.	Mucosa highly folded. Small amount of mucus in vestibule.	2 days postestrus.
5	Large heavy walled follicle on right ovary. Four small corpora on left ovary.	Normal. Open. Irrigated.	Poorly vascularized. Right horn filled with watery fluid	Congested with clear, transparent, stringy mucus.	Clear, transparent mucus in vestibule	9 days postestrus.
83	One large, golden yellow corpus luteum covering approximately 2/3 right ovary. Three small degenerate corpora on left ovary.	Right larger and longer than left. Irrigated.	Left horn small. Right extremely large, highly vascularized, sloughing mucosa, filled with bloody pus.	Relaxed. Blood present.	Edematous, engorged with blood.	1 day postpartum.

TABLE 19. (Continued)

Cow No.	Gross Anatomy of: Ovaries	Oviducts	Uterus	Cervix	Vagina	Stage of Cycle at Slaughter
165	Large, red corpus, 4 developing follicles on right ovary. Orange-yellow corpus and one small purplish-red developing follicle on left ovary.	Normal. Irrigated.	Right horn large, highly vascularized. Cotyledonary areas well defined. Left horn small and poorly vascularized.	Good tone, closed tightly. Filled with rubbery, yellowish mucus.	Vestibule much folded, covered with glistening, watery, stringy mucus.	40 days post-partum. No heat periods since calving.
114	Approximately 20 developing follicles and 1 large corpus right ovary. Several small developing follicles with 1 deep seated follicle on left ovary.	Normal. Irrigated.	Right horn extremely vascularized. Left normal. Both filled with mucus and pus.	Large, highly dilated. Filled with greenish-yellow pus.	Several blister-like pimples near the external os.	5 days post-partum.
499	8 developing follicles and 1 large blood colored corpus right ovary. Two large follicles and 5 small follicles, 1 golden red corpus left ovary.	Normal. Irrigated.	Small but apparently normal	Double external os that opened into 1 passageway at 2nd annular ring. Filled with clear, thick mucus.	Vestibule not folded, small, hyperemic.	3 days post-estrus.

A similar histological picture prevailed during estrus. The stratum germinativum became more pronounced and the nuclei stained more intensely, were more crowded, tended to become elongate in shape, and were arranged with their long axis perpendicular to the membrana propria. Mitosis, observed throughout the cycle, was most frequent during estrus. The polyhedral cells of the middle zone constituted the bulk of the vaginal epithelium. They contained larger amounts of faintly staining cytoplasm than did the cells of the basal and superficial layers. The nuclei were oval and distinct. The small cysts or epithelial follicles were more concentrated in the middle zone than in the other epithelial zones. Lymphocytes and leucocytes were present in all epithelial layers. The average epithelial height during estrus was 54 micra as contrasted to 46 micra during proestrus.

Two days postestrus the epithelial height had increased markedly to an average of 84 micra. This increase was due largely to the increased size of the polyhedral cells of the middle zone. There was a tendency for the superficial cells to be more flattened than they were during estrus and for the lymphocytic and leucocytic concentration to be somewhat reduced. The stromal density and congestion of the smaller blood vessels of the superficial stroma were markedly increased over the estrus conditions.

To about 10 days postestrus the superficial layers of epithelium tended to become more squamous in character. True cornification was not observed. Superficial epithelial desquamation, although evident throughout the cycle, was maximum between 3 to 10 days postestrus.

The mucoid epithelium approximately 2 to 3 cm. from the os uteri is distinctly characteristic of the cow, and differs considerably from the vaginal epithelium of most species. Instead of epithelial cornification during estrus, as is common in most species, the superficial epithelium is composed of tall columnar, highly active, mucus-secreting cells. Below the superficial layer the epithelium is of the stratified squamous type and varies considerably in thickness at different stages of the cycle.

The histological changes at this level of the vagina follow a rhythmic pattern during the estrual cycle. During estrus the superficial layer was at its maximal height due to enormous distention of the cells with mucus. In many areas the epithelium was reduced to a single layer of mucus cells, while in others it consisted of 2 to 5 layers of polyhedral or squamous type cells in small clusters adjacent to the membrana propria. Since desquamation was not observed in this region of the vagina it was difficult to account for the reduced number of layers of epithelial cells unless possibly the cells were "used up" in the process of mucus secretion. The nuclei of these mucus-secreting cells were greatly compressed and stained intensely with hematoxylin. They showed little, if any, nuclear differentiation. The basal portions of the cytoplasm of the mucus cells stained more intensely than did the apical portions.

One day postestrus the epithelum resembled closely that of estrus. The columnar cells had a tendency to be reduced in height and there was a slight increase in the number of layers of squamous or polyhedral cells. Occasional leucocytes were observed in the superficial stroma and basal epithelial layers.

Two days after heat the superficial epithelium was reduced still further in height, had changed to a cubical form, and the luminal borders of the cells were serrated and irregular. The number of layers of squamous cells cells reached a maximum at this stage of the cycle. In contrast to estrus, the stroma was slightly denser, the congestion of the blood vessels reduced, and the concentration of leucocytes decreased.

From 3 days postestrus the superficial epithelial cells varied from low columnar to cuboidal and remained so during the rest of diestrus. The number of layers of squamous cells decreased gradually to become minimal at estrus.

Following parturition the epithelium was comparable to the interestrual condition. The superficial cells were cuboidal to columnar in type, the stroma was dense and fibrous, and stromal vascularization was slightly less than during the interestrual period.

The cyclic changes that occurred in various regions of the bovine vagina are illustrated in Figures 6 through 15 (pages 42, 43, 44 and 45).

Cyclic changes in the cervix. Anatomically the cervix is part of the uterus, but it will be discussed separately because it is both morphologically and physiologically a distinct organ. The mucosa of the cervix was thrown into many plica that had a central core of cell-poor connective tissue. These plica were lined by a single layer of mucoid epithelium which formed sim-

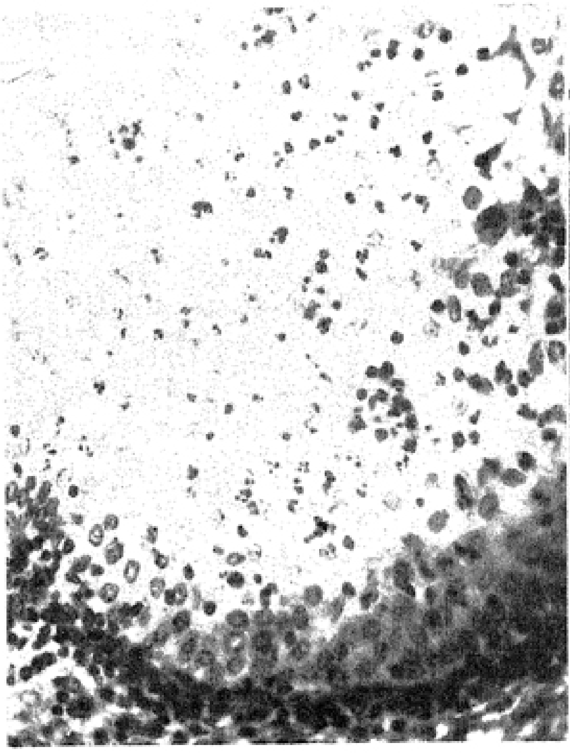

Figure 6.—Vagina near urethral orifice at 6 hrs. in estrus. Note epithelial height and leucocytes subjacent to epithelium. Cow 509 x 352. Hematoxylin and eosin.

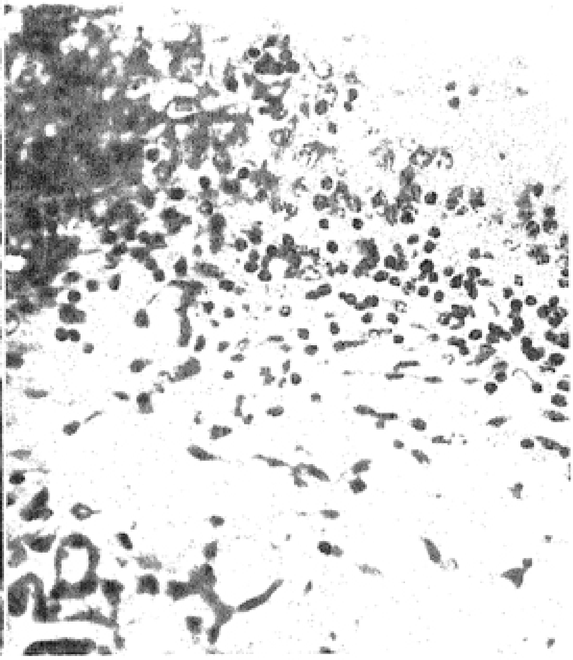

Figure 7.—Vagina near urethral orifice at 2 days postestrus. Note heavy concentration of lymphocytes in superficial stroma and crowding of germinal layer of epithelium. Cow 14 x 352. Hematoxylin and eosin.

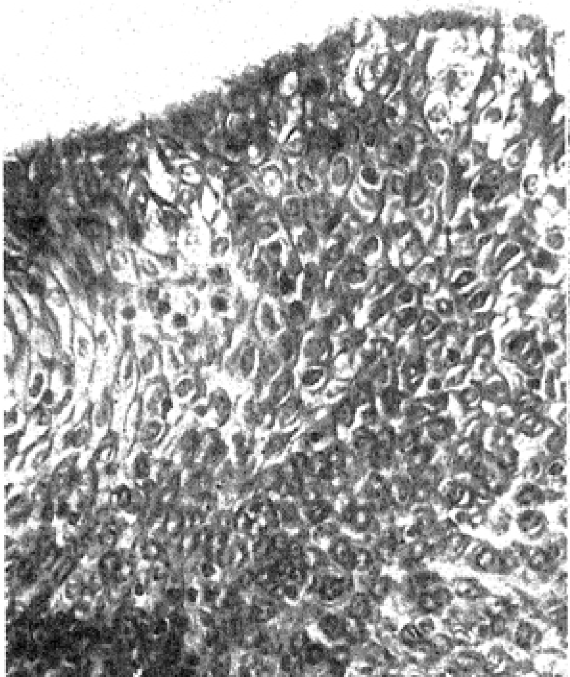

Figure 8.—Vagina near urethral orifice at 8 days postestrus. Note honeycomb appearance of polyhedral cells in middle zone of epithelium and tendency toward cornification of superficial cells. Cow 513 x 352. Hematoxylin and erythrosin.

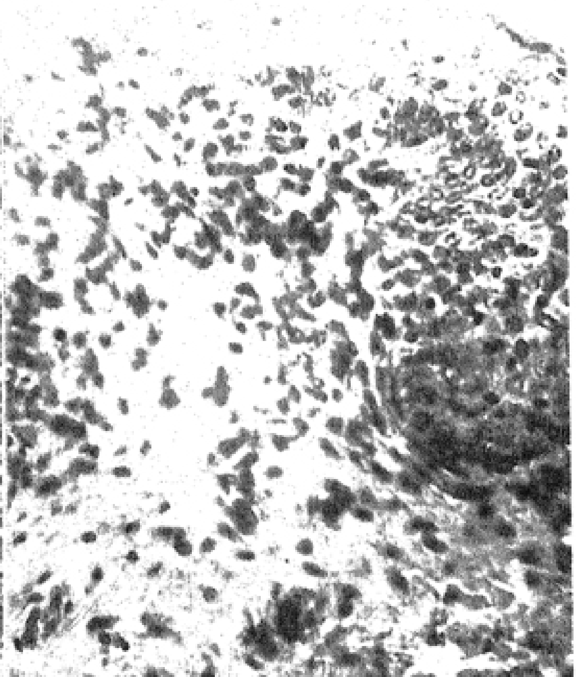

Figure 9.—Vagina near urethral orifice at 9 days postestrus. Observe squamous character of superficial cells and absence of leucocytes in epithelial layers. Cow 5 x 352. Hematoxylin and erythrosin.

ple sacculated and/or branched tubular glands. The character of the epithelium varied considerably in different stages of the cycle.

Evidence for secretory activity by the columnar epithelium of the cervix was found at all stages of the cycle. During estrus the cells were tall columnar with basally crowded and elongate nuclei that were arranged with their long axis perpendicular to the basal membrane. The epithelial height was typically 15 to 24 micra, 10 to 16 micra staining intensely with mucicarmine. Lymphocytes were noted in both the stroma and epithelium, but leucocytes were lacking. The stroma was loose and slightly edematous.

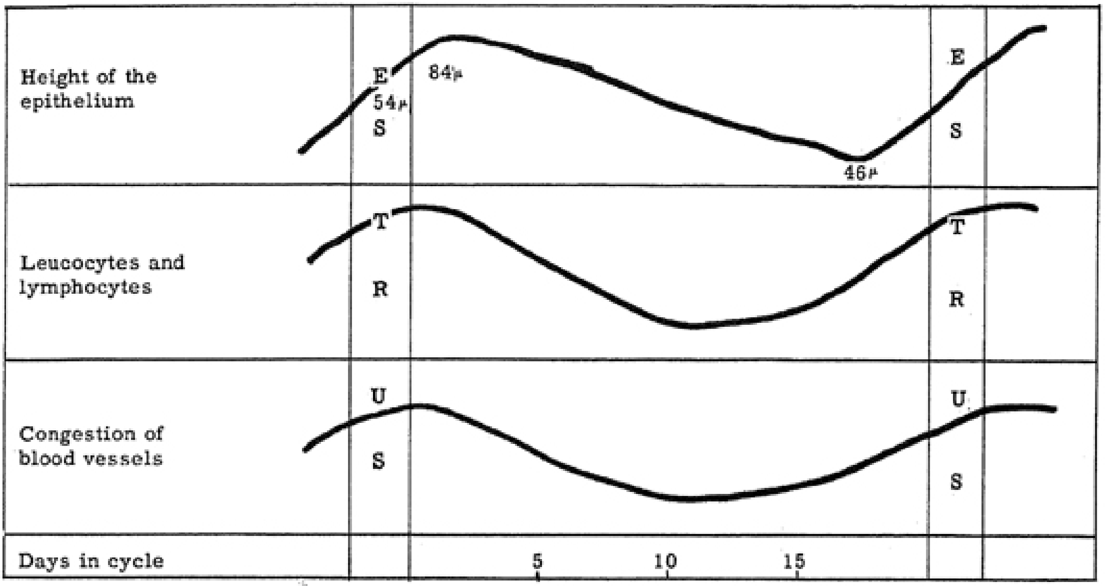

Figure 10.—Schematic diagram illustrating the histological changes in the bovine vaginal vestibule near the urethral orifice during the estrual cycle.

Two days postestrus the height and form of the superficial epithelium was quite variable. In some areas the epithelium was tall columnar with crowded, basal, and elongate nuclei, whereas in other regions the cells had become cubical, the nuclei being oval and less basally located. The stroma had become denser and the smaller blood vessels less congested than during estrus.

At 6 to 10 days postestrus the epithelial cells were greatly reduced in size and stained only lightly at the periphery with mucicarmine. In the deeper crypts, however, some of the cells were columnar and stained intensely with mucicarmine. The height of the epithelium at this stage of the cycle was characteristic of the larger part of the interestrual period and varied from 12 to 20 micra.

During proestrus the epithelium increased slightly in depth, the cells changing from cuboidal to columnar form. Mucicarmine staining showed that the cells started secreting mucus at this time. The stroma was loose and edema slight. Figures 16 through 20 show the cyclic changes in the cervical epithelium (see pages 46 and 47).

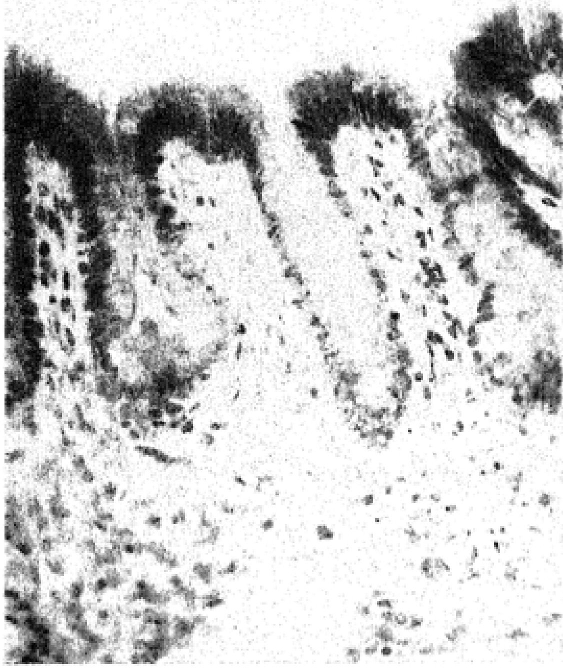

Figure 11.—Vagina near cervix during estrus. Note distension of tall, columnar cells with mucus and basal, elongate, dark-staining nuclei. Cow 509 x 352. Hematoxylin and mucicarmine.

Figure 12.—Vagina near cervix at 6 days postestrus. Note serrated appearance and reduced height of superficial columnar cells. Cow 507 x 352. Hematoxylin and eosin.

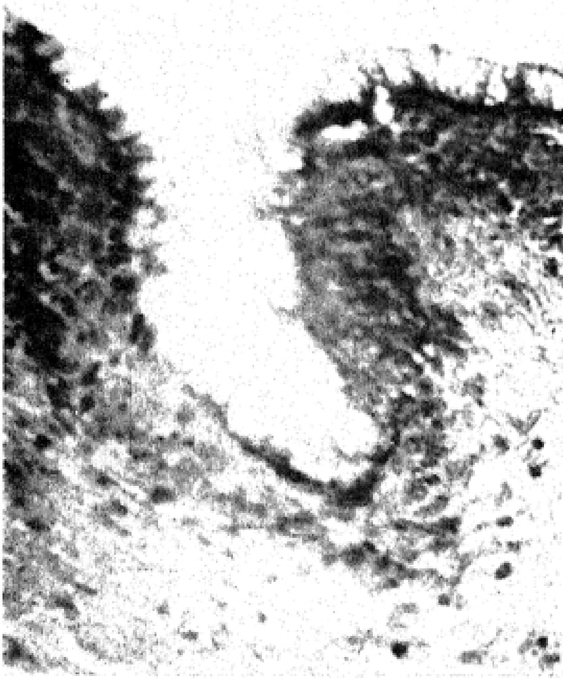

Figure 13.—Vagina near cervix at 2 days proestrus. The mucus-secreting cells have increased in height and rest upon deeper layers of polyhedral cells. Cow 969 x 352 Hematoxylin and erythrosin.

Figure 14.—Vagina near cervix at 1 day proestrus. Note increased height of superficial columnar cells and single layer of epithelium. Cow 104 x 352. Hematoxylin and erythrosin.

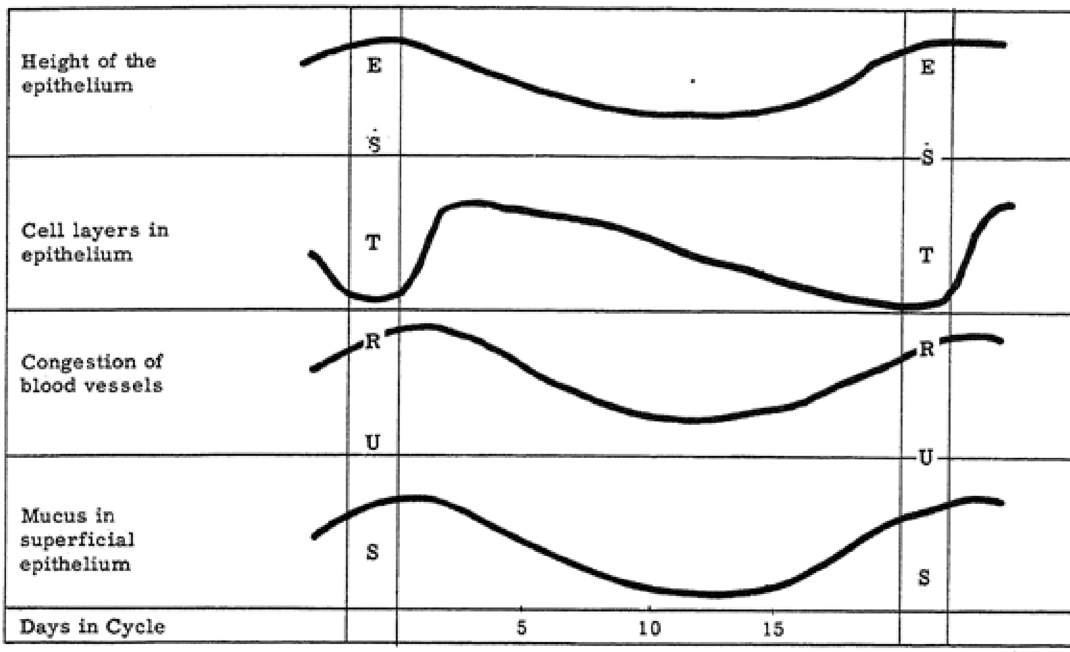

Figure 15.—Schematic diagram illustrating the histological changes in the bovine vagina near the cervix during the estrual cycle.

Cyclic changes in the uterus. The mucosa of the bovine uterus is not homogenous like the majority of animals but, in common with ruminants, is differentiated into cotyledonary and intercotyledonary areas. The intercotyledonary area resembles the uterine surface of most animals and is the surface onto which the uterine glands open. Beneath the epithelium there was a thin, dense layer of connective tissue followed by a loose layer of connective tissue in which the uterine glands ramified. The cotyledonary areas consisted of raised button-like projections arranged in rows parallel to the long axis of the uterus. No uterine glands opened onto their surface. They were covered by a single layer of epithelium beneath which was a mass of dense connective tissue richly supplied with blood vessels.

The cyclic histological changes occurring in the uterus during the estrual cycle are shown in Figures 21 through 28.

During estrus the nuclei of the epithelial cells lining the uterine lumen and the gland ducts were elongated and situated basally. The glandular lumina were small due to the height of the epithelial cells composing their bodies. The stroma was edematous and cell-poor.

At 2 days postestrus the cytoplasm of the surface epithelium stained more intensely than during estrus. The stroma was considerably increased in density and less edematous. The glands had become more coiled at their base and the lumina were, for the most part, filled with a secretion.

Nine days after heat the superficial stroma was highly vascularized and congested, but little extravasation was observed. The nuclei of the epithelial cells had become oval and stained poorly. Glandular hypertrophy was very marked and the epithelial height appeared to be maximum.

Figure 16.—Cervix at 2 days postestrus. Note the elongated nuclei which are crowded basally. Cow 14 x 352. Hematoxylin and mucicarmine.

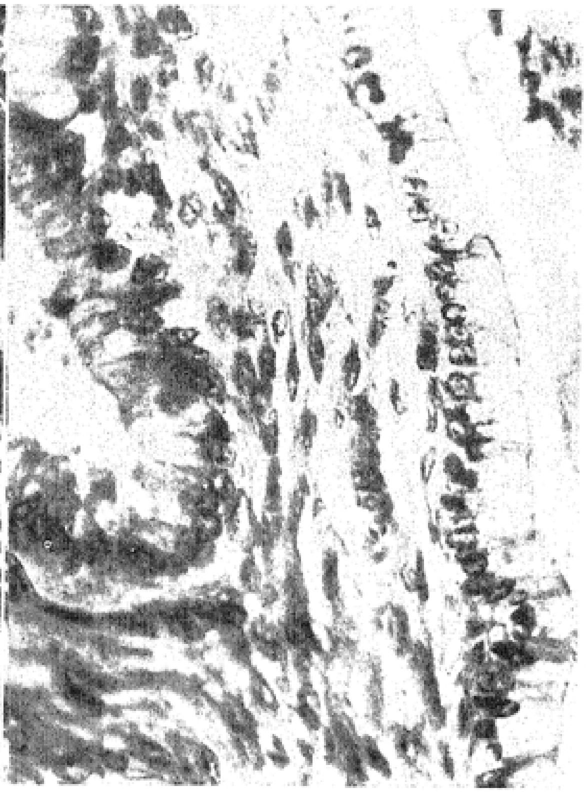

Figure 17.—Cervix at 3 days postestrus. Note the nuclei are more oval and less basally located. Cow 499 x 352. Hematoxylin and mucicarmine.

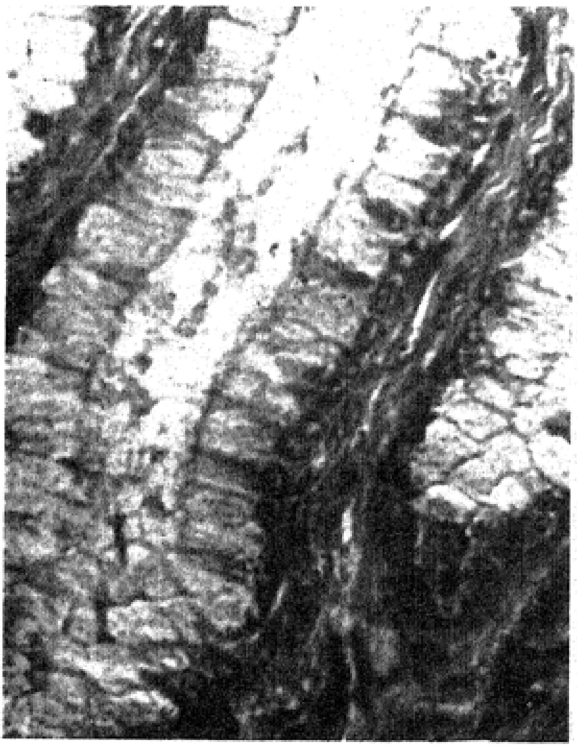

Figure 18.—Deep cervical crypt at 7 days postestrus. Note mucus streaming from the tall columnar cells. Cow 90 x 352. Hematoxylin and erythrosin.

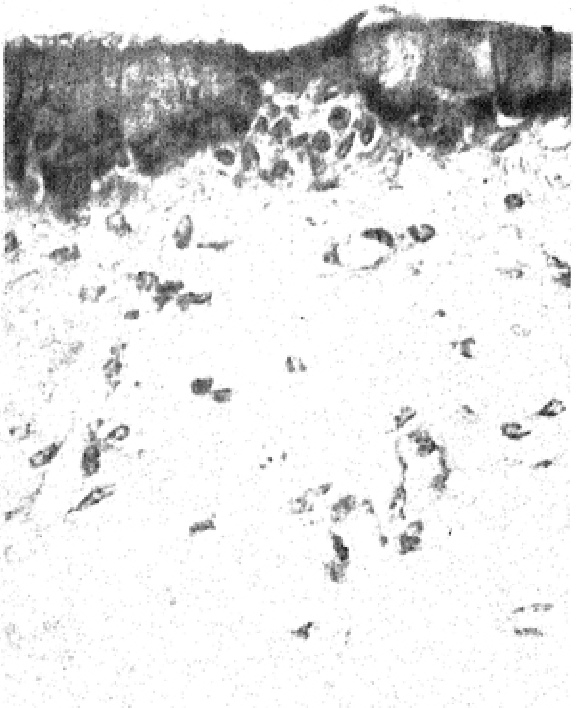

Figure 19.—Mucoid epithelium of cervix at 2 days proestrus. Note transition from cubical to columnar cells. Cow 969 x 352. Hematoxylin and mucicarmine.

During proestrus the epithelial cells were tall columnar and pseudostratified and the nuclei tended to be located basally. The glands were spiral in shape and branched freely in the superficial stroma. Leucocytes, which were present throughout the cycle in the stroma and epithelium, were present in greatest numbers during proestrus and estrus.

Variations in epithelial height, stromal density, and vascularization of the cotyledonary areas were similar to, but less pronounced than, those occurring in the intercotyledonary areas.

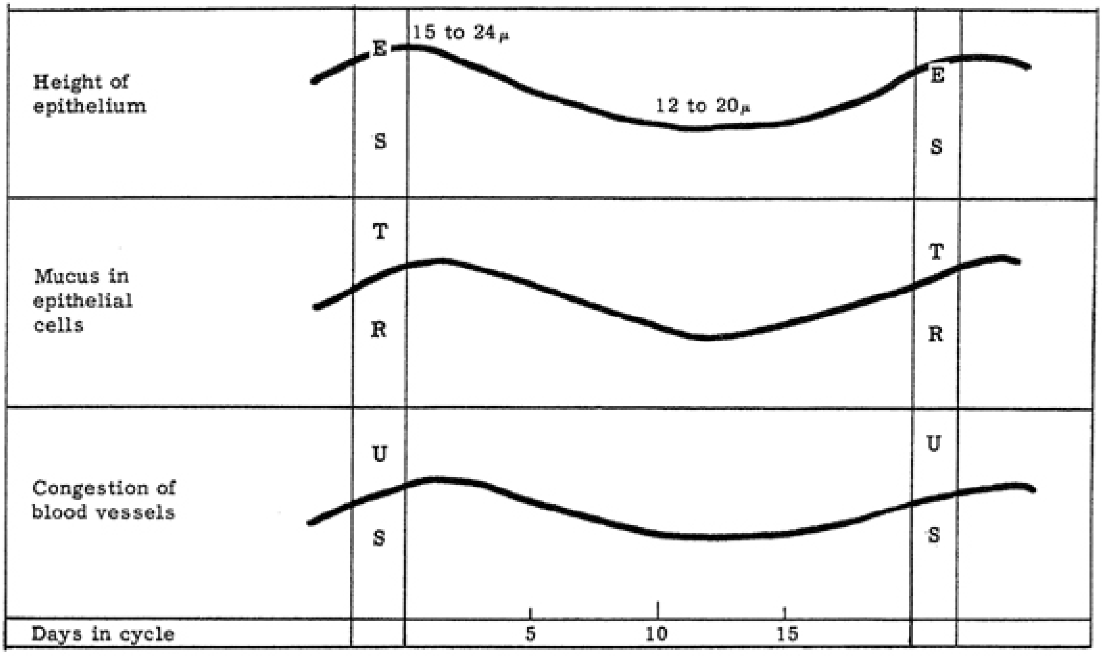

Figure 20.—Schematic diagram illustrating the histological changes in the bovine cervix during the estrual cycle.

Cyclic changes in the oviducts. For descriptive purposes, the oviducts were divided into 3 regions. Beginning with the ovarian end these are the fimbriated portion, the mid-region, and the uterine segment. The epithelium lining the oviducts was chiefly columnar in type; some cells were ciliated and others non-ciliated. The pseudostratified condition was often noted. The epithelial height and the ratio of the ciliated to non-ciliated cells, although variable in neighboring regions of the tube, showed changes associated with the estrual cycle.

The mucosa of the oviducts consisted of thin longitudinal folds, which progressively decreased in height and intensity from the fimbriated to the uterine end. The stroma was composed of richly cellular connective tissue which decreased in density from the uterine to the ovarian end of the tube.

The epithelium of the fimbriated ends of the oviducts reached maximal height during and shortly after estrus with a variation between 35 and 47 micra. The epithelial surface was more nearly level during estrus than at succeeding stages of the cycle. Cytoplasmic projections, which were present

Figure 21.—Intercotyledonary mucosa of uterine horn during estrus. The stroma is highly vascular and the vessels congested. Cow 509 x 80. Hematoxylin and eosin.

Figure 22.—Intercotyledonary uterine mucosa at 2 days postestrus. Note stromal density and extravasated blood. Cow 14 x 80. Hematoxylin and eosin.

Figure 23.—Intercotyledonary uterine mucosa at 3 days postestrus. Observe slight glandular hypertrophy and density of the stroma. Cow 499 x 80. Hematoxylin and eosin.

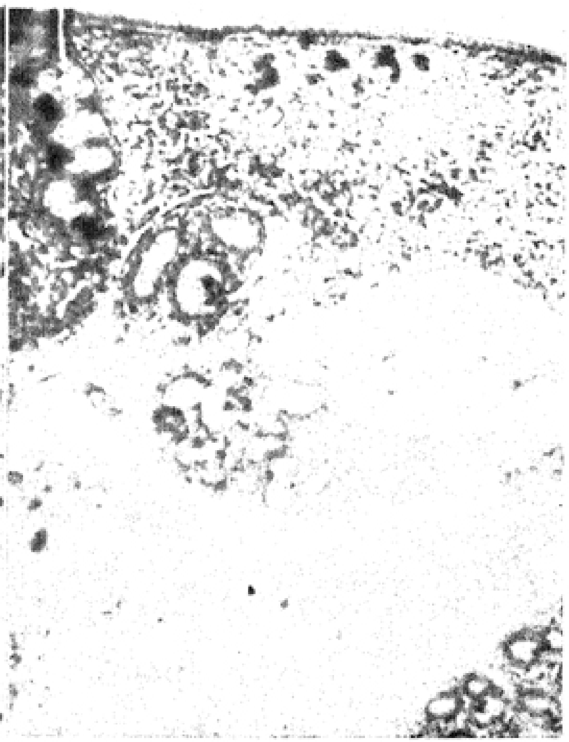

Figure 24.—Intercotyledonary uterine mucosa at 9 days postestrus. Note marked glandular hypertrophy and epithelial height. Cow 5 x 80. Hematoxylin and mucicarmine.

Figure 25.—Intercotyledonary uterine mucosa at 18 days postestrus Note atrophy of glands. Cow 759 x 80. Hematoxylin and eosin.

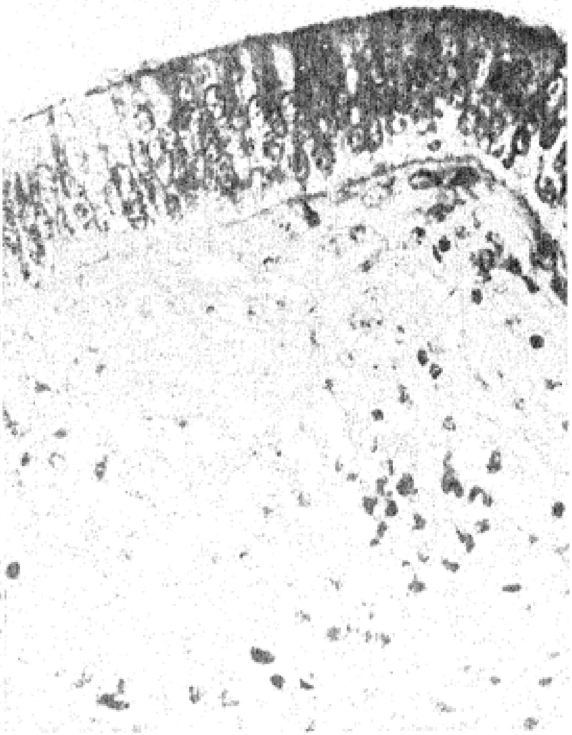

Figure 26.—Intercotyledonary uterine mucosa at 18 days postestrus. Observe distinct basement membrane and vacuolar changes in the epithelium. Cow 759 x 352. Hematoxylin and eosin.

Figure 27.—Thick-walled blood vessels of uterine cotyledonary area at 2 days proestrus. Cow 696 x 352. Hematoxylin and erythrosin.

Figure 28.—Cotyledonary uterine mucosa at 2 days proestrus. Note distinct basement membrane, vacuolar degeneration, pseudostratified appearance of epithelium. Cow 969 x 352. Hematoxylin and mucicarmine.

in variable numbers throughout the cycle, were least prominent during estrual and early postestrual stages.

The epithelial height was lowest at 8 to 9 days postestrus and ranged from 21 to 38 micra. The cell limits were most distinct at this period, the nuclei being elongate and perpendicular to the membrana propria. The maximal number and height of cytoplasmic projections were noted at this time. Their height was typically 5 to 10 micra. These projections often appeared as globules lying free on the epithelial surface and it was impossible to distinguish whether they arose from ciliated or non-ciliated cells. Extrusion of nuclei into the cytoplasmic projections was maximal about 9 days postestrus.

In late luteal and proestrual stages, the epithelium of the fimbriated segment of the oviduct was intermediate in height, ranging from 28 to 32 micra. The change from the ragged epithelial surface covered with projecting and extruded cells, to the high level condition of estrus and early postestrus apparently took place about 2 days proestrus.

The appearance of the mucosal epithelium of the midregion of the oviducts and its cyclic changes were similar to those of the fimbriated segment.

Cyclic changes were not evident in sections from the uterine segments of oviducts. Plica were less extensive and the stroma was denser than in the upper portions of the oviducts.

The cilia on the epithelial cells were more distinct and longer during estrus than at other cycle stages. During estrus they varied from 7 to 10 micra high and were 5 to 8 micra at other stages of the cycle. No glands were found in the oviducts, nor could mucus be detected by mucicarmine staining at any period of the cycle. Leucocytes were observed at all stages of the cycle, but a clear-cut cyclic pattern in their concentration was absent. The cyclic changes occurring in various segments of the oviducts are shown in Figures 29 through 37 (pages 51, 52 and 53).

Discussion

The histological changes in the vagina are confined to a period of rapid growth near estrus which is followed by regressive changes during the remainder of the cycle. The growth period is likely the result of the action of estrogenic hormone produced at estrus in increased quantities. Regressive changes during the luteal phase may reasonably be attributed to lowered levels or absence of estrogenic hormones.

In this study the vaginal superficial epithelium was polyhedral and sometimes flattened squamous, but true cornification, as reported by Murphey (1926), was not observed at any stage of the cycle. Maximal size was noted earlier in the epithelial cells than reported by Cole (1930).

Leucocytic infiltration of the vaginal mucosa was observed in fairly large quantity during estrus, in the superficial epithelium 1 day postestrus, and was practically absent at 2 days postestrus. Migration of leucocytes through the epithelium may be responsible for the increased number of leucocytes in mucus and for its changes in consistency and appearance following estrus (Section II).

Figure 29.—Fimbriated end of oviduct at 6 hours in estrus. Note scarcity of cytoplasmic projections. Cow 509 x 352. Hematoxylin and eosin.

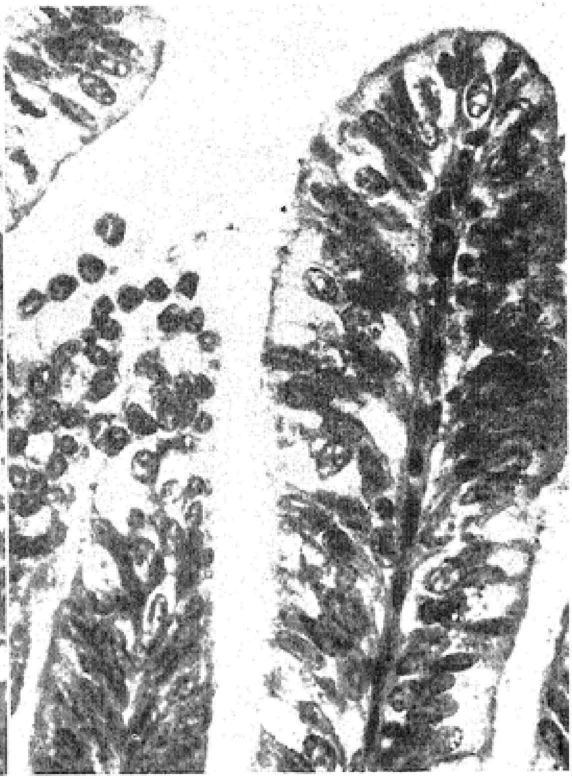

Figure 30.—Fimbriated end of oviduct at 3 days postestrus. Note levelness of epithelium. Cow 499 x 352. Hematoxylin and eosin.

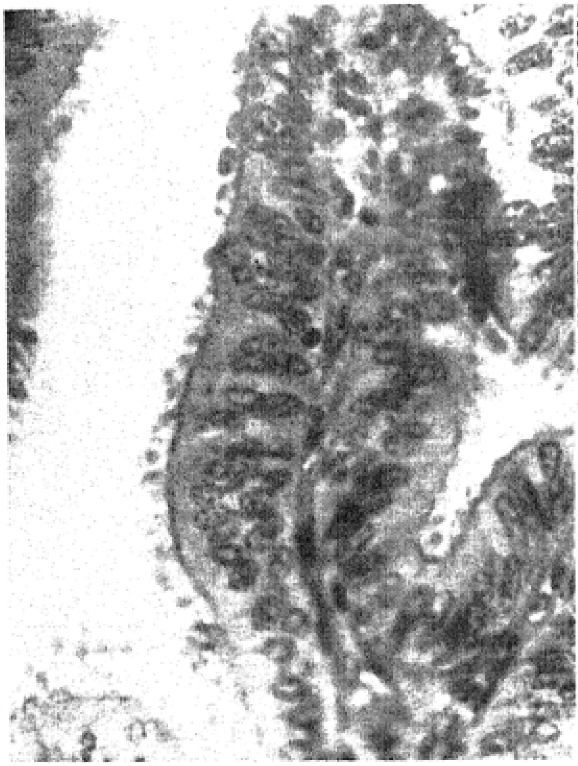

Figure 31.—Fimbriated end of oviduct at 8 days postestrus. Note increased number and height of cytoplasmic projections and nuclei in process of extrusion. Cow 513 x 352. Hematoxylin and erythrosin.

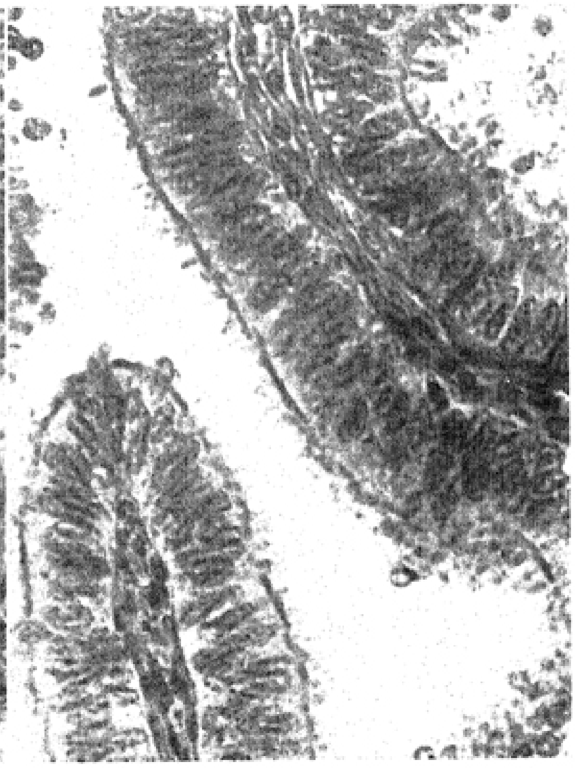

Figure 32.—Fimbriated end of oviduct at 18 days postestrus. Note the high, level condition of epithelium. Cow 969 x 352. Hematoxylin and erythrosin.

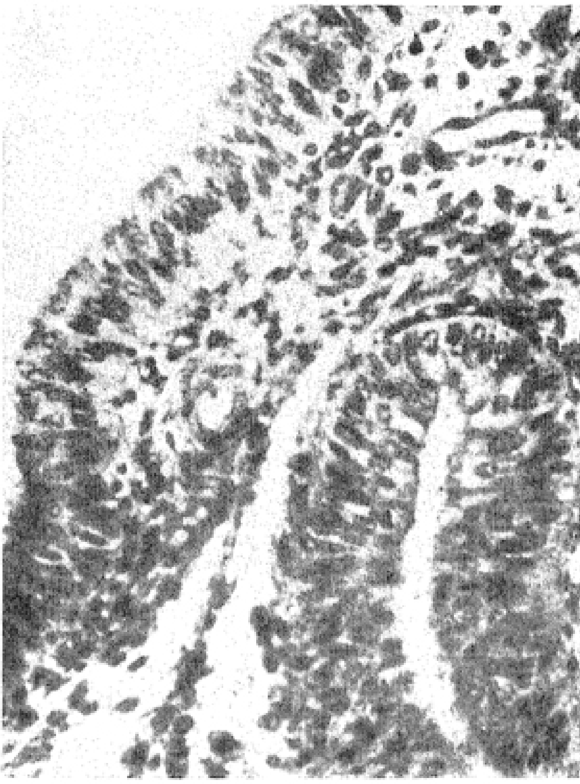

Figure 33.—Mid-region of oviduct at 6 hours in estrus. Note that this segment of tube has fewer cilia and cytoplasmic projections than the fimbriated segment. Cow 509 x 352. Hematoxylin and eosin.

Figure 34.—Mid-region of oviduct at 3 days postestrus. Note mucosal plica. Cow 499 x 80. Hematoxylin and eosin

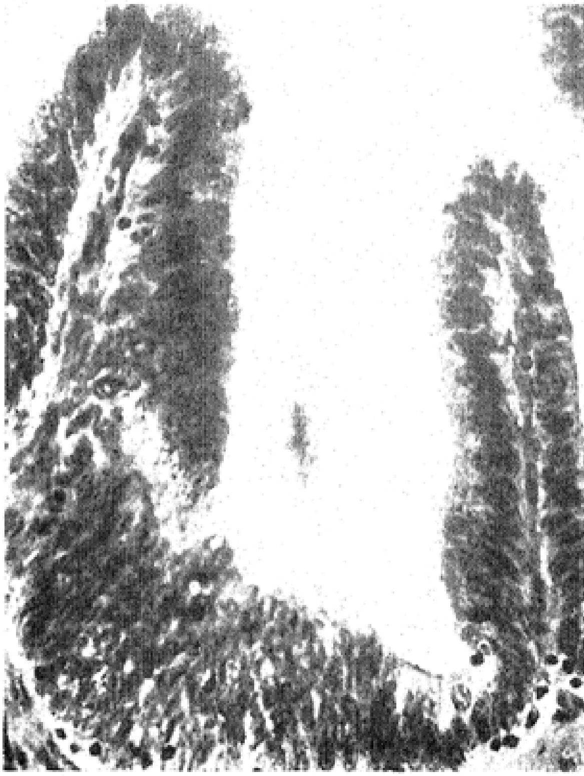

Figure 35.—Mid-region of oviduct at 9 days postestrus. Note central location of nuclei and irregular appearance of epithelial surface. Cow 5 x 352. Hematoxylin and eosin.

Figure 36.—Uterine end of oviduct at 2 days proestrus. Cyclic changes not evident in this segment. Cow 759 x 352. Hematoxylin and erythrosin.

The occurrence of the extremely tall columnar epithelium in the form of goblet cells and its reduction to a minimal number of layers at estrus give an unmistakable designation of the cycle stage. The changes that were observed in this region of the vagina agreed closely with the findings of previous investigators. Results of this study, as did those of Cole (1930), indicate that the vagina may play an important role in the secretion of mucus.

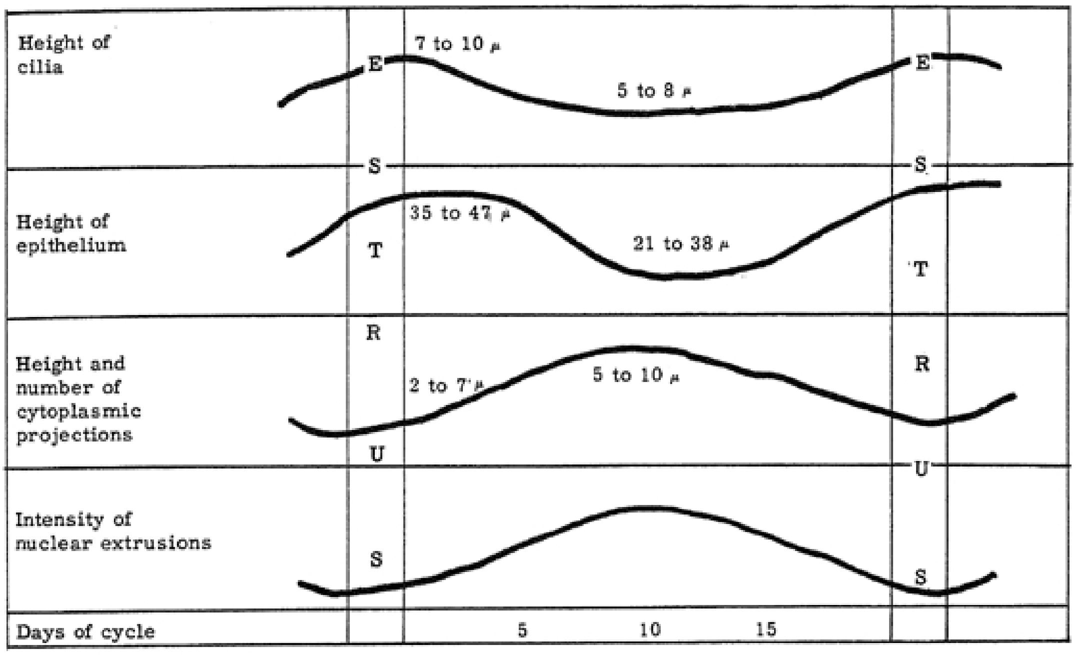

Figure 37.—Schematic diagram illustrating the histological changes in bovine oviducts during the estrual cycle.

As in most species, the bovine vaginal mucosa near the os uteri was thickened during estrus, but the increased thickness resulted from the increased height of the columnar cells rather than from growth of the stratified layers of epithelium. Shortly after estrus when the mucosa of most animals is receding as a result of sloughing of the superficial layer of epithelium, the same effect is gained in the cow by a reduction in the height of the columnar mucus-secreting cells, even though the underlying epithelium is increasing in thickness.

The changes which occurred in the cervix during the estrual cycle were similar to those that occurred in the vagina near the os uteri. The epithelial cells increased in height due to an accumulation of mucus during proestrus and reached maximum height and distention about the time of ovulation. Since the mucus-secreting epithelial cells changed from columnar to cuboidal in type with a corresponding decrease in the amount of mucus about 2 to 3 days following heat at a time when the corpus luteum appears, it is reasonable to assume that progesterone produced by the corpus luteum tends to abate the secretion of mucus. About 3 days prior to the onset of estrus when estrogen from the developing follicle may physiologically override progesterone, the epithelial cells gradually change from the cubical form of diestrus to a columnar type and actively begin anew the secretion of mucus. The change in form of the epithelial cells and in the intensity of mucus secretion closely parallels the follicular phase of the estrual cycle. The exact mechanism of this apparent hormonal control of mucus secretion in the reproductive tract is, at present, not known.

Sections taken at various levels of the cervix, though similar histologically, indicate that the secretion of mucus begins in the anterior portions of the cervix and progresses posteriorly through the cervix and into the mucoid epithelium of the upper vagina.

Although some changes occurred in the surface epithelium of the uterus they were not clear-cut. The height of development of the uterine epithelium and glands was reached about 10 days postestrus. This was earlier in the cycle than Zeitschmann (1921) and Cole (1930) reported. Since glandular hypertrophy has been shown to be associated with the development of the corpus luteum in pseudo-pregnant rabbits, it is believed that the corpus luteum through the secretion of progesterone is also the cause of the cyclic changes in the uterine glands of the cow.

In the oviducts, the cilia and epithelium reached maximal height shortly after estrus or near the time of ovulation in the cow. This period of growth probably results from the action of estrogenic hormone and may serve to facilitate the passage of ova and spermatozoa through the tube.

The process of nuclear extrusion which had a direct relation to the number and height of the cytoplasmic projections and an inverse relation to the height of the cilia and epithelium appears to be a process of cellular regression. Courrier (1924) and Bourg (1931) referred to nuclear extrusion as "intercalary cells" and suggested that this phenomenon might in reality be a holocrine type of secretion as a means of cell removal. Asdell (1946) contends that these extrusions are secreted protein which is imbibing fluid at the periphery, thus staining less intensely in this region than in the center. The specimens comprising this study cast doubt upon this contention since extruded nuclei with clearly defined nuclear membranes and nuclear inclusions, as well as intact cells in the process of extrusion, were frequently noted. The origin of these cells and the manner in which the low ragged epithelium of diestrus is transformed into the high, level epithelium of estrus has not been determined and well deserves extensive study.

The periods of growth and regression appeared to begin in the fimbriated end and progress as a wave toward the uterine end of the tube with approximately a 1-day lag in the maximum changes at the various levels of the oviducts.

SECTION IV.—SPERMATOZOA PENETRABILITY AND SURVIVAL IN BOVINE MUCUS IN VITRO

Review of Literature

Mechanisms by which sperm are directed toward the cervix and by which they pass through have long been considered by physiologists and gynecologists. Miller and Kurzrok (1932) studied the penetration of sperm in human cervical mucus *in vitro* with a microscope. They noted that mucus sometimes formed an impenetrable barrier to sperm, and this mucus they called "infected." In normal mucus, sperm oriented themselves to the mucus and then invaded it in a triangular shape with the apex being farthest removed from the semen-mucus junction. These workers related penetration and advance of sperm in mucus to a "mucolytic" enzyme which they had described previously (Kurzrok and Miller, 1928).

Lamar, Shettles, and Delfs (1940) studied penetration of cervical mucus by sperm using micro-tubes and a barrier-marker air bubble. They correlated maximal penetrability, which was observed at midcycle, with the peaks in freedom from leucocytes, pH (except during the menstrual flow), amount of mucus secreted, and greatest longevity of sperm in the mucus.

Within 48 to 72 hours following estrogenic administrations to bilaterally oophorectomized and hysterectomized women, Abarbanel (1946) recovered a clear, glairy, watery cervical mucus through which sperm could penetrate. Using a modification of Lamar's *et al.* (1940) penetrability method in which the barrier-marker air bubble was eliminated, he found the rate of sperm progression to be about 1 mm. per minute and that motility lasted as long as 72 hours. Administration of 5 to 10 mg. of progesterone rendered the mucus impenetrable or very slightly penetrable to sperm. He concluded that the production of cervical mucus easily penetrable by sperm was under hormonal control of estrogen.

Barton and Wiesner (1946) studied the receptivity of cervical mucus to spermatozoa. They found that 3 factors determined the suitability of mucus for fecundity: (1) capacity to permit forward motility of sperm, (2) capacity to sustain sperm life for extended periods, and (3) the invadability of mucus.

Herman and Horton (1948) reported that the penetrability of bovine cervical mucus by sperm was greatest during the first 6 to 10 hours of estrus.

Harvey and Jackson (1948) have published a method of investigating the penetration of cervical mucus by spermatozoa devised to minimize interference from surface tension. As yet results of penetration studies using this method have not been published.

The rate of sperm travel and the survival of sperm in the female genital tract may influence the results obtained from breeding. In most mammals sperm live only a few hours in the female genital tract. Kirillov (1937) recovered live sperm from bovine cervices up to 15 hours after insemination; however, 22 hours after insemination only dead sperm were found. Beshlebnov (1938) inseminated cows about 10 hours after ovulation and killed them at intervals from 40 minutes to 40 hours after insemination. In the cervix, 50 per cent motility was observed after 24 hours and slightly motile sperm were found 40 hours after insemination. Sperm in the vagina became immobile within 4 hours. In one case, sperm were found in the fimbriated end of the oviduct 1.75 hours after insemination. Sperm reached the infundibulum of cows inseminated during diestrus, but motility ceased sooner than in cows inseminated during estrus. Brewster, May, and Cole (1940) found the minimum time required for sperm to reach the upper third of the oviducts was 4 hours and 15 minutes in heifers and 5 hours and 30 minutes in mature cows. They found no significant correlation between the age of the animal and sperm travel. The difference in time was due to differences in the length of the genital tracts which averaged 52.70 cm. in heifers and 64.95 cm. in cows. Nalbandov and Casida (1942) found live sperm in the upper end of the oviducts of 3 cows killed at 6.5, 8.5, and 13.0 hours after insemination.

Sergin *et al.* (1940) believed the conditions prevailing in the genital tract of the cow, the predominantly alkaline reaction and the high electroconductivity of the secretions, stimulated the activity of sperm and had an unfavorable effect upon their survival. They suggested that in the cervix conditions were more favorable to sperm survival due to a weakly acid reaction and the absence of leucocytes. Here motility was reduced and the sperm survived for 2 to 3 days. Counts of sperm in the cervix at various intervals after mating showed that the number reached a maximum in 2 to 3 hours and at 5 hours the number had fallen to about half the 2 to 3 hour number.

Very few data are available on the survival time of bovine spermatozoa at high temperatures. Weber (1936) reported a mean survival time of 8 hours at 38°C. Beck and Salisbury (1943) found that semen diluted with egg yolk-citrate maintained motility not longer than an hour or so when stored at 45° or 47.5°C., but for longer periods when held at temperatures ranging from 37.5° to 42.5°C. Frank, Smith, and Eichorn (1941) reported no motility in semen diluted with egg yolk-phosphate after 3.5 hours storage at 37.5°C. Lohmann (1943) incubated a mixture of equal parts of bovine semen and vaginal mucus at 38°C. until all motility ceased. He found a highly significant negative correlation between the pH of this mixture and the initial progressive motility of the semen.

Materials and Methods

This phase of the study was designed to determine the correlation between mucus characteristics at various stages of the estrual cycle and spermatozoa penetrability and survival *in vitro*.

Penetrability of bovine mucus by spermatozoa was determined by using the method of Lamar *et al.* (1940) with slight modification. Slides were prepared by placing 2 slides together and standing them on edge in the center of another slide. Melted paraffin was then poured on each side of the 2 slides standing on edge. After the paraffin had cooled, the 2 slides were warmed enough to allow them to be removed from the base slide, thus leaving a smooth, narrow, paraffin groove. The ends of this groove were sealed with a drop of melted paraffin. A drop of mucus was placed in this groove so as to fill all but about one-half inch at either end. A drop of good quality semen, either undiluted or diluted, was placed in this space.

The slide was then placed in a constant temperature chamber at 38°C. and clamped to a graduated mechanical stage on a microscope. In checking the distance penetrated, with the aid of a stop watch, one could locate an individual sperm and follow it for 1, 2, 3 minutes, etc. The distance traveled in the mucus was then noted on the graduated scale and the average penetrability per minute was computed for 5 sperm.

Motility longevity studies were made on a 1:1 semen-mucus mixture. One portion of semen, which served as a control, was placed in a small test tube. Another portion was added to an equal volume of mucus and also placed in a small test tube. These were immersed in a water bath and incubated at 37 to 39°C. Motility observations were made on these samples, usually at 2-hour intervals, so long as motility persisted.

Results

Penetrability of bovine mucus by spermatozoa. Semen and mucus did not mix; they maintained their separate identities. A distinct interface was formed where the semen and mucus contacted each other. Even macroscopic inspection showed clearly the sharply defined boundary between the mucus and semen. Microscopically, it was again this boundary between semen and mucus which dominated the picture. This boundary, rather than being obliterated by diffusion of semen into mucus, became more pronounced on standing.

Spermatozoa tended to congregate in dense masses at the interface with most of the heads pointed toward the mucus. Sperm distant from the interface moved in their usual random fashion. A small portion of the highly active sperm passed into the mucus, some almost instantaneously, so that a few seconds after the slide had been charged with sperm they were found in the mucus. The subsequent fate of these sperm varied greatly. Sperm moved about in seminal fluid with a wide lashing of the tail. In mucus, however, the lashings are faster, with less amplitude, and greater forward motion was observed. Many sperm swam about at high speed and, since collisions were less frequent than in densely populated semen, their path was less devious, but appeared random with many sperm passing back into the semen. Other sperm that had passed through the interface continued to lash their tails but failed to show any forward movement. These "oscillatory" sperm were most numerous in the field adjacent to the boundary. Yet other sperm soon lost all motility. Thus a gradient developed; the total density of the sperm decreased, but the proportion of motile ones increased, with distance from the interface. For the first hour or so after the slide had been set up the penetration of mucus by sperm continued. A larger number of sperm penetrated the mucus than returned to the semen; thus, the population of sperm within the mucus increased gradually. After varying periods (1 to 3 hours) a point of culmination was reached. At this stage the seminal fluid contained comparatively few motile sperm since it had been depleted by continuous emigration of the most active ones. At the interface the seminal fluid was bounded by a dense mass of sperm, too weak to penetrate the interface, and forming as they assembled a growing obstacle to the more active sperm. On the mucus side there was a mass of oscillatory or immobile sperm near the interface, the more distant mucus being uniformly penetrated by motile sperm. This condition characterized the penetration test.

Neglecting minor deviations, 4 fairly definite types of mucus, with regard to sperm penetration, were distinguished. These were:

1. Sperm penetrated the interface and the whole depth of the mucus column readily, but lost progressively motility within 10 to 60 minutes.

2. Sperm penetrated the interface but invaded the mucus for only a short distance (less than 1 mm.). This type of mucus showed reasonably dense penetration limited to the boundary zone.

3. Sperm failed to penetrate the mucus; they congregated at the interface. Movement along the semen side of the interface appeared normal.

4. Abnormal mucus not only prevented penetration but appeared to affect the boundary region of the semen, since sperm soon lost their motility upon arrival at the interface.

TABLE 20. PENETRABILITY OF BOVINE MUCUS BY SPERM AT VARYING INTERVALS DURING THE ESTRUAL CYCLE

Period of Cycle	No. Cases	Average Penetrability in mm/min.	Range of Penetrability in mm/min.
Hours in estrus			
0- 4	236	2.79	0.5 to 5.0
4- 7	148	2.81	0.0 to 4.0
7-10	142	2.81	0.2 to 6.0
10-13	60	2.87	1.0 to 4.7
13-19	55	2.75	0.7 to 4.2
19-25	21	2.66	0.8 to 4.0

These types were, of course, not sharply distinct from each other. Some specimens of mucus were virtually impenetrable even though isolated sperm did penetrate.

Not all specimens of semen penetrated a given sample of mucus with equal facility. In some instances sperm, morphologically normal and of good motility, would not penetrate a given mucus specimen, whereas sperm from a different male and of equal quality would penetrate with ease. Preliminary trials indicated no essential difference in the penetration rates of undiluted and diluted semen; however, the total number of sperm effecting a penetration was greatest in the undiluted semen.

The average distance that sperm penetrated mucus collected at varying intervals during estrus is shown in Table 20. The average rate of penetration was 2.79 mm. per minute at the start of estrus and gradually increased to a maximal average penetration of 2.87 mm. per minute at 10 to 13 hours in estrus. The range in penetration during estrus was 0 to 6 mm. per minute.

Motility survival of sperm in mucus at 37 to 39°C. The data are presented in Table 21. In all cases sperm motility was maintained longer in the semen-mucus mixture than in the control sample of semen; the average difference was 3.30 hours with a range of 1.85 to 4.82 hours. The physiological basis for this observation has not been established. It is of interest to note that upon incubation sperm motility was preserved longer in mucus collected from 7 to 19 hours after the start of estrus than that at the other periods studied. Motility survival time was maximum in mucus collected at 10 to 13 hours in estrus with a longevity of 10.08 hours.

TABLE 21. LONGEVITY OF SPERM MOTILITY IN SEMEN AND SEMEN-MUCUS MIXTURE IN VITRO AT 37 TO 39°C

Period of Cycle	No. Cases	Av. Motility Preserved in Control Semen Hrs.	Range in Motility Preserved in Control Semen Hrs.	Av. Motility Preserved in 1:1 semen-mucus mixture Hrs.	Range in Motility Preserved in 1:1 semen-mucus mixture Hrs.	Difference Hrs.
Hours in estrus						
0- 4	206	4.57	2- 8	7.01	2-16	2.44
4- 7	174	5.12	2-10	8.61	2-19	3.49
7-10	64	5.12	2-10	9.46	5-18	4.34
10-13	46	5.26	1- 8	10.08	2-17	4.82
13-19	45	5.37	2-10	9.28	4-16	3.91
19-25	14	5.07	3-10	6.92	3-19	1.85

Relationship of the properties of mucus to sperm penetration and survival. An attempt was made to ascertain what factors in the properties of mucus might be related to and perhaps be responsible for variations in sperm penetration and longevity. Figure 38 shows the relation of the volume, leucocytic concentration, flow elasticity, surface tension, pH, and water content of mucus to sperm penetrability and motility longevity.

There was a definite positive correlation between sperm penetration and motility longevity in mucus collected at varying intervals during the estrual cycle. No striking correlation was observed between the water content of mucus and sperm penetrability and longevity of motility.

During the first 12 hours of estrus there was an inverse relation between the relative amount of mucus secreted and sperm penetration and longevity. As the amount of mucus gradually decreased the rate of penetration and hours of longevity preservation increased. However, from 12 to 24 hours after the start of estrus there was a positive relation of the amount of mucus to sperm penetration and longevity.

Investigation of the number of leucocytes in mucus revealed an inverse correlation with penetrability and longevity. The average penetration and

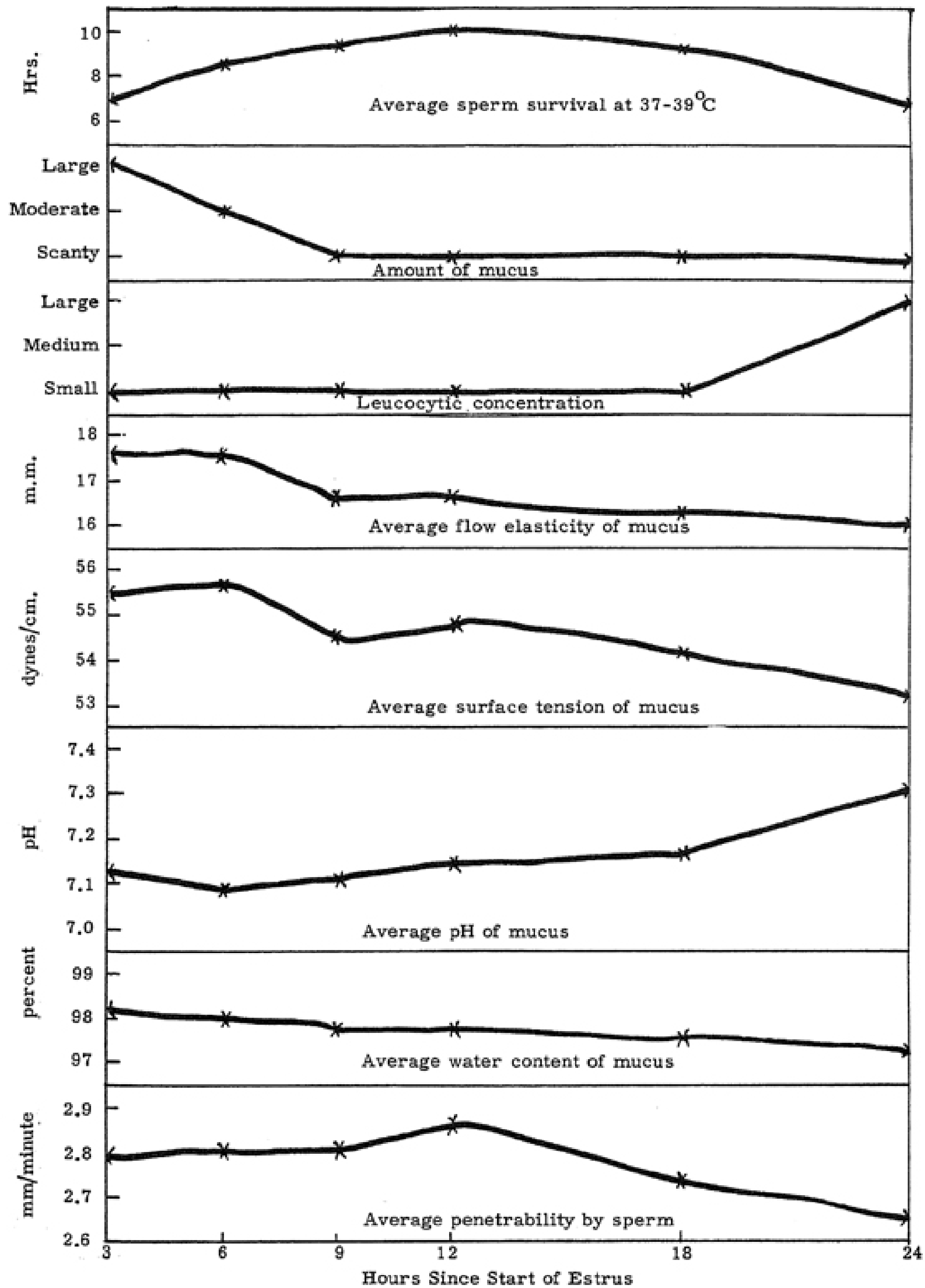

Figure 38.—Relationship of various mucus characteristics to sperm penetrability and survival.

longevity were greatest when leucocytes were few and lowest when leucocytes were most abundant.

Observations on flow elasticity with relation to penetrability and longevity showed a slight correlation between low flow elasticity and low penetrability and reduced longevity.

The surface tension of mucus appeared to be more closely correlated with penetration than the other physical properties studied. When the surface tension increased or decreased there was a corresponding rise or fall in penetration and longevity.

A study of the pH of mucus showed an inverse correlation with sperm penetrability and longevity. During the first 12 hours of estrus when the mucus was most acid, it was most penetrable to sperm and sperm maintained motility for longer periods. As the pH became more alkaline, the rate of penetration and the hours motility was maintained decreased.

Discussion

The relative immiscibility of semen and mucus is so pronounced that an interface is formed where semen and mucus contact each other. This interface may be likened to an invisible membrane and the passage of sperm across such an interface doubtless requires an appreciable amount of energy. Suitable *in vitro* preparations, in which good quality semen is brought in contact with normal mucus, reveal the significance of this interface. Many sperm on approaching the interface are retarded or prevented from penetrating. On the other hand some sperm pass through the interface, invade the mucus, and survive therein for varying periods of time.

Observations on the penetration of mucus by sperm bear upon certain problems of the physiology of reproduction. In this study there was no evidence that mucus "attracts" spermatozoa. The gradually increasing density of sperm at the interface is adequately explained by chance movements. In fact, the interface may be likened to a biological membrane and the sperm to particles endowed with kinetic energy but moving at random. Though the assembly of sperm at the interface might suggest positive attraction by mucus, it is more likely that the congregation consists of weaklings unable to extricate themselves from the conditions prevailing at the phase boundary. Similar congregations can be seen at interfaces between air bubbles and semen, emulsion globules and semen, etc. Inanimate particles may congregate in a similar manner (Rideal, 1930; Getman and Daniels, 1943).

The highest average rate of penetration was 2.87 mm. per minute in mucus collected at 10 to 13 hours in estrus, with some diminution in penetration rate as the collection of mucus departed on either side from the peak penetration. Essentially the same relationship existed with respect to sperm motility survival time in a 1:1 mixture of semen and mucus incubated at 37 to 39°C. These observations indicate that the interface, which is apparently influenced, in part, by the cyclic changes in the physical properties of mucus, exerts a variable effect on the behavior of spermatozoa. The fact that sperm of equal quality, according to present methods of evaluation, penetrated the same mucus specimen with unequal facility is not understood.

The highest *in vitro* penetration and survival time of sperm in mucus collected at various stages of estrus agree closely with conception results from insemination at various stages of estrus. Penetration and survival time of sperm were maximal in mucus collected during full and late estrus. It appears that if cows are inseminated when sperm penetrability and survival are maximal that breeding efficiency would also be maximal. Further study is needed to determine the best practices in breeding cows that secrete a heavy, viscid mucus during estrus as well as to determine the optimum time for insemination under these conditions. If conditions are such that sperm cannot survive the necessary length of time, or travel the required distance to reach the ovum, the obvious result is failure to conceive.

Sperm require utilizable sugars for their prolonged activity and glycolysis. Since they probably carry with them no appreciable extracellular nutritive material when they penetrate mucus, it is suggested that they find in mucus a suitable medium for their metabolism and activity. The observation that sperm survival time was longer in a 1:1 semen-mucus mixture than in control semen samples substantiates this suggestion. Enzymes may play an important role in the utilization by sperm of substances in mucus, a conjecture which well deserves study.

The penetrability and survival time of sperm in mucus evidently depend on the intimate structure and possibly on chemical factors of the mucus which are not wholly reflected by the comparatively gross characteristics revealed to the eye or measured by present physical methods. The *in vitro* technics for testing mucus for sperm penetrability and survival time may be useful in the evaluation of semen and in studies of the conditions prevailing at the cervix at the time of breeding in cases of "shy" breeding cows or unexplained "sterility" in cattle.

SUMMARY

A study of the physiological and histological phenomena during the bovine estrual cycle was made on more than 100 cows of the Guernsey, Holstein, and Jersey breeds in the Missouri Station herd. Particular attention was given the properties of vaginal-cervical mucus at varying stages of the estrual cycle.

Observations on the estrual behavior of 68 cows are described. The beginning and end of estrus were sometimes abrupt and in other cases gradual and could be distinguished by the character and consistency of the mucus secretions. Data on the 1503 estrual periods on 1182 cows showed that the majority of the periods began in the A. M.

The average length of 504 estrual cycles was 21.41 days with a range of 11 to 35 days. Eighty per cent of the cycles was within the range of 18 to 24 days. Analysis of variance for length of estrual cycles showed significant differences between breeds, but there were no significant differences between seasons and age groups.

Vaginal temperatures and heart rates were slightly higher during estrus than during diestrus and pregnancy.

Over 500 examinations per speculum of the reproductive tracts of cows during various stages of the estrual cycle and gestation showed that the color and vascularity of the vulva, vagina, and cervix tended to be maximal at the approximate time of ovulation. There was a gradual relaxation of the cervix 2 to 3 days prior to heat with maximal relaxation coinciding with the approach of ovulation. Cervical muscle tone was maximal at 5 to 15 days postestrus. Following parturition the cervix was completely relaxed and the contracted interestrual state was gradually reached within 4 to 5 weeks after calving.

The volume of mucus secreted, its flow elasticity, surface tension, and water content decreased as estrus progressed, but the number of leucocytes increased.

The pH of mucus in both *in vivo* and *in vitro* determinations varied during the estrual cycle; the lowest pH usually was observed in early estrus. Pregnancy eliminated the usual decrease of pH at estrus. *In vivo* vaginal pH was significantly lower than *in vitro* pH of withdrawn mucus. In 30 paired determinations the *in vivo* pH averaged 6.57 and the *in vitro* 7.45. This difference did not appear due to carbon dioxide loss.

Mucus was collected from the cervix and vagina of 10 cows at slaughter. In all cases, the cervical mucus was more acid than vaginal mucus; the former averaged pH 6.89 and the latter 8.01.

Color reactions were obtained from mucus that were characteristic of glycogen, peptide linkage, and the amino acids tyrosine, cystine, tryptophane, and phenylalanine. The color tests were faintest during early heat and tended to become more intense as ovulation approached.

Histological studies revealed that the height of the epithelium lining the vagina proper was maximal at 2 days postestrus. Congestion of the stromal blood vessels was greatest during the follicular phase of the cycle and least during the luteal phase. True cornification of the superficial epithelium was not observed, but the maximum tendency toward cornification was from 3 to 10 days postestrus. Infiltration of leucocytes and lymphocytes was noted in the superficial stroma during proestrus, in the deeper portions of the epithelium during estrus, and in the superficial epithelium at 1 day postestrus.

The vaginal epithelium near the cervix consisted of a superficial layer of mucoid epithelium resting upon a variable number of stratified squamous cells. At estrus the number of layers of stratified squamous cells was minimal; however, the total epithelial height was maximum due to an accumulation of mucus in the mucoid epithelium. During diestrus the mucoid epithelium changed from columnar to cubical in type.

Mucicarmine staining of cervical sections showed the least amount of mucus in the mucoid cells during midcycle and the greatest during estrus. Sections at various levels of the cervix indicated that mucus secretion begins anteriorly and progresses posteriorly. Congestion of blood vessels and density of cervical stroma were greatest during the follicular phase of the cycle and least during the luteal phase.

Cyclic changes in the uterus were more pronounced in the stroma and glands than in the epithelium. Glandular hypertrophy was maximum at 9 to 10 days postestrus. The greatest number of leucocytes was present in the uterine epithelium and stroma during proestrus and estrus. The stroma was edematous and cell-poor during estrus, but it became denser and less edematous during diestrus.

The height of the tubal epithelium and cilia was greatest during estrus or early postestrus and least during diestrus. Cytoplasmic projections appeared shortly after ovulation and increased in prominence as the epithelium decreased in height. The greatest number and maximum height of the cytoplasmic projections were reached about midcycle. The process of nuclear extrusion, which is probably a method of cellular regression, appeared to be maximal about 9 days postestrus. The periods of growth and regression appeared to begin in the fimbriated end and progress as a wave toward the uterine end of the oviducts.

A method of observing the penetration of bovine mucus by spermatozoa *in vitro* is described. The significance of the semen-mucus interface is discussed. Mucus was grouped into 4 fairly definite types with regard to sperm penetration. The average penetration rate in mucus collected during estrus was 2.81 mm. per minute, the range was 0 to 6 mm. per minute, and the average maximal penetration was in mucus collected at 10 to 13 hours in estrus. Not all specimens of semen penetrated a given mucus sample with equal facility.

When incubated at 37 to 39°C. sperm motility was maintained an average of 3.30 hours longer in a semen-mucus mixture than in control semen samples. Sperm motility survival was maximal in mucus collected during full and late heat.

Spermatozoa penetrability and survival *in vitro* in mucus appeared to be correlated directly with surface tension and flow elasticity and inversely to leucocytic concentration and pH of mucus.

The *in vitro* technics for testing mucus for sperm penetrability and survival time may be useful in the evaluation of semen and in the evaluation of mucus in cases of unexplained "sterility" in cattle.

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