

Resumen por el autor, Raymond M. Selle.

Cambios en el epitelio vaginal del conejillo de Indias durante el ciclo éstrico.

El ciclo éstrico del conejillo de Indias presenta cuatro periodos o estados bien definidos, además del intervalo, que corresponde a estados semejantes de la rata. En el primer estado, que en apariencia no ha sido notado por Stockard y Papanicolaou, el frote vaginal contiene solamente grandes células epiteliales granulosas y vacuolares. Estas células epiteliales derivan de las capas superficiales de la mucosa, debajo de las cuales ha tenido lugar la cornificación. La mucosa alcanza su mayor altura en este periodo. En el estado 2, las células epiteliales superficiales han desaparecido, dejando como capa más externa el estrato córneo. Este último mediante descamación susministra las células típicas, anucleadas, en forma de copos, del frote. Hasta este momento la semejanza con la rata es muy estrecha. Sin embargo, en el conejillo de Indias toda la capa córnea, no todo el epitelio, con algunas de las células no cornificadas pueden ser expulsadas en masa. Mientras que en el estado 3 de la rata el material granular y caseoso consta de elementos del estrato córneo, descamado rápidamente, aisladamente o en pequeños grupos, hasta desaparecer por completo, en el conejillo de Indias la masa caseosa descrita por Stockard y Papanicolaou está constituída por las células epiteliales no cornificadas situadas más profundamente, las cuales se desprenden en grandes números. En el último estado (4) los leucocitos descritos por Stockard y Papanicolaou en su estado 3 aparecen. El autor no ha hallado estado alguno que corresponda a su estado 4. No existe prueba alguna sobre la importante contribución del útero a los elementos celulares de la vagina, pues la última es prácticamente idéntica en los conejillos normales y en los hysterectomizados.

CHANGES IN THE VAGINAL EPITHELIUM OF THE GUINEA-PIG DURING THE OESTROUS CYCLE

RAYMOND M. SELLE

THREE PLATES (ELEVEN FIGURES)

INTRODUCTION AND LITERATURE

The last few years have witnessed a considerable advance in our knowledge of the cyclical changes in the reproductive organs of the mammalian female, chiefly as the result of the introduction of a new method by Stockard and Papanicolaou; a method by which the course of the cycle may be followed in the living animal. The results obtained during the preceding years involved the sacrifice of animals at various times with relation to some definite event such as parturition or heat (when the latter is easily determined) and the microscopic study of sections. Obviously, it was not possible to study successive cycles in the same animal.

Nevertheless, data of some importance have been accumulated from the time of Lataste, Morau, and Retterer to the present. Since the literature of the period is dealt with more completely by Long and Evans ('22), it is unnecessary here to do more than call attention to those papers dealing more directly with the guinea-pig and the development of the method.

As early as 1892, Retterer observed that regular changes occurred in the vaginal mucosa of non-pregnant guinea-pigs. Following parturition he killed animals at regular intervals and was the first to describe a stratum cornium in the guinea-pig, although such a layer had been described in mice by Morau in 1889. He found that on the fifteenth day postpartum a layer of cornified cells was beginning to form under the superficial layers of the stratified vaginal mucosa.

In a number of papers Leo Loeb reported his results on the study of ovulation and the formation of corpora lutea in guinea-pigs. He came to the conclusion that it recurred at intervals of

about twenty-one days. Similar investigations on the incidence of ovulation, etc., in rats and mice were being carried on in this laboratory by Doctor Long and several students.

No further work of importance on the vaginal cycle of the guinea-pig was recorded until 1917, when Stockard and Papanicolaou published their paper on "The existence of a typical oestrous cycle in the guinea-pig with a study of its histological and physiological changes." They studied the vaginal cycle by taking samples of the contents of the vagina at regular intervals. They obtained these samples by introducing a small nasal speculum into the vagina, the arms of which were held apart by means of a thumb-screw, allowing them to observe the entire lumen of the vagina. They were thus able to recognize an oestrous rhythm consisting of four stages, each having a characteristic vaginal fluid which contained cells from the walls of the vagina.

The vaginal fluid during the first period contained a great amount of mucous secretion, many desquamated epithelial cells, and non-nucleated, or cornified cells, which appeared toward the end of the stage. The second stage could be recognized by the thick, cheese-like contents of the vagina due to the accumulation of the desquamated epithelial cells. During the third stage the vaginal fluid became thinner because of the solvent action of leucocytes which invaded the epithelium and entered the lumen. The fourth stage, during which a small amount of blood was found in the vagina, was of short duration and did not always occur. These stages were followed by the dioestrus, or intermenstrual period, during which the vaginal fluid contained mucus, atypical squamous cells, and many leucocytes. They then correlated these stages with the conditions of the uterus and ovary at corresponding periods.

A second paper by Stockard and Papanicolaou appeared in 1919 in which they described the vaginal closure membrane and the time of copulation in relation to the oestrous cycle. They found that there was formed regularly a very delicate epithelial membrane which closed the orifice of the vagina soon after the 'heat period' and remained closed until the next cycle or until parturition in case of pregnancy.

As the result of following the suggestions contained in Stockard and Papanicolaou's earlier paper, the cycle in the rat was worked out (Long, '19) and later elaborated and verified (Long and Evans, '22). Smears taken from the vagina of the rat by means of a small spatula were studied and correlated with sections of all parts of the reproductive system. By means of the smears, it was possible to recognize four definite stages besides the interval. Smears taken during the first stage contained nucleated epithelial cells of uniform size; during the second stage, few, large, cornified cells; in the third stage they were made up of a great abundance of cornified cells; in the fourth stage, by many leucocytes admixed with cornified cells. Throughout the interval between stage 4 and stage 1, or the dioestrous pause, the vaginal fluid contained leucocytes with only a few epithelial cells.

The sections revealed a cyclical growth and desquamation of the vaginal epithelium. The epithelium is lowest during the interval, increasing in thickness just before the advent of stage 1. One of the striking features is the occurrence, during this stage, of the processes of cornification not in the superficial layers but at a depth of almost two cells, the upper two layers of cells retaining their epithelial character, later to be shed as the cells of the smear of stage 1. The cornified stratum so denuded becomes superficial, increases in thickness, and is later detached, thus furnishing the cells of stages 2 and 3. Leucocytes, of which the epithelium is devoid during stages 1, 2, and 3, now invade the mucosa and escape into the lumen in stage 4.

Long and Evans attempted to correlate the cycles in the rat with the cycles in the guinea-pig as found by Stockard and Papanicolaou and as observed by themselves in twenty-two guinea-pigs for a couple of months. In a section of the vagina of the one guinea-pig which they killed, Long and Evans observed what they believed to be the cornified layer under a layer of epithelial cells. Since they found that such a condition occurred regularly in the rat, they conjectured that an epithelial layer superficial to the cornifying layer must be normal in the guinea-pig, and if such a condition were true, then it was evident that Stockard and Papanicolaou had overlooked it.

With these results in mind, it seemed worth while to study the changes in the vaginal epithelium of the guinea-pig in order to ascertain whether or not a layer of epithelial cells occurred superficial to the stratum cornium as observed by Retterer; to supplement the work of Stockard and Papanicolaou, and to correlate the rat and guinea-pig more exactly. The problem was undertaken at the suggestion of Prof. J. A. Long and was carried out under his general direction in the Zoölogical Laboratories.

METHODS

The guinea-pigs chosen for these experiments were common white, brown, and mixed colored virgin females about a year old. They were kept at a relatively constant temperature averaging 68° to 72°F. and were fed a constant, daily ration of green grass or alfalfa hay, carrots, and rolled barley, because there is reason to believe that a lower or a higher temperature as well as variation in the ration tends to affect the span of the oestrous cycle.

Since the determination of the state of progress of the cycle, as shown by the previous work cited on the guinea-pig and rat, is dependent on our knowledge of the character of the contents of the vagina, the method of sampling the contents becomes a matter of some importance. Several methods of taking samples were employed. For reasons explained later, the method employed by Stockard and Papanicolaou was discarded, and because of the extreme length of the vagina in the guinea-pig the spatula method as used by Long and Evans on the rat was not of much value.

The pipette method, suggested by Doctor Long and developed by Miss E. Fisher in this laboratory for making vaginal smears from mice, was tried, and after several improvements a satisfactory method for taking samples was perfected. The instrument used was made by inserting into a 25-cc. oval, rubber bulb a piece of thin-walled, glass tubing 14 cm. long and 1 cm. in diameter, about 5 cm. of which was drawn down to a diameter of 5 mm.

When a sample was to be taken, the animal was held ventral side up in the left hand allowing it to lie on its back along the left forearm with its head toward the elbow. The left thumb

was placed in front of the animal's left hind leg and exerted pressure just in front of the vulva; at the same time with the left forefinger between the vulva and the right leg, it was easy slightly to open the orifice of the vagina. The syringe, containing about 1 cc. of warm, normal, salt solution, was then introduced into the vagina to a depth of 25 to 40 mm. By squeezing the bulb and forcing the contents of the syringe into the vagina once or twice, it was possible to get a characteristic sample of the cells in the lumen. If, while withdrawing the syringe, pressure was applied anterior to the vulva with the thumb of the left hand, the sample could be drawn up readily into the syringe. Enough of a sample was obtained at one operation by this method to make several smears. This proved a most satisfactory method, because each time a sample was taken the vagina was washed out and the cells which were free in the lumen were removed; consequently, it was reasonable to suppose that all of the cells which were found in any one sample had been shed during the interval immediately following the preceding sample.

In order to study the cycle, samples were taken at 8 A.M., 12 M., 4 P.M., and 8 P.M., and the results recorded. Animals were then killed during each stage as revealed by the character of the smears. A small amount of 0.75 per cent solution of table salt immediately followed by Bouin's fixing fluid was injected under pressure posteriorly into the aorta just anterior to the diaphragm. By this method the vagina in situ was fixed in its normal position at the earliest possible moment and its excision greatly facilitated. After removal from the body the vagina was immersed twenty-four hours longer in the fixing fluid. Most of the picric acid was extracted in 50 per cent alcohol containing lithium carbonate. Portions of the vagina including the cervix were then dehydrated, cleared, and imbedded in paraffin and sectioned at $7\frac{1}{2}\mu$. The condition in the vaginae as revealed by these sections was then correlated with the smears taken just before killing the animal.

The uterine glands in the guinea-pig secrete an abundance of mucus which flows down into the vagina (Stockard and Papanicolaou, '17), often obscuring the nature of the smear. Indeed, Stockard and Papanicolaou found uterine epithelial cells thus

carried into the vagina. In order to avoid any confusion that might be caused by the presence of uterine mucus and cells, in seven of the twenty females the uterine horns were tied off posterior to the oviducts and anterior to the cervix and the intermediate pieces excised. The value of this operation was not sufficient to warrant its recommendation. Although the contents of the vaginae in the animals which had been hysterectomized were freer from mucus and consequently more easily diagnosed, there was not a great difference between smears made from these animals and smears made from normal guinea-pigs.

A careful scrutiny of successive vaginal smears of twenty guinea-pigs over a period of seven months disclosed a succession of cell changes in the contents of the vagina substantially similar to that described by Stockard and Papanicolaou for the guinea-pig and by Long and Evans for the rat. The enumeration of the stages in this paper follows that employed by Long and Evans.

CHANGES IN VAGINAL SMEARS AND HISTOLOGY OF VAGINAL EPITHELIUM

Interval of dioestrus

If the vaginal smears of a number of guinea-pigs were to be examined, it would be found that most of them would contain many leucocytes and few, small, round epithelial cells; in fact, this condition would be found to be characteristic during three-fourths of the time in any one pig. While the nature of the smear does not seem to vary during this period there are, however, histological changes in the mucosa.

The lining of the vagina of a guinea-pig consists of a stratified epithelium, the upper surface of which is nearly even, while the side in contact with the submucosa, from which it is clearly demarcated, appears in section to be deeply lobed as though furrowed, the effect being produced by tongue-like projections of the submucosa extending up into the epithelium. The epithelium thus seems to vary in thickness (figs. 1 and 8). The whole mucosa is further thrown into larger longitudinal folds.

At about the middle of the interval the epithelium was found to be thinner than at any other time, being only one or two layers of cells high at the thinnest places. At the deep ends of the downward projections of the epithelium were numerous mitoses—a condition which apparently substantiated Stockard's and Papanicolaou's statement that the vaginal epithelium was regenerated from the base of the infoldings.

Towards the end of the interval the epithelium rapidly increased in height until it reached a maximum of ten to twelve cells. The uppermost of these cells which bordered the lumen had become enlarged and vacuolated, with their nuclei usually at their bases. The cells lining the inner surface of the vagina were the most vacuolated, while the layers beneath were smaller and gradually approached the normal size of the epithelial cells at about three or four layers below the surface (figs. 2 and 9).

Stage 1. Large irregular epithelial cells only

(No corresponding stage described by Stockard and Papanicolaou)

Stage 1 marks the beginning of the oestrous cycle. A smear made early in this stage exhibited, in marked contrast to that of the interval, first, numerous, large, round or odd-shaped, epithelial cells containing many vacuoles, and, secondly, very few leucocytes. The latter undoubtedly remained over from the interval which, as just described, was characterized by the presence of many leucocytes and a very few epithelial cells. These epithelial cells appeared in the smear singly or in groups of three or more.

A section of the mucosa early in stage 1 showed the epithelium to be practically as high as at the end of the interval, i.e., about ten to twelve cells; also that a characteristic transformation had already made its appearance. This transformation was exhibited in cornification of certain of the epithelial cells as shown in figures 3 and 10. It will be observed that the most superficial cells were enlarged, were odd-shaped, and contained vacuoles; that the next deeper cells were somewhat smaller and more spherical, and that the next deeper levels showed the beginning

of the process of cornification. The former of these were the cells which were cast off and furnished the cells found in a sample taken during this stage. They are called superficial epithelial cells for the reason that they were above a region in which cornification later took place. The cornifying process began rather suddenly and continued at a rapid rate simultaneously throughout the entire vagina. It began two to four cells below the surface of the superficial epithelium, and involved four to six cell layers. In some cases it extended up into the superficial epithelial cells before the latter had been entirely cast off. This is shown in figure 4. Beneath the stratum cornium may be distinguished the typical layers of stratified epithelium.

A little later in this stage the leucocytes had entirely disappeared from the vaginal fluid and the only cells found in the lumen were the superficial epithelial cells. As a result of the sloughing off of these cells, the vaginal mucosa became reduced in height and the cornified layer exposed.

It usually happened that the shedding of these superficial epithelial cells occurred while the entrance to the vagina was closed by the vaginal closure membrane described by Stockard and Papanicolaou, often making it necessary to break the membrane in order to get a sample.

Stage 2. Cornified cells only

(Stockard and Papanicolaou's stage 1)

A vaginal sample taken during this stage contained many flattened, horny, non-nucleated or cornified cells. No leucocytes were found in the lumen. Stockard and Papanicolaou in their first paper mentioned two kinds of cornified cells. These are apparently two different stages of the same process of cornification. The various intermediate degrees of cornification can be demonstrated both in smears and sections counterstained with eosin, the amount of stain taken by the cells varying with the extent of cornification.

Because of the fact that there were almost always a number of cornified cells at the external orifice of the vagina, smears of the

succeeding stages were constantly contaminated by them, especially in those obtained by means of a nasal speculum or a spatula; hence the value of the syringe method.

As the process of cornification continued, the cornified cells became loosened from the underlying layers and were cast off into lumen singly or in flakes of many cells.

An interesting occurrence not described by any writer was the shedding of the entire cornified layer as a cast which showed the natural shape of the lumen of the vagina. It was possible in several animals by the use of the syringe to get several succeeding cornified casts at regular intervals of sixteen days. When pieces of the casts were examined under a microscope, the structure was readily recognized as continuous layers of superimposed cornified cells. Some very perfect casts were obtained, even showing the folds about the cervix. Fourteen complete cornified linings or casts were obtained and preserved, besides a great many more casts broken in the process of taking the sample. This shedding of the entire lining of the vagina occurred at the end of stage 2.

The shedding of the entire cornified layer would seem to have been mistaken by Stockard and Papanicolaou to be the whole vaginal epithelium separated from the underlying connective tissue, for, according to them ('19, p. 234), "the epithelium is now expelled as one continuous tube forming the cover around the vaginal plug instead of sluffing off in smaller pieces as occurs during the fourth stage when copulation has not occurred. However, the vaginal epithelium may occasionally be shed en masse without copulation. . . . It is clear, therefore, that what was termed by Lataste the 'enveloppe vaginale' is the layer of epithelium separated from the underlying connective tissue. . . ."

That the entire vaginal epithelium did not separate from the connective tissue, but that only the whole cornified layer became loosened from the underlying epithelium and lay free in the lumen, is shown in figure 5. The condition of the vaginal epithelium immediately after the shedding and expulsion of the cornified cast is shown in figures 6 and 11.

Stage 3. Cornified cells and small epithelial cells

The characteristic smear for this stage consisted of cornified cells, and of nucleated, small, epithelial cells. There are as yet no leucocytes found, but as the stage progressed fewer cornified cells and increasing numbers of epithelial cells appeared. Some of the latter contained granules in the cytoplasm. In case the cornified layer had been shed in a cast, this stage lasted only a short time during which the epithelial cells were cast off.

The active shedding of these two kinds of cells reduced the height of the stratified epithelium to from four to seven cells.

This stage was called the cheesy stage by Stockard and Papanicolaou, because of the appearance of the mass of epithelial cells. Since in this investigation the vagina was regularly washed out with warm saline solution in the process of taking samples and was thereby kept clean, the cells could not accumulate to form such cheesy masses.

During this stage leucocytosis began in earnest. Leucocytes invaded the submucosa and the epithelium, in the latter of which it was common to see aggregates of three to ten leucocytes. In many cases leucocytes had become imbedded in the epithelial cells, as many as five having been counted in a single cell. Cornified cells had completely disappeared from the smears and did not appear again until the next cycle.

Stage 4. Small epithelial cells and leucocytes

(Stockard and Papanicolaou's stage 3)

This marks the advent of leucocytes in the lumen of the vagina. In the previous stage the leucocytes were migrating into the vaginal epithelium, but had not yet reached the lumen. Smears made during stage 4 contained the nucleated epithelial cells of the preceding stage and also increasing numbers of polymorphonuclear leucocytes. At first the epithelial cells predominated, but gradually ceased to become detached; while at the same time the number of leucocytes rapidly increased until they were in a majority.

At the end of this stage the epithelium was only two to four cells high over the finger-like processes of the submucosa (fig. 7).

Following this stage, Stockard and Papanicolaou described another, which they called the fourth, during which blood was found in vaginal samples. In these investigations only two cases were observed in which blood was found in the smears, and in both cases it occurred at the end of stage 4. It is hardly probable that this is a common occurrence. Because of the thin epithelial covering over the submucosa at this stage, it seems highly probable that an instrument inserted into the vagina might injure some of the many blood vessels in the submucosa and thus permit blood to escape into the vagina.

Because of the differences in the stages of the cycles of the guinea-pig as found by Stockard and Papanicolaou and in these investigations, the following table (1) has been constructed in

TABLE 1
A comparison of the stages in the oestrous cycles in the guinea-pig and rat

GUINEA-PIG (STOCKARD AND PAPANICOLAOU)	GUINEA-PIG (NEW)	RAT (LONG AND EVANS)
<i>stage</i>	<i>stage</i>	<i>stage</i>
1. First period: Squamous epithelial cells Second period: Cornified cells	1. Large irregular epithelial cells only. (Superficial epithelial cells)	1. Superficial epithelial cells only
2. Cheesy mass, epithelial cells	2. Cornified cells only	2. Cornified cells only
3. Leucocytes and epithelial cells	3. Cornified cells and small epithelial cells, or small epithelial cells only	3. Many cornified cells (cheesy)
4. Appearance of blood in lumen	4. Leucocytes and epithelial cells	4. Leucocytes and cornified cells (sometimes epithelial cells)
5. Interval: Leucocytes and epithelial cells	5. Interval: Leucocytes (few epithelial cells)	5. Interval: Leucocytes and epithelial cells

order to point out the similarities as well as the differences. It will be seen that these newly determined stages in the guinea-pig correspond more closely to stages in the rat as described by Long and Evans.

Interval

At the end of stage 4 or at the beginning of the interval the vaginal closure membrane began to form as described by Stockard and Papanicolaou, growing very rapidly and, if not disturbed, closing the vagina in one to three days. This membrane remained intact until mechanically destroyed or until the next cycle, when it was normally broken during the cornified stage by the tension produced by the swollen vulva and accumulated fluid in the lumen. When samples were taken daily, the membrane was prevented from forming for an indefinite period.

It will be recalled that toward the end of stage 4 and the beginning of the interval the rapid rate of desquamation of the epithelial cells gradually diminished, while the leucocytes increased until they were in the majority. The transitional period between stage 4 and the interval is indicated by the decreasing numbers of epithelial cells and increasing numbers of leucocytes in the smears. Samples taken up to the middle of the interval contained a few epithelial cells, but for the next week or more only leucocytes. From this time on until the beginning of stage 1 vaginal smears were found to exhibit leucocytes and some atypical cells.

A section through the vagina during the middle of the interval showed the epithelium to be only one or two cells high. This reduction in height was no doubt caused by the continued dropping off of the epithelial cells which were found in the vaginal fluid following stage 4.

Toward the end of the interval and immediately before the beginning of stage 1 the epithelium was rebuilt to a height of ten to twelve cells (fig. 2). The uppermost of these cells became vacuolated as described under the interval at the beginning of the discussion on stages.

It is interesting to note that, although there was the above rapid transformation in the epithelium, such a condition could

not be suspected by a mere study of the smears. Consequently, the method of obtaining material for histological study at this critical time involved a departure from the ordinary procedure. In the ordinary procedure it was only necessary to keep records of smears made from the animals under operation and to kill the pig at the desired period in order to correlate the smear with the condition in the vagina as shown in sections. In the above exception, the one killed for the high epithelium shown in figures 2 and 9, the method used was to study the length of consecutive cycles in all the animals and then to select the animal with the most regular recurring cycles and to kill it twenty-four hours before the calculated cornified stage.

In this connection the question arises, may not frequent douching of the vagina with warm saline solution affect the length of the cycle, and also may not the operation for hysterectomy affect the following cycles in the animal. By referring to table 2, the following facts will be made clear. The average length of the one hundred eleven cycles observed in twenty-four guinea-pigs was found to be 15.87 days. This corresponds to the length of the cycle as found by Stockard and Papanicolaou who found the average length of the cycle to be 15.73 days for sixty-seven cycles. There was no perceptible difference in the length of cycle in the normal and hysterectomized animals (table 2).

SUMMARY

1. The syringe method for taking vaginal samples is more satisfactory than any other method yet described for the guinea-pig.

2. The length of the oestrous cycle of the guinea-pig was found to be 15.87 days, the same as determined by Stockard and Papanicolaou (15.73).

3. The oestrous cycle has four well-defined periods or stages besides the interval.

4. Stage 1. The epithelium is at its greatest height at the beginning of this stage, ten to twelve cells. Cornification has been going on beneath the superficial layers. Vaginal smears contain large, vacuolated, granular, odd-shaped, epithelial cells.

5. Stage 2. This is the stage of desquamation of the flattened, scale-like, non-nucleated cells—cornified cells. The inner cornified portion of the vaginal mucosa loosens and may be shed as a cast.

TABLE 2

Showing the length of consecutive cycles in twenty guinea-pigs from August, 1920, to January, 1921

ANIMAL	LENGTH OF CONSECUTIVE CYCLES IN DAYS	AVERAGE LENGTH OF CYCLE
199	16, 17, 16, 17, 16, 17	16.50
200 ¹	15, 14, 15	14.66
206 ¹	15, 16, 16, 16, 16, 17, 15, 16	15.75
208	17, 16, 15, 16, 16, 17, 17, 16, 16	16.22
211	16, 16, 16, 16, 17, 15, 16	16.00
213 ¹	17, 16	16.50
220	15, 16, 16	15.66
232	15, 15, 15, 15, 15, 16, 17	15.42
233	16, 17, 16	16.33
246	14, 14, 16, 16, 16, 16, 16, 15	15.37
262 ¹	15, 16, 15, 16, 16, 15, 15, 16, 17	15.66
272 ¹	17, 16	16.50
277	16, 17	16.50
279	16, 16	16.00
280	15, 16, 15, 16, 16, 16, 15, 15, 15, 15	15.40
281 ¹	16	16.00
282	17, 16, 17	16.66
285	15, 16, 16, 17, 16, 17, 17, 17, 17	16.44
296 ¹	17, 15, 16, 16, 16, 16, 16, 16, 16, 16	16.00
298	16, 15, 15, 14, 15, 16, 16	15.28
Number of 14-day cycles.....		4
Number of 15-day cycles.....		28
Number of 16-day cycles.....		57
Number of 17-day cycles.....		22
Total number of cycles.....		111
Mode.....		16.0 days
General average.....		15.87 days

¹ Animals having both horns of the uterus excised.

6. Stage 3. Vaginal smears made during this stage contain round, nucleated, epithelial cells and some cornified cells which have remained from the preceding stage. Leucocytosis begins during this stage but leucocytes do not enter the lumen.

7. Stage 4. During this stage leucocytes appear in the lumen of the vagina for the first time since the beginning of the cycle. Smears contain epithelial cells and leucocytes.

8. Stage 5 or interval. Vaginal smears contain leucocytes and mucus, but very few or no epithelial cells. The epithelium has become reduced to its lowest condition, one to two cells. The epithelium is rapidly regenerated at the end of the interval immediately preceding the next cycle.

9. Stage 4 of Stockard and Papanicolaou, marked by the appearance of blood, is very doubtful.

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PLATE 1

EXPLANATION OF FIGURES

1 (Microphotograph.) Longitudinal section of the vagina at the middle of the interval, showing the finger-like processes of the submucosa projecting up into the epithelium. The submucosa contains many blood vessels. The epithelium is thinnest at this time, being only one or two cell layers. $\times 115$.

2 (Microphotograph.) Longitudinal section of the vagina near the cervix at the end of the interval, about twenty-four hours before stage 1, showing the epithelium at its greatest height, about ten cells. The uppermost cells are greatly enlarged and vacuolated and are the superficial epithelial cells found in vaginal smears during stage 1. Leucocytes are seen in the lumen. $\times 115$.

3 (Microphotograph.) Longitudinal section of the vagina during stage 1. Some of the large irregular epithelial cells characteristic of stage 1 are seen in the lumen along with a few leucocytes. The uppermost layers of cells have begun to slough off. About three or four cells below the surface of these superficial epithelial cells the cornifying layers are seen as flattened and more darkly stained cells. $\times 115$.

4 (Microphotograph.) Longitudinal section of the vagina during stage 1, showing the well-formed cornified layers splitting from the stratum granulosum below and the process of cornification extending up into the superficial epithelial layers above. $\times 115$.

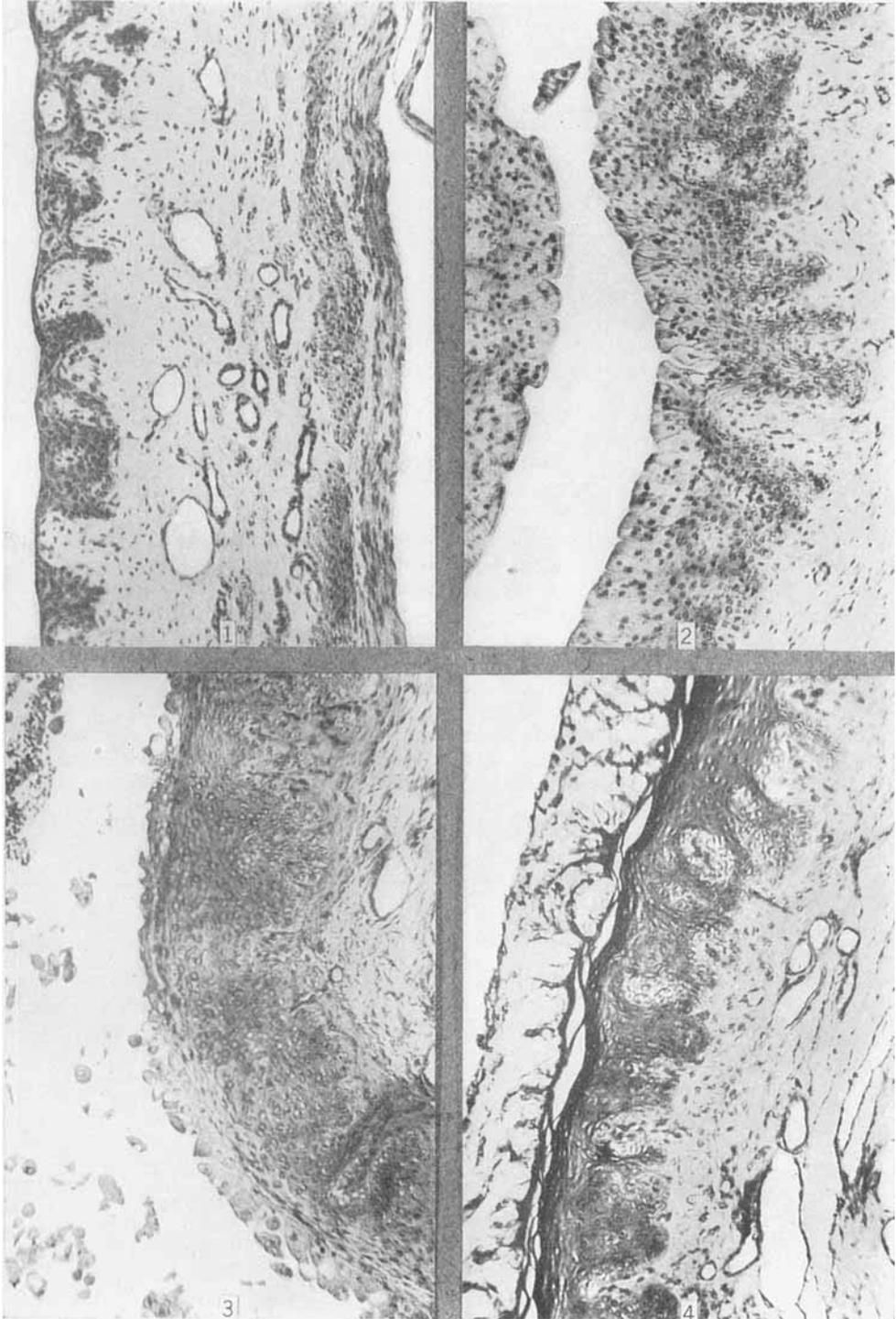


PLATE 2

EXPLANATION OF FIGURES

5 (Microphotograph.) Transverse section of the vagina near the cervix, showing the cornified layers free in the lumen. The animal was killed at the beginning of stage 2. Notice the few superficial epithelial cells between the two cornified layers. $\times 115$.

6 (Microphotograph.) Longitudinal section of the vagina near the cervix, showing the condition of the epithelium immediately after the entire cornified layer was shed as a cast. The epithelium is only four to seven cells high. Stage 3. $\times 115$.

7 (Microphotograph.) Longitudinal section of the vagina near the cervix, showing the condition of the epithelium during stage 4 when epithelial cells and leucocytes are found in the lumen. The epithelium is about three to four cells high. $\times 115$.

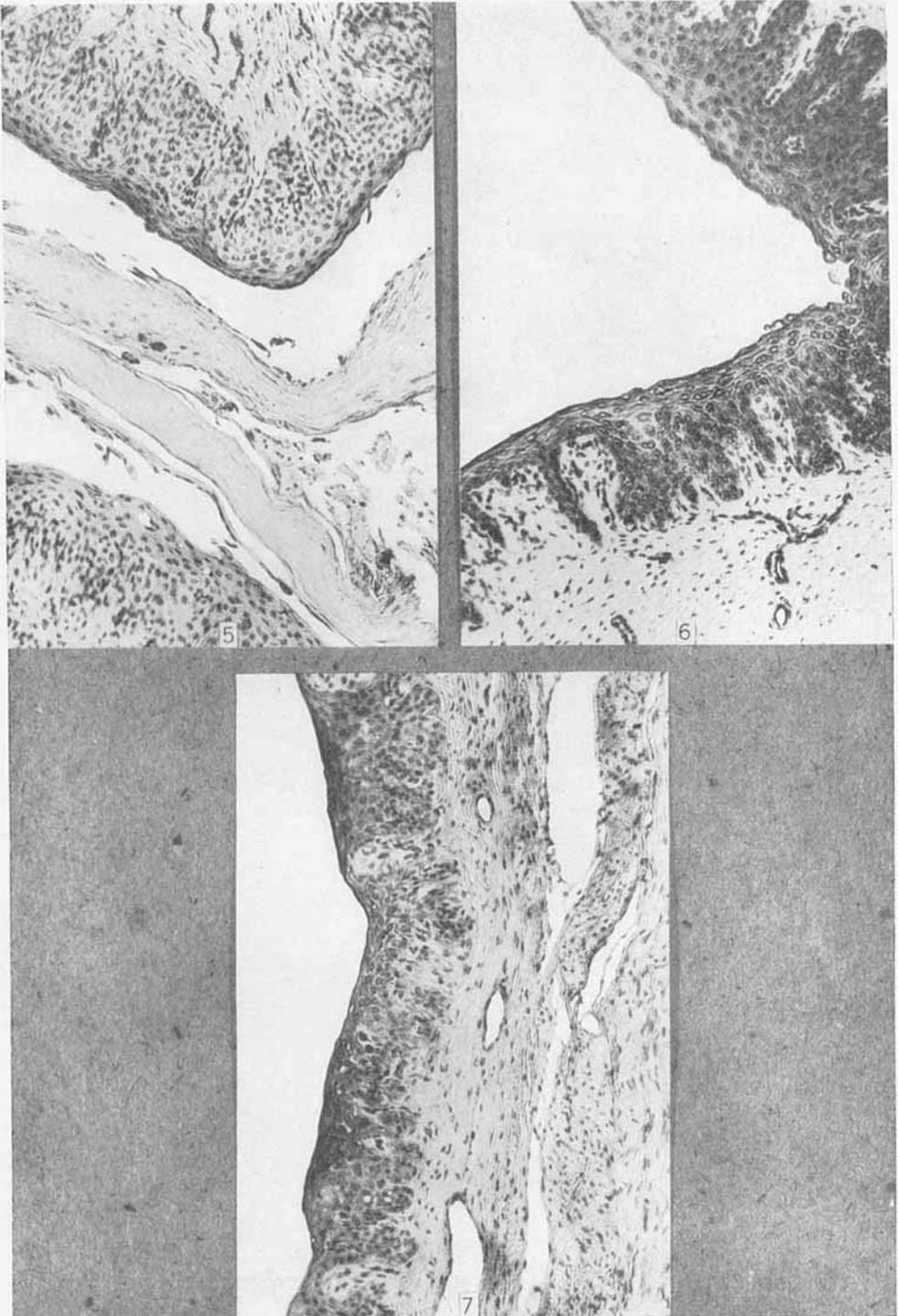


PLATE 3

EXPLANATION OF FIGURES

- 8 Part of mucosa in figure 1 enlarged. Middle of interval. $\times 624$.
- 9 An enlarged portion of the epithelium in figure 2. End of interval. Note the enlarged superficial epithelial cells. $\times 572$.
- 10 The upper part of the mucosa in figure 3 more highly magnified. Stage 1. The superficial epithelial cells are vacuolated. Under them is the beginning of the cornified layer. $\times 624$.
- 11 More highly magnified portion of epithelium in figure 6. After the shedding of the cornified layer. Stage 3. $\times 624$.

(Drawn by Miss Edna Fisher)

