Resumen por el autor, Edgar Allen

El ciclo éstrico del ratón.

El autor compara las observaciones llevadas a cabo en animales vivos con los cambios histológicos en el tracto genital y los ovarios. Estos cambios son de naturaleza cíclica y requieren un periodo medio de cuatro a seis dias. Los estados están representados por quiescencia, crecimiento, el climax éstrico y degeneración. Se manifiestan en la vagina por la formación cíclica y la degeneración de una capa córnea. Los cambos degenerativos en el epitelio uterino no siguen a su extirpación; como consecuencia de esto la hemorragia uterina es rara. La extrusión de los núcleos en el epitelio ciliado del oviducto es paralela a las fases degenerativas del útero y la vagina.

En le ovario existen grandes folículos durante la fase anabólica y son reemplazados por cuerpos amarillos en la fase catabólica. Por consiguiente, la ovulación separa a estas fases y tiene lugar durante el estro. La clasificación del ratón entre las especies de ovulación espontánea es errónea. Algunos ovulan con regularidad, otros tan solo de un modo esporádico, y otros tan solo ovulan con un estímulo sexual adicional. Diferentes modos en la longitud del ciclo son peculiares de diversas razas de ratones. Puesto que el ciclo es corto y la ovulacón puede ser espontánea o no serlo, los ovarios pueden contener tres o cuatro series de cuerpos amarillos voluminosos o no contener ninguno. En ambos casos se presentan ciclos normales. A consecuencia de esto parece justificada la conclusión de que los cuerpos amarillos del estro no poseen función causativa en los procesos anabólicos o catabólicos del cico éstrico. Las pruebas acumuladas indican la presencia de óvulos en los folículos grandes como causa del crecimento del tracto gental y el estro, y su ausencia o atresia como la causa de la fase degenerativa.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MARCH 27

THE OESTROUS CYCLE IN THE MOUSE

EDGAR ALLEN

Department of Anatomy, Washington University School of Medicine

TWENTY-FOUR FIGURES

CONTENTS

1. INTRODUCTION AND LITERATURE

Although the mouse, Mus musculus, has been used for embryological purposes for nearly a century, no exact knowledge of its oestrous cycle is available. One reason for this is that the mouse, like other rodents, comes into 'heat' and receives the male within from six to twenty-four hours after parturition, and most investigators have timed their collection of embryological material from this 'heat' moment. Another reason for our lack of knowledge of the oestrous cycle in this form is the fact that external signs of 'heat' have been relied upon for diagnosis. Concerning this, Heapa states: "It is difficult to determine the length of the prooestrum and oestrus in rodents, since the external signs which characterize these conditions are comparatively slight." In many animals I have found them to be either entirely absent or so slight as to make an accurate diagnosis impossible.

Discussing the sexual phenomena of rodents, Marshall says: "Mus decumanus and M. musculus are known to experience a recurrence of the dioestrous cycle for more than nine months of the year in the absence of the male." The other three months are probably winter. No observations on the length of this cycle are given.

Sobotta ('95) is the only embryologist supposedly timing his stages from an oestrous period other than that following parturition, and he believed the duration of the cycle to be equal to the gestation period, i.e., 20+ days. Apparently all writers up until as late as 1916–17 have followed Sobotta in this particular.

Danforth in unpublished observations in 1914–15 attempted to check the length of the cycle by tabulating the number of days between two successive litters and computing the greatest common divisor of these intervals. In his records of sixty-six animals, twenty-three were excluded because of the possible complication due to the mother lactating while pregnant. The greatest common divisor of the modes of his curve was found to be 4 to 6 days. This evidence is inconclusive, but it at least casts doubt on the existence of a uniform oestrous cycle of 20+ days.

H. P. Smith ('17) attempted to approach the problem from a consideration of the ovarian cycle. This method permits of only one reliable observation, other than that of parturition, on one animal, namely, that made on histological examination of the ovaries after death. Conclusions must be drawn from a series of animals on each of which only one observation is made. The individual variation among even litter mates is so great as to make this method very inaccurate although a large number of animals be used. By this method Smith was led to conclude that the oestrous cycle is one of great variability, averaging seventeen and one-half days. He began the collection of his series at parturition, and his results are therefore really the recovery time of the ovaries—the time required for a resumption of ovulation after the interference of pregnancy. The variation in this period is known to be great even within a single species, therefore the conclusions drawn can scarcely be applied to the normal ovarian cycle.

Estimates of previous oestrous periods arrived at from histological comparisons of the corpora lutea are not reliable since, as will be shown in the present paper, many mice when isolated from males do not ovulate spontaneously during oestrus.

Daniels ('10) and King ('13) have shown, in the mouse and rat, respectively, that if an animal becomes pregnant at the oestrous period following parturition and suckles her litter as well, the gestation period of her second litter may be lengthened, in some cases to from twenty-four to thirty days, an increase of 20 to 50 per cent. Kirkham ('16–'17) showed this delay to be due to a failure of the embryos to implant in the uterine mucosa. The embryos apparently remain free in the uterus in a state of inhibited growth for a time equal to the extension of the gestation period. No histological differences have been reported between the uterine mucosa of pregnant mice and those pregnant and also lactating. Long and Evans ('20), working on the rat, have reported histological difference between corpora lutea under these two conditions and ('21) a limiting influence of combined pregnancy and lactation on the growth of the vaginal epithelium. For several reasons, therefore, it seems better to time embryological material from a mating during a heat period not immediately preceded by parturition.

Further literature on the oestrous cycle in the mouse is limited to isolated observations made incidentally during investigations of other problems.

Concerning the literature on other rodents Marshall ('10) accepts Heape's statement that "dioestrous cycles recur for five or six months in the domestic rabbit, and that if oestrus is experienced in winter it may occur independently of the possibility of pregnancy. While some animals exhibit oestrus every three weeks fairly regularly, others do so every ten days; on the whole, I think 10–15 days is the usual length of their dioestrous cycle."

This is the sort of observation on which so much of the literature of oestrus is based.

Lataste has done considerable careful work on the vaginal plug of rodents and in this connection notes that the dioestrous cycle is usually about ten days.

Loeb ('11 a) reported a sexual cycle in the guinea-pig recurring every twenty to twenty-five days and described the histological changes in the uterus and ovaries at intervals. In a later paper ('11 b) he concludes the duration of the cycle to be from fifteen to twenty days.

In 1917, Stockard and Papanicolaou described a method of diagnosing the stages of oestrus in animals showing only slight external signs of their condition by a histological examination of the cell contents of the vaginal fluid. The cellular content of this fluid changes characteristically as the cycle progresses. This method offers the advantage of providing a complete history obtained from the repeated observation of reliable criteria upon the same living animal. It thus permits the study of individual variations and gives a record of the events taking place before killing the animal to study histologically the internal genital organs. By this method they obtained an oestrous cycle in the guinea-pig of remarkable regularity averaging 16+ days. This method also permitted Stockard and Papanicolaou to locate very exactly the moment of ovulation in a living guinea-pig. The rupture of the follicle occurs when the vaginal smear shows a definite cellular picture.

In the rat, Heape placed the duration of the oestrous cycle at ten days. Long and Evans ('20), using the above-mentioned vaginal fluid examination method, have shown this to be from four to six days, or actually only one-half as long as Heape supposed.

Of course much valuable information concerning certain phases of the sexual cycle in the mouse is already at hand in the work of Sobotta, Kirkham, Long and Mark, Smith, and others; this information, however, may be added to when approached from another viewpoint.

2. MATERIAL AND METHOD

Variation in the duration of the oestrous cycle has been reported in nearly all mammals studied, not only in closely allied forms, but within the limits of the same species. It seemed desirable, therefore, to include some individuals from each strain of mice in our colony. Several variations in other phenomena peculiar to certain strains had formerly been noticed, the most striking being the difference of reaction to ether anaesthesia: Albinos in our stock are very susceptible, while brown and black are quite resistant. It was thought important to find if there was any variation in the oestrous cycle typical of different strains. The stock chosen included brown, black, albino, dominant white with black eyes, gray, agouti, and yellow, as well as hybrids of these strains. More than ninety animals have been used in this work, the majority being young virgin mice. A few who had born litters were included for comparison, but in all cases records were not begun until the animals had been separated from males for at least a month after the removal of their young.

It has long been known that to maintain the oestrous rhythm the animals must be healthy, well fed, and under uniform environmental conditions. To eliminate crowding, no more than four animals were placed in one cage. Cages had a floor space of 850 sq. cm. and a capacity of 17,000 cc.

A constant supply of water was accessible to the mice through small holes in the ends of test tube containers. The food consisted of oats, cracked corn, sunflower seed, and dog biscuits.

Usually a little hay was added as nest-building material. During the winter the animals were kept in a heated room.

Methods

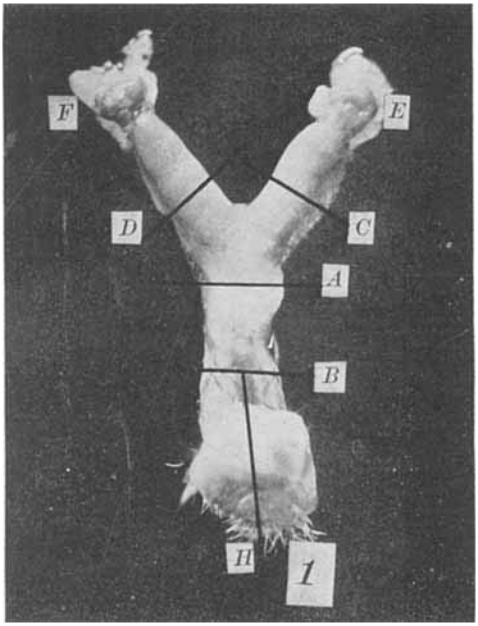
Stockard and Papanicolaou ('17) have shown the most reliable criterion of the condition of heat in the guinea-pig to be the cell content of the vaginal fluid. Their technique, slightly modified because of the smaller size of the mouse, was followed. Some animals were examined three times daily to get the exact time relations of the various phases of the cell changes, but for the accumulation of data as to cycle length, examination once daily was found to be adequate. The condition of the vulva, the degree of opening of the vagina, and the nature of the vaginal contents were noted. A histological examination of the smears was always the deciding factor in the diagnosis. Smears were made by the usual bacteriological technique. Haematoxylin and aqueous eosin were used as stains. A block with a converging trough which could be quickly covered was devised for holding the animal while smears were being taken.

Animals used for correlating the conditions of different parts of the genital tracks with each other and with the cellular changes in the smears were examined and smears collected for several cycles before they were killed. These repeated examinations gave some indication of their 'degree of sexuality.' Smears were invariably taken immediately before killing. The animals were killed instantly by a sharp rap at the base of the skull, the abdominal cavity opened, and the conditions of the uterine cornua noted as to size, transparency, blood supply, and contractility. Shortly after the body cavity is opened, the uterus contracts to such an extent that the marked differences present at various stages in the cycle cannot be detected; therefore, an immediate inspection is necessary. After examining the fresh uterus the bladder was clipped off, the symphysis pubis sectioned, the skin was cut around the vulva, and the phallus, vagina, uterus, oviducts, and ovaries were dissected out and placed on a glass slide. After a minute's drying they adhere to the slide and can be fixed without contortion. Bouin's fluid was used as a fixing reagent and proved satisfactory for both uteri and ovaries. After hardening in the lower alcohols, the organs were cut to facilitate orientation and imbedding. Serial sections 10 μ thick were made of the ovaries and oviducts. In trimming these organs it is important that the peritoneal ovarian sac be left intact to protect the surface of the ovary during dehydration and imbedding. Several transverse serial sections of the middle of each uterine cornu, the body of the uterus, the vagina, and the cervix, as well as several serial sagittal sections through the phallus and lower vagina, were cut at a thickness of 6 to 8 μ.

Cut surfaces were oriented so that the same regions in different animals could be compared (text fig. 1).

3. POINTS OF IMPORTANCE IN THE ANATOMY OF THE GENITAL ORGANS OF THE MOUSE

There are some points in the anatomy of the genital organs of the mouse which need emphasis since they have an important bearing on certain aspects of this work.



Text fig. 1 Genital organs before fixation, showing the planes of histologica sections. *A*, body of uterus; *B*, vagina; *C*, middle of left cornu of uterus; *D* middle of right cornu; *E*, left ovary and oviduct; *F*, right ovary and oviduct *H*, sagittal section of phallus and lower vagina.

The vagina in the mouse opens directly on the vulva without the protection of any structures homologous to the labia minora. Its stratified epithelium is devoid of glands. The phallus is large and prominent and is traversed in its entire length by the urethra. The cervix is relatively short, the division into the lumina of the uterine cornua occurring 1 to 3 mm. above the opening of the external os. A stratified epithelium, similar, but considerably lower than that of the vagina, is continued up into the cervix in virgins as well as in multiparae, but beginning at the branching of the cervical canal, it becomes simple, non-ciliated, cuboidal or columnar. Glands are uniformly distributed throughout the uterine mucosa except along the line of attachment of its broad 'mesentery-like' ligament. The entrance of the oviduct into the uterine cornu is surrounded by valve-like folds of the mucosa, which make it very difficult to inject the oviducts from the uterus. These valves are in a position to guard against a back flow of fluid from the distended uterus into the oviducts, which might be fatal to the passage of ova down the tubes. The oviducts are ciliated only at the ovarian end where the mucosa is thrown into high folds. Throughout the rest of their extent the epithelium is simple columnar. The uterine end can be distinguished from the middle segment by the low folds of its mucosa, those in the middle segment being relatively high.

The ovaries are situated just caudad to the kidneys and are completely surrounded by closed sacs of peritoneum from which the openings of the oviducts lead. Therefore, the number of ova in the tubes and periovarian sacs is always the number ovulated, since none can escape into the peritoneal cavity.

4. EXTERNAL MORPHOLOGICAL CHANGES DURING THE OESTROUS CYCLE

a. Evidence from the external genitalia and cell contents of the vagina. Among many rodents there is very little or no discharge during 'heat.' The degree to which congestion and reddening occur in the vulva is also variable, often being totally absent or so slight as to be a very poor criterion of oestrus. In a total of 355 cycles chosen at random to decide this point in the mouse, only 190, or 53.5 per cent, showed well-marked external signs. And seventy-three, or 23.3 per cent, showed the vulva and vagina in an apparently resting condition, although by the cell contents of the vaginal fluid oestrus was shown to be present. A few animals evidenced continued external signs of 'heat' during the metoestrum and the dioestrous interval, so that here again external signs as criteria of a condition of oestrus are not reliable. That such signs are due primarily to congestion and consequent poor tonus of the muscle layers of the vagina is indicated by their rapid disappearance coincident with the relaxation of the urethral sphincter following death. However, to settle the question as to their value in diagnosis, the external signs were always noted when taking smears, and as they were typical in 53.5 per cent of cases, a description is desirable. Heape's terminology is followed in describing the phases of the cycle, with emphasis on the following points: 1) that growth and congestion continue through oestrus; 2) that the metoestrum may be further subdivided into two periods.

To eliminate a too frequent use of 'oestrum' in its various forms, the stage will be designated as shown below:

D, dioestrum, period of relative quiescence.
P, prooestrum, period of augmented growth and congestion.
O, oestrum, period of sexual excitement, the climax of prooestrous conditions.
$\left.\begin{matrix}M_1\\M_2\end{matrix}\right\}$ metoestrum, period of return to the dioestrous condition.

Stage D. In the mouse during the dioestrous interval the vulva is very inconspicuous and the orifice of the vagina is usually tightly closed. There is a little fluid which is viscous and stringy. The smear shows epithelial cells, usually only a few, in various stages of nuclear degeneration and cytoplasmic shrinkage, and always some polymorphonuclear leucocytes.

Stage P. During the prooestrum, if external signs are marked, the vulva is pink or red and swollen, the vagina gapes open, the fluid in its lumen is serous, and the smear shows only nucleated epithelial cells (fig. 2).

Stage O. During the oestrous period, the vulva may still be swollen, the orifice of the vagina open and a dull white in color, the vaginal mucosa almost dry, and the smear shows only cornified, non-nucleated, red eosin-staining cell remains (fig. 3). In animals where external signs are absent or slight, the finding of only cornified cells in the smear makes a diagnosis of 'heat' possible.

Stage M_1. In the first half of the metoestrum, the vulva has usually lost most of its swelling, the orifice of the vagina still gapes open and is whitish, occasionally showing small granulations, the lumen is still dry, and the smear shows red cornified elements of the previous periods, always very numerous, and now bunched or caked (fig. 4).

Stage M_2. This stage is evidenced by a normal vulva and a tightly or partly closed vaginal opening. The vaginal contents change as the period progresses from a pasty or viscous to a fluid consistency, and the smear shows polymorphonuclear leucocytes among the red horny elements. A few polymorphs and many cornified cells is an early M_2 stage, and a heavy leucocytic infiltration and decrease in number of the cornified cells is a late one (fig. 5).

This stage then merges into D with a decrease in leucocytosis and the appearance in the smear of small numbers of nucleated epithelial cells. By standardizing the technique of smear preparation, stages O and M_1 are easily differentiated, although the basis for this division is the number and clumping rather than any change in the character of the cells themselves.

The separate phases as well as the duration of the whole cycle show great variation under the most uniform conditions of food and environment. Further discussion may be postponed until the histological condition of the various genital organs can be described.

5. ANIMALS STUDIED HISTOLOGICALLY

A series of twenty-seven carefully timed animals was prepared for histological study. They consisted chiefly of four strains: albino, black, brown, and gray. Observations were made daily for a period of from three to five weeks before killing, in order to obtain an accurate record of the number and duration of oestrous periods as an aid to the interpretation of ovarian conditions. The smear made just before killing usually followed the routine smear for that day by three to six hours, so that two observations at short intervals were available for diagnosis.

The criteria used in placing the animals are the following:

1. The cell contents of the vaginal smear at death.
2. The number of layers of vaginal epithelium unaffected by cornification or leucocytic infiltration.[1]
3. The position, with regard to the free surface of the epithelium, of the granular and horny layers when present.
4. If ova are present in the uterine tubes, the segment in which they lie indicates the time of ovulation with regard to oestrus.
5. The stage of development of the corpora lutea when present aids in a correct arrangement of the series. Their age is estimated by the position of the ova in the tubes, and compared with corpora lutea of pregnancy (recorded in column 7, table 1).

The following table of animals killed for histological examination represents a complete, closely spaced series. It seems convenient to begin at the middle of the dioestrous interval and return to the same point in the next cycle.

The results of the histological examination of this series of organs can be most satisfactorily presented by means of a detailed description of typical animals (marked # in table 1) representing the midpoints of the five stages of the cycle.

The dioestrous interval

Stage D is best represented in this series by animal #2, a virgin mouse seven months old. She was killed after 53 days, observation, during which time nine complete cycles were recorded, making the average duration 5.9 days. In six of these nine cycles pronounced external signs were apparent. The last four oestrous periods occurred 3 to 5, 11, 14 to 15, and 19 to 20 days before her death. The record shows her to have been in an early day of the dioestrous interval after a cycle of from 7 to 8 days' duration marked by external signs and containing a three-day oestrous period.

A gross examination of the internal genital organs was made immediately after opening the body cavity before there was any

---

[1] These underlying layers are very difficult to count in some of the phases, so that two numbers are used to express a limit of error.

TABLE 1

Animals examined histologically arranged in order of progressive phases of the oestrous cycle as diagnosed by vaginal smears and microscopical examination of the genital organs. (For key to table, see footnote.)[2]

CYCLE SEQUENCE	COLLECTION NUMBER	NUMBER OF LAYERS OF VAGINAL EPITHELIUM	POSITION OF GRANULAR AND HORNY LAYERS	SEGMENT OF OVIDUCT CONTAINING OVA	AGE OF MOST RECENT CORPORA LUTEA IN DAYS	AGE EQUIVALENT OF CORPORA LUTEA OF PREGNANCY	SMEAR	STAGE
1	26	5–6	Gone	2nd–3rd	2+	30–50 hrs.	0NE, 0P	D
#2	*24*	5–6	Gone	3rd	3	3+ days	1NE, 3P	D
3	8	5-7	Gone	3rd+	4	3+ days	1NE, 2P	D
4	11	5–9	Gone	None	?		0NE, 1P	D
5	13	3–5	Gone	None	5		0NE, 1P	D
6	15	5–6	Gone	None			0NE, 0P	D
7	6		Gone	None	?		0NE, 0P	D
8	3	7–10	Before formation	3rd	3		0C, 1NE	P
#9	*10*	12–13	Granular, no horny	None	3–4	3+ days	1NE, 0P	P
10	12	11–13	Under 2–4	None	3–4	3+ days	0NE, 0P	P
11	14	11–13	Under 2–4	None	?		0C, 1NE	P
12	9	8–10	Under 3–5	None	5		0C, 1NE	P
13	23	9–10	Under 3–4	*None	None	*	1C, 1NE	P
14	1	10–11	Superficial	None	5		1C	O
#15	*4*	8–12	Superficial	None	5		1C	O
16	2			None	5+		1C	O
17	16	12–13	Superficial	None	7–8		1C	O
18	21	9-12	Superficial	1st–2nd	1	3- 7 hrs.	3C, Bu	M_1
#19	*19*	11–12	Superficial	2nd	2	30–35 hrs.	3C, Bu	M_1
20	18	10–12	Superficial	*None	None	*	2C, Bu	M_1
21	7	4–6	In lumen	2nd–3rd	2	20–40 hrs.	2C, Bu	M_1
22	20	6–7	In lumen	2nd-3rd	2	40–50 hrs.	3C, Bu	M_1
23	25	4–7	In lumen	3rd	3	50–60 hrs.	2C, 1P	M_2
24	17	7–9	In lumen	3rd	3	60–70 hrs.	1C, 1P	M_2
25	22	7–8	In lumen	3rd	3	50–60 hrs.	2C, 2P	M_2
#26	*27*	6–8	In lumen	2nd–3rd	2 & 3	30–50 hrs.	1C, 3P	M_2
27	5	5–6	In lumen	3rd	3	72+ hrs.	1C, 2P	M_2

---

[2] *Key to table 1.* Column 1 represents the progressive order of the series beginning with an early phase of the dioestrous stage. #, Animals described in detail as typical of the five stages of the cycle; * (in columns 5 and 7), ovulation not spontaneous; *0, 1, 2, 3* (in column 8), represent relative numbers of cell types; *C*, cornified epithelial cells; *NE*, nucleated epithelial cells; *P*, polymorphonuclear leucocytes.

obliteration through contraction of the characteristics which differentiate between the O and D uterus. The anastomosing uterine and ovarian vessels were small, the uterus was of medium size and anemic.

Histological examination of cross-sections of the vagina midway between the cervix and the vulva shows the lumen to be small in diameter, with the walls thin, folded, and collapsed. Free nucleated epithelial cells and leucocytes are present in considerable numbers. There is no basement membrane under the epithelium and the deepest layer of the stratum germinativum shows no distinct cell membrane on the side adjacent to the stroma. The epithelium not extensively infiltrated with leucocytes is only five or six layers deep and shows no signs of cornification (fig. 6). Very few mitoses are to be found. Scattered leucocytes are abundant in the one or two superficial layers, and in several places small clumps are gathered in clear lacunae.

In general the description of the vaginal epithelium holds true for that of the lower cervical canal; there is no basement membrane beneath the epithelium, which is five or six layers high, and no sign of cornification.

Cross-sections through the uterine cornua midway between their junction and the attachments of the oviducts show narrow, slit-shaped lumina indicative of a lack of distention. There are very few cells of any sort free in the lumina. The epithelium has no basement membrane, the lower parts of its cells stain lightly, and cell walls are not readily distinguishable. In many places the cells are piled up four to eight layers deep in poorly staining syncytial masses. Mitoses are only occasional, being present in a few instances in the syncytial masses. The epithelium is intact everywhere, although it is quite heavily infiltrated with leucocytes. Some of the glands show functional activity, as evidenced by slight distention.

The oviducts were adherent to the periovarian sacs and were sectioned serially with the ovaries.

The oviducts are moderately distended throughout, and are entirely free from leucocytic infiltration. The non-ciliated epithelium, lining the second and third portions, shows few signs of degeneration, but the ciliated cells covering the highly folded mucosa of the part leading from the ovisac show all stages of the extrusion of their nuclei. Some of these extruded nuclei still adhere to the free surface of the cells and are similar to nuclei of normal cells. Others show varying degrees of pycnosis. Many small regions are to be found devoid of cilia, apparently marking cells from which the nuclei have been extruded. A further description of this process will be undertaken later.

The two oviducts in this animal contained eight ova, still in good condition, although in none was the zona pellucida prominent. (This may be due to the fixing reagent as stated by Sansom, '20.) In most of the ova the second maturation spindles were still intact, but rarely were polar bodies recognizable. The ova were bunched in the last segment of the tubes. Therefore, ovulation occurred three days previously (H. P. Smith, '17).

The ovaries contain eight medium-sized follicles, all superficially located. The primary liquor folliculi is beginning to form, but has not yet reached a stage far enough advanced to make possible the distinction of a cumulus. The nuclei of the ova are in the resting stage. In this pair of ovaries, there are more than thirty atretic follicles in all stages of degeneration, most of them deeply situated in the stroma. There are at least three sets of corpora lutea present.[3] The most recent corpora lutea are easily distinguishable from the older ones by their blue color, the latter staining more heavily with eosin. There are eight of this last set. They are similar in degree of development, so that a description of one will suffice for all. The central lake is almost obliterated, chiefly by the hypertrophy of the granulosa cells which do not at this stage take eosin readily. The theca interna has entirely disappeared and the ingrowth of connective tissue has reached the edges of the lake and woven a fine reticulum about the inner walls of the luteal cells, at the same time beginning an arrangement of these cells into cords. Slight vascularization is evident, but there are erythrocytes in

---

[3] It should be understood here that all corpora lutea referred to in this paper are corpora lutea of oestrus, as no pregnant nor lactating animals have been used.

the central cavity in only one of the eight corpora lutea of this set. From the position of the ova in the tubes, it was concluded that ovulation has occurred three days previous to killing. This set of corpora lutea of oestrus is, therefore, three days old. They are surely the equivalent of the seventy-two-hour corpora lutea of pregnancy.

The next older corpora lutea are greatly degenerated. They are small, deeply placed, irregular in outline, and poorly demarcated from the surrounding stroma. The proportion of connective-tissue cells to luteal cells is about equal. As stated above, the cytoplasm of the luteal cells stains red with eosin, but it is a faded or blotchy red. No distinct large blood vessels are present as in the first set described. A third set consisting of only a few still more poorly defined corpora lutea is present. These are very small, staining a light pink, and are almost completely obliterated by a connective-tissue ingrowth.

Of the three sets of corpora lutea distinguishable, the age of the youngest can be placed at three days, with a limit of error of about twenty hours. From the results of the examination of other ovaries of this series, the second set is at least ten days old, and the third still older.

There are a few cells present in these ovaries which could be defined as interstitial.

The prooestrum

Animal #9 was chosen as typical of the stage P condition. A routine examination was made at 1 P.M. and another just before killing at 4.30 P.M., an interval of three and one-half hours, in which time many of the leucocytes had disappeared from the vaginal contents, indicating an early stage P. Previous to killing she had been observed for sixteen days, during which time four complete cycles had been recorded, making the average duration four days. External signs were marked during all four of the oestrous periods. These occurred 4, 7 to 8, 11 to 12, 15 to 16 days before death. Her record is one of minimum cycle duration and perfect regularity.

The uterine and ovarian vessels were congested (two or three times larger than during the D period), and the uterine cornua were much distended. When the animal was killed the uterus did not expel this fluid (as the bladder expels the urine) and when held up between the observer and the light, the uterine cornua are so transparent that the folds of the mucosa are easily visible. The ovarian capsules were not distended.

Cross-sections of the vagina show a fairly large lumen containing some nucleated epithelial cells and a very few apparently degenerate polymorphonuclear leucocytes. The epithelium shows a basement membrane in a few restricted regions. There are twelve to thirteen layers of epithelial cells, of which the outer four to five layers stain very lightly with eosin. This demarcation is made still clearer by a well-formed granular layer which serves as a line of division. The layers superficial to this are not so flattened as those immediately underlying the stratum granulosum. There are as yet no other signs of cornification (fig. 8). Mitoses are abundant in the germinativum. The epithelium is free from leucocytes except for a very few in the most superficial layer of cells. The nuclei of the stroma are loosely packed and intercellular spaces are everywhere evident. The sagittal section shows the same conditions, except that a thin cornified layer still remains on the ventral side of the vagina at its opening on to the vulva.

The cervical epithelium just before it merges into the simple epithelium of the uterus, although at this point being only from three to five layers high, is yet divided into two regions, a deep, darkly staining, and a superficial, lightly staining one. It is similar in most respects to that of the vagina except as regards height.

The lumina of the uterine cornua are large. The fluid with which they are distended is not coagulated by Bouin's reagent. There are no cells of any sort free in the lumina. The epithelium is low columnar and has a distinct basement membrane in all but a few regions. Mitoses are frequent. No leucocytes are to be found in the epithelium and only an occasional one is in the subepithelial zone which is most heavily infested by them during the D stage. The gland ducts are distended and their cell outlines are clearly defined. Mitoses are absent, which indicates little growth activity in the glands during hyperfunction. Glands are distributed evenly throughout the mucosa except opposite the line of the attachment of the broad ligament. The nuclei in the stroma are densely packed, possibly because of distention of the lumen.

The oviducts are slightly distended. Their epithelium is in good condition in the non-ciliated portions. In the ciliated segment, all stages in the process of extrusion of nuclei are apparent, but to a less degree than in the D stage animal previously described. No leucocytes are present.

There are no ova to be found in either oviduct. Therefore, if ovulation occurred at the last O period, that should have been at least four days before death.

Sections of the ovaries show a marked hyperemia. There are two sets of follicles containing liquor, the first one composed of ten follicles, which are large and distended with considerable amounts of liquor. They are all superficially located. The cumuli are intact and still solid masses of cells. The nuclei of the ova are in a resting condition. The second set of follicles is less mature, being medium sized, with the liquor still confined to small pools at the poles of the follicles.

There are several medium-sized atretic follicles in which the cells of the cumuli have entirely degenerated, leaving the ova free in the liquor. The granulosa cells also show marked signs of atresia.

The ovaries of this animal are unusually large, which may be partly accounted for by their hyperemia, but the unusual number of corpora lutea is probably the main reason. At first glance the ovaries appear to be just large masses of corpora lutea. Three sets are easily distinguishable. Those of the first set are superficial, medium sized, and stain dark blue. Their central lakes are completely ingrown, but two of them show small patches of red blood corpuscles at their centers. They are at least four days old.

The corpora of the second set are larger than those of the first, are superficially located, and deep red in staining reaction. The cells are arranged in 'cords,' but their walls are not clear-cut and the cytoplasm has a blotchy appearance. The edges of these corpora lutea in section are clearly defined from the surrounding stroma. Theca interna cells as a distinct layer no longer exist. This set probably corresponds to the second ovulation before death, occurring at some time during the O period; therefore, seven to eight days previously.

A third set of corpora lutea, less clearly defined from the stroma, red staining, and about the size of the first set, is present. The stroma has begun to invade their surfaces and the proportion of connective-tissue cells to luteal cells throughout has increased. The record of this mouse shows an O period eleven to twelve days before death, so that this third set must be at least that old. There are a few cells clearly definable as interstitial in these ovaries. They are restricted in distribution to limited areas near the periphery.

The oestrous period

Animal #15 shows a typical oestrous condition. She had been examined for twenty-eight days previous to killing, in which time only three complete cycles had been recorded, making the average duration 9⅓ days. Her oestrous periods were 6, 16 to 17, and 28 days previous to death. Her last cycle was of five days' duration. Her first and second cycles had long dioestrous intervals, seven to eight days in each case, while the other four stages required only three to four days.

Upon opening the body cavity, the uterine and ovarian vessels showed some congestion, though less than that reported during the prooestrous period. The uterus was moderately distended, but less transparent. Gross examination shows the same conditions as found in the prooestrum to prevail, although to a less degree.

The lumen of the vagina in cross-section is much folded and collapsed, as would be expected from the record of the examination before death. It contains a small number of detached cornified epithelial cells which are very thin in cross-section. A distinct basement membrane clearly marks off the epithelium from the stroma. The epithelium is eight to twelve cells deep under the granular and horny layers, which are now superficially placed in all regions. A few shrunken blue staining cells with pycnotic nuclei still remain in the deeper parts of the crypts between folds in the mucosa. They represent the last stages of degeneration of the cells of the prooestrous smear. Mitoses are frequent. There are no leucocytes present in the epithelium (fig. 10).

The cervical epithelium is six to eight layers high, but shows no cornification as yet. Except for these two points, the description of the vaginal epithelium holds good for that of the cervix; i.e., it has a clear-cut basement membrane, contains frequent mitoses, and is free from leucocytes.

The lumina of the uterine cornua are moderately distended, but contain no cells of any sort. The basement membrane of the epithelium is distinct and heavy and the cells are columnar (fig. 15). Mitoses are moderately frequent. No leucocytes are to be found in the epithelium. Gland lumina show a slight distention pointing to moderate functional activity, but the scarcity of mitotic figures indicates little growth. Cell borders are distinct and the cells show no degenerative changes. Although a few leucocytes are distributed through the stroma, only an occasional one is found in the gland epithelium. In section, the uterus shows slight hyperemia.

The oviducts are moderately distended. In the ciliated portions nuclear extrusion is still apparent, but to a less degree than during the P stage. Consequently, larger surfaces present unbroken ciliation. There are no leucocytes present. No ova are to be found in the tubes; therefore, if ovulation occurred at the last oestrus, this must have been at least four days previous to killing.

There are two sets of normal follicles far enough matured to show liquor folliculi. The ten follicles of the first set are the largest found in this series of animals. They are all superficially placed, and although the cumuli are still intact, the secondary liquor folliculi is present in them in small pools. The ova show resting nuclei centrally placed. The second set of follicles are medium sized and show only an early beginning of liquor formation. There are several atretic follicles present in various stages of granulosa degeneration.

There are from twenty to thirty corpora lutea in this pair of ovaries, among which at least three sets can be defined. The most recent ones, ten in number, are superficially placed and quite large. These corpora stain a light blue in contrast to the red-staining older sets. They are clearly defined from the stroma, and their constituent cells which are arranged in cords show distinct cell walls. Although the ovaries are hyperemic, there are few erythrocytes to be seen in these corpora lutea, which is in marked contrast to the small vessels crowded with erythrocytes in the four-day corpora of the stage D animal described above. They must correspond, therefore, to the O period recorded on the sixth day before death. The other two sets of corpora lutea are distinguishable chiefly by size, position in regard to the surface of the ovary, and proportionate ingrowth of stroma cells at their surfaces. The cytoplasm of the 'luteal' cells of both sets stains a blotchy red. There are a few interstitial cells in these ovaries.

The early metoestrum

Animal #19, representing a typical M_1 stage, was one of three females of a litter of homozygous brown mice separated from males at the time of weaning, and examined daily for the appearance of her first oestrus. An open vagina was first noted on July 12th, at the age of three and one-half months, the membrane closing the orifice having ruptured during the night of the 11th. Routine smears were started on the 13th, and continued for forty days, during which time five complete cycles were recorded, making the average duration eight days. If, however, the first two longer periods are excluded, it brings this average down to seven days. At only one of these periods did she evidence external signs of oestrus. Previous 'heat' periods are recorded

2 to 3, 9 to 10, 14 to 16, 23 to 24, and 34 days before she was killed. A gross examination showed the ovarian and uterine vessels to be relatively small and inconspicuous. The uterine cornua were small in diameter and quite opaque. The periovarian sacs were slightly distended.

Cross-sections of the vagina (fig. 11) show a semicollapsed lumen crowded with masses of non-nucleated, cornified, deeply red-staining, epithelial cells. The epithelium has no basement membrane and the deepest layer of the germinativum is in intimate relation with the connective-tissue cells of the stroma. There are eleven or twelve layers of epithelial cells under the granular and horny layers, which are everywhere superficial and intact except for the delaminated masses in the lumen. There are very few or no mitoses present in the germinative layers. Leucocytes are also absent.

The lumina of the uterine cornua are not entirely collapsed, but contain in several of the sections a few nucleated epithelial cells. The basement membrane of the uterine epithelium is entirely lacking and in its place is a broad red-staining band. The deeper edges of the epithelial cells and the most superficial stroma cells are involved in it, and their structures contained in this band become blurred and take the stain poorly. Small vacuoles are apparent in and among the epithelial cells. A very few leucocytes have already entered the degenerate band or zone under the epithelium.

The oviducts are moderately distended. Vacuoles are present in the non-ciliated epithelium of the segments adjacent to the uteri and in the ciliated regions extrusion of nuclei is general (fig. 19).

Eight ova are present in the second segments of the tubes. Membranes and maturation spindles are intact in every case, and polar bodies are still adherent. The animal was killed then during the second day after ovulation.

There are from ten to fourteen medium-sized follicles in which the liquor folliculi is beginning to form at the poles. There are none sufficiently distended to indicate the approach of an ovulation. A very few small and medium-sized atretic follicles are present.

There are over thirty corpora lutea in these two ovaries. The most recent set number eight, which corresponds with the number of ova in the tubes. They are blue staining and have large central lakes of liquor. Theca interna cells are still evident at the periphery and connective-tissue sprouts have not yet grown completely through the granulosa cells. No erythrocytes are included, indicating that vascularization is not far advanced. Judging by the position of the ova in the tubes, these corpora lutea are in their second day of development.

The other corpora lutea are divisible into at least three sets. They are all red-staining, solid masses of cells, differing chiefly in size, location, and clearness of demarcation from the surrounding stroma. The second oldest set are much larger than the recent ones, clearly defined, and peripherally situated. The third oldest set are smaller than the second, but larger than the first. They are separated from the germinal epithelium by several layers of connective-tissue cells. The second and third sets correspond to the ovulations during the oestrous periods nine to ten and fourteen to sixteen days previous to death. This mouse has apparently ovulated spontaneously at every oestrous period.

The late metoestrum

Animal #26 had been examined for twenty-two successive days before killing, during which time five complete cycles had been recorded, giving an average duration of $4\frac{2}{3}$ days. External signs were marked at only two of these five periods, and in one of these instances they extended into the late metoestrum. Her previous oestrous periods were 2 to 3, 6 to 7, 11, 16 to 17, and 20 to 21 days previous to her death.

The last smear shows all degrees of epithelial degeneration and cytolysis. The cornified cells present a varied staining reaction to eosin, some being bright red, others a faded pink, and a few almost colorless. Where one cell is isolated in a group of leucocytes its exoplasm is a clear colorless zone, suggesting the extraction of the eosin staining cytoplasm.

Immediately after killing, gross examination showed her uterus to be anemic, small, and opaque.

The lumen of the vagina is collapsed and the walls much folded. It is crowded with polymorphs, among which lie big fragments of delaminated cornified elements. They are very thin in section. The epithelium is without any vestige of a basement membrane, pointed shoots of the stroma penetrating into the lower layer of the germinativum. There remain only four to seven layers of healthy epithelial cells beneath a broad region which has been reduced almost to the appearance of a fine reticulum by the infiltration of enormous numbers of leucocytes which lie in its meshes. In regions less affected, small clear lacunae containing several leucocytes are distributed throughout the epithelium. No signs of a granular layer exist, and the cornified layer has been freed from its attachments in most places. This action is just as marked in the crypts of the mucosa as on the ridges. Leucocytosis is at its maximum in this animal. In spite of this extended destruction of the epithelium an occasional mitosis may be found.

The sagittal section shows that the process of leucocytosis does not extend far through the vaginal orifice, for as it approaches the vulva the epithelium still appears normal and its granular and horny layers are superficial but intact. As these layers are traced back into the lumen, the eleidin granules begin to disappear and the cells gradually to stain pink and then red until they merge into a fully formed cornified layer.

The lumen of the uterus is small in diameter and contains an occasional small mass of free cells. The epithelium in most regions is quite completely degenerated. There is no basement membrane, but in its place a broad, blurred red or pinkish band involving the lower part of the epithelial cells and the adjacent stroma. The epithelium consists for the most part of several layers of nuclei, which might lead one to classify it as pseudostratified. But cell outlines are lacking or very indistinct, so that it represents the appearance of unorganized masses of nuclei in poorly staining cytoplasm. In a very few restricted areas occasional mitoses are evident, but for the greater part no signs of growth activity are apparent. The epithelium and, to a greater extent, the subepithelial zone are heavily infiltrated with leucocytes (fig. 16).

The glands show a minimum of function, as judged by the slight distention of their ducts. A few mitoses are scattered through their epithelium. Leucocytes in small numbers are present between and beneath their cells, but seem to exert little cytolytic action.

The oviducts, especially the segments adjacent to the uterus, are distended. The epithelium is high columnar and contains a few vacuolated cells in the non-ciliated portions, while in the ciliated section the extrusion of nuclei is marked.

There are seven ova present in the tubes. Some of them show signs of degeneration. The membranes are lacking and the chromatin material has disappeared. Two ova in the right oviduct still show the second maturation spindle and one has a polar body in which separate chromosomes can be distinguished. These two ova are in the second segment of the oviducts, while all the rest from this ovulation are in the third.

Most noticeable in the examination of the left ovary was the unusually numerous follicles of moderately large size, ranging from an average diameter of 0.32 mm. to 0.4 mm. There are twelve of these in the left ovary and seven in the right. The liquor folliculi is well formed, the cumuli are intact, and the nuclei of the ova resting. One of these large normal follicles in the right ovary is sausage shaped and contains two ova, each with its separate cumulus and its resting nucleus.

Twenty to thirty corpora lutea are present in both ovaries. The most recent set are blue staining, but are not all at the same stage of development. Two in the left, and three in the right ovary have small central lakes, and two of those in the right ovary contain erythrocytes plainly indicative of "bleeding into the central cavity." The shoots from the cells of the theca interna have grown completely through the layers of the granulosa in these five corpora and have formed five connective-tissue reticula around the central lakes of liquor. In the other two recent corpora in the right ovary, the central lakes are much larger and the connective-tissue sprouts have not yet grown through the granulosa cells. There is no distinguishable histological difference in the luteal cells of the two stages. Five of this set of corpora lutea are in their third and two in their second day of development. There are two older sets of red corpora lutea in these ovaries corresponding to the oestrous periods six to seven and eleven days previous to killing. The large number of these in the right ovary as contrasted with the few in the left would point to a hyperfunction of the former, at least during the last three oestrous periods. The unusual number of normal, moderately large follicles in the left ovary indicates the shifting of the major function for the next oestrus.

There are a few interstitial cells in these ovaries. Leucocytes do not appear in significant numbers, although leucocytosis is at its height in the vagina and uterus.

6. SUMMARY OF STAGES BY ORGANS

1. External signs

Although certain external signs may occur during the prooestrous and oestrous periods in the mouse, these periods may be present without them or the signs may continue after 'heat' has passed. When present, the external signs consist of a swelling and coloration of the vulva due to congestion and the gaping open of the vaginal orifice.

2. Vaginal content

Changes in the cells and the fluidity of the vaginal contents are a much more reliable criterion of the condition of oestrus. During the prooestrum and oestrum there are no leucocytes present in the vaginal contents. At all other times they are found there in varying numbers. During the dioestrous interval (D) epithelial cells (chiefly nucleated) and leucocytes are present; in the prooestrum (P) only light staining cells with pycnotic nuclei; during oestrus (O) single, non-nucleated, eosin-staining cornified elements constitute the smear; these are clumped in masses in the early metoestrum (M_1), and invaded by leucocytes as this period progresses until most of the cornified elements disappear and a dioestrous condition again prevails (figs. 2 to 5). In stage D the vaginal content is viscous or stringy; in P it is serous. As the O stage begins the epithelium may become dry, and then granular in the M_1 period. As the leucocytes invade the masses of C cells during the M_2 stage, the contents of the lumen become pasty, then milky, and finally stringy again as this stage merges into the dioestrous interval.

3. Histology of the vagina

During the D interval the epithelium is low (three to seven layers), has no clear-cut basement membrane, and is freely infiltrated with polymorphonuclear leucocytes. At the end of this interval, a basement membrane begins to be evident, mitoses become more frequent, new layers of cells are added, and leucocytosis ceases. In the P stage growth processes reach a maximum which thickens the epithelium to ten to thirteen layers. A distinct basement membrane is present in most places. Then the outer three to five layers begin to degenerate, as is shown by their loss of affinity for cytoplasmic stains and the pycnosis of their nuclei. These two areas become clearly separated by the formation of a granular layer. This is converted into a cornified layer (stratum lucidum), which is not superficial as would be supposed, but underlies three to five layers of nucleated cells. As the P merges into the O stage, these superficial layers disappear (probably through autolysis or continued cornification) until the cornified layer becomes superficial, at which time oestrus is evident. By this time continued growth has piled up the epithelium to a thickness of twelve or thirteen cell layers under the stratum granulosum which first formed at about the eighth layer. The definition of the lower layer of the germinativum from the adjacent stroma is very clear-cut. As the M_1 period sets in the cornified layer begins to be delaminated and the lumen of the vagina is filled with fragments or masses of C cells. The basement membrane becomes thinner and mesenchymal papillae indent the lower layers of the epithelium. Leucocytes filter in from the stroma and collect in the superficial layers of the germinativum. After they have accumulated in considerable numbers here, they pass on into the cornified masses in the lumen, stage M_2, and in a day's time may completely dissolve them.

Their continued action in the superficial part of the germinativum reduces these layers to the appearance of a reticulum containing large clumps of leucocytes. This process gradually declines until the vaginal epithelium returns to a typical dioestrous condition (figs. 6 to 13).

Growth activity seems practically at a standstill in the early D stage. Toward the end of this interval the growth curve begins to rise until it attains its maximum in a late stage P, after which it gradually falls during O and M_1 to its minimum in the late M_2 and early D stages.

4. The uterine changes

Although the uterus goes through a cycle of changes, they are far less striking than those occurring in the vagina. Gross changes consist of a marked hyperemia and distention during the P stage, which diminishes gradually during oestrus and disappears as the M_1 period progresses. During the D interval the uterus is anemic.

Histological changes in the uterus consist of periodic growth, degeneration, and leucocytosis of the epithelial cells. These phases coincide with those in the vagina. A hyperfunction of the uterine glands (judged by the distention of their ducts) is the evident cause of the distention of the uterine cornua during the P and O stages, at which time they are very transparent. Uterine glands are distributed everywhere throughout the uterine mucosa except along the line of attachment of the broad ligament. The glandular epithelium escapes much of the degeneration and destruction common to the other epithelial tissues. Mitoses are most frequent in the glands during the late D and early P stages, becoming less in number as functional activity increases. In other words, the growth wave in the glands slightly precedes that in the uterine and vaginal epithelium.

The shape of the uterine epithelial cells varies chiefly with the degree of distention of the cornua, and is consequently a poor criterion of growth. Degenerative processes are first apparent in this tissue in the fading of the basement membrane (which is so clear-cut during the P and O stages) into a pink-staining band which includes the basal sides of the epithelial cells and the superficial stroma. This is quite marked in the M_1 stage before leucocytosis has begun, which would indicate that the destruction of the epithelium by leucocytosis is secondary to degenerative changes in that tissue. It is in this subepithelial zone that the leucocytes collect in greatest numbers and from which they further invade the epithelium. A few places appear to be exempt, retaining their healthy appearance. Seldom is a region found entirely denuded of its epithelium, but this tissue becomes markedly degenerate (figs. 14 to 16). This material does not show greater numbers of mitoses in the uterine epithelium near the openings of the gland ducts than in other regions.

5. *Changes in the oviducts*

The oviducts apparently escape entirely the periodic leucocytosis, so extreme in the rest of the genital tract. They do exhibit definite cyclic changes, however. Earlier in this paper the oviducts have been divided into segments distinguishable by the presence or absence of cilia, the height of the folds of the mucosa, and the thickness of the muscle layers. The segment leading from the periovarian sac is ciliated, the remaining portion has simple non-ciliated columnar epithelium.

During the P and O stages the nuclei of the ciliated portion are ranged in a quite regular row. As the M_1 phase of the cycle advances, some of them migrate to the free ends of the cells which loose their cilia and through which these nuclei are extruded. They may retain the appearance of normal nuclei or become pycnotic before they are extruded, but when lying on the free surface of the epithelium, are shrunken and dark staining. This process reaches its height during the M_2 and early D stages, at which time the epithelium may become greatly vacuolated. It is therefore of degenerative significance (figs. 18 and 19).

The non-ciliated portions of the uterine tubes also show varying degrees of vacuolization, which seems to result from a hypersecretion of this epithelium. As yet it has not been possible to correlate this with definite phases of the oestrous cycle.

Ova may be found in the tubes during the early D interval, and if this is very short, may still be present in the succeeding P stage. They remain in good condition in the oviduct, with the second maturation spindles and sometimes the polar bodies intact, for two and even three days. During the third day, in the segments proximal to the cornua, they may begin to fragment. Their degeneration here must be by autolysis, as no leucocytes are present in the lumen of the oviducts. If occasionally they remain intact and pass into the uterine cornua on the fourth day after ovulation, phagocytosis may be their fate, for leucocytes are present there if the mouse has not passed the dioestrous interval.

6. *The ovaries*

In a consideration of the ovarian cycle, two main subdivisions will be made for animals that do and those that do not ovulate spontaneously (i.e., without the added stimulus of sexual contact). Those only occasionally ovulating spontaneously are obviously intergrades and will not be considered separately.

Where ovulation is spontaneous, three ovarian structures are involved: the follicles, the corpora lutea forming after their rupture, and possibly interstitial tissue. Where ovulation is not spontaneous there are, of course, no corpora lutea present, but atretic follicles take on an added significance.

Large, normal follicles are always present in the P and O stages, while none are to be found in the M_1 period. This, with the added evidence deduced from the position of the ova in the tubes, checked by standardized corpora lutea, shows ovulation to occur at the end of oestrus. The follicles usually rupture synchronously, but this is not necessarily so. In twenty-seven animals studied histologically three showed almost a day's difference between the position of the ova in the tubes, which is checked by a difference in the degree of development of the corresponding corpora lutea. It takes a period equal to at least one oestrous cycle for the maturation of the follicle from a medium sized stage with primary liquor folliculi forming at the poles to a large one greatly distended with a single lake of liquor (fig. 20).

Corpora lutea of oestrus in the mouse differ in no details distinguishable histologically from those of pregnancy during the first four days of development at least. In only a few cases is "bleeding into the central cavity" found. Three days are required for the hypertrophy of the former granulosa cells and the ingrowth of the theca interna to completely fill the central lake of tertiary liquor folliculi. For five or six days (unless another ovulation intervenes) these newly formed corpora stain blue with haematoxylin, and are therefore distinguishable until the next oestrus. After this time they have an affinity for eosin. If size is taken as a criterion of development, corpora lutea of oestrus in the mouse do not attain their maximum until an age of from ten to fourteen days is reached. This usually corresponds with the second ovulation after the one initiating their growth as corpora lutea. Even at the third ovulation following their start they may be equal in size to five-day corpora (fig. 24). Thus, an ovary containing from ten to sixteen large, clearly defined corpora lutea, the result of three ovulations between the fifth and sixteenth days preceding, may again ovulate. After twenty days, at which time they are usually not superficially located, ingrowth of cells from the stroma obliterates their outlines.

In animals of the first class, then, there are always present in the ovaries one recent set of blue-staining and two, three, or four older sets of red-staining corpora lutea, and normal follicles of medium to large sizes. In the O period follicles attain their largest size, and the youngest set of corpora are solid and blue staining. In the M_1 stage the former corpora stain red and a more recent set of blue-staining ones are developing in the ruptured follicles. At this time (one day after ovulation) they contain large central lakes of tertiary liquor folliculi (fig. 22). The largest follicles are medium sized and liquor is forming at their poles. During the M_2 stage the ovary is not subject to the general leucocytosis occurring in the uterus and vagina at this time. In the middle of the D interval the blue corpora are solid and the largest follicles contain one fairly large lake of primary liquor folliculi. During the P stage the follicles become more distended and an increase in size is apparent in the corpora lutea.

In the second class of animals (those which do not ovulate spontaneously) the follicles attain their largest size during oestrus, which may be considerably prolonged. The follicles failing to rupture, the cumuli disintegrate and atresia begins in the granulosa cells, continuing until the follicular epithelium is entirely gone. Contained ova may fragment in the late stages of follicular atresia or they may persist with maturation spindles intact until most of the granulosa cells have disappeared. The finding of several distinct sets of abnormal follicles in progressively later stages of atresia when no corpora lutea are present in the ovaries, but several oestrous periods have been recorded for the animal, makes it possible to approximately estimate the time required for a certain degree of follicular atresia. In late stages of degeneration, former large follicles are reduced to medium and even small size. Record of previous oestrous cycles is necessary to properly evaluate the conditions of follicular atresia in the ovary of the mouse.

Interstitial tissue is present in the ovaries in varying but usually small amounts in the majority of the animals in this series. Its distribution is for the most part peripheral. Its presence or amount is not peculiar to any stage of the cycle.

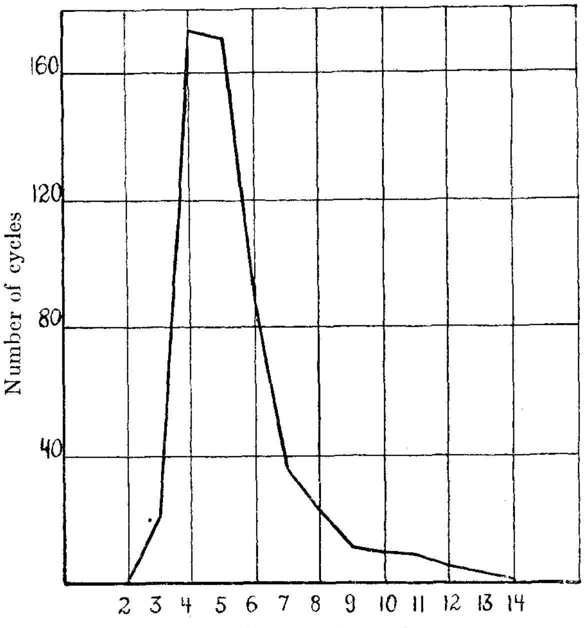
7. TIME RELATIONS OF THE CYCLE

The duration of the cycle and of its various stages shows great variability. This is represented in the curve (graph 1) obtained by plotting the number of cycles against their duration in days. A total of 563 cycles is included. The mode of the curve falls at 4½ days. An average duration of four to six days therefore represents the findings.

The dioestrous interval shows a greater variation than any other stage, lasting from less than a day to as long as fourteen days. Its length is usually from one to three days, however. In several cases of an extremely long D interval, the smears may at times show considerable amounts of cornified cells but the presence of leucocytes makes evident the diagnosis of the stage D.

Stage P, as diagnosed by the smear method, may be less than one day, because leucocytes may not entirely disappear from the superficial vaginal epithelium until after growth in the deep layers is well under way. Stage O usually lasts one or two days, but in several cases unbroken O smears have continued for nine days, and four days of heat are not uncommon. M_1 and M_2 stages usually last a day each and show little variation.

Curve of cycle length



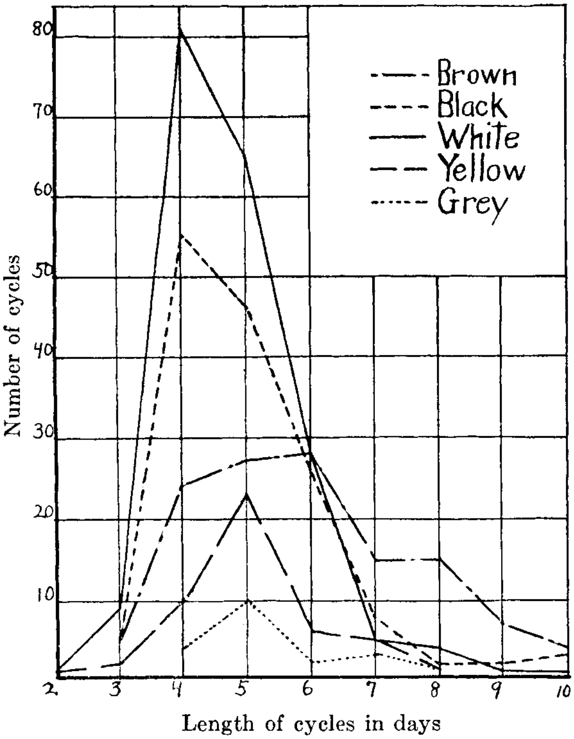
Length of cycles in days

Graph 1

The data accumulated in this study indicate no correlation between the number of ova produced at an oestrus and the length of the cycle.

While variability of cycle length is great in the same animal, it is still greater in different strains. Plotting the number of cycles against their duration in mice of different coat color gave the following curves. The mode of the curve for brown coat color is six days. This strain has the greatest number of unusually long cycles. That for black and albino is four days. Albinos in our stock are browns minus the color factor. The mode for yellow and gray falls at five days. The identity of yellow and gray is more significant, since these grays are recessive derivatives of the yellow strain. The stock used included a black-eyed, dominant white mouse which has a lethal factor. Her

Variations in cycle length in different strains



Graph 2

average for seven cycles was 7.7 days, which, compared to the mode of four days for the albino strain, is a striking difference. This is only one case, but it raises the question of the relation of this lethal factor to the duration of the oestrous cycle. These findings indicate that a genetic factor is accountable for some of the variation in cycle length among different strains of mice.

As to variation in the same litter, my material is too meager to warrant any statement. However, the following data concerning four albino mice from the same litter are of interest. These animals were examined for 146 days, making from twenty-four to thirty cycles for each animal. The average cycle length is as follows:

TABLE 2

	ANIMALS			
	53 W	53 WNL	53 WNR	53½ E
Average, total	4.8	5.7	4.1	5.2
Number of unusually long cycles	0	4 (9, 11, 11, 10 days)	1 (8 days)	2 (10, 10 days)
Average minus long cycles	4.8	4.8	4.9	4.9

Reference to the first line shows considerable variation, but if the seven longer cycles, which are approximately twice the average length, be excluded, there is a difference of only 0.1 of a day, which is well within the limit of error.

It is difficult to generalize on the variation with age of the individual. The first and second cycles following puberty are usually longer than the average during later life.

Four mice were kept in a cold room at a temperature varying from 40° to 55°F. for five weeks during the middle of summer to see if continued low temperature would retard the cycle. It resulted only in their building an elaborate, covered 'house-nest' in which they spent most of the time. The duration of their oestrous cycles was not affected.

Tumors appeared in two mice which were undergoing routine examination, which was continued in one case until the tumor grew to an inconvenient size. The first cycle after the appearance of the tumor contained a seventeen-day dioestrous interval succeeded by a one-day oestrus. This was followed by an eight-day D interval, after which time she was mated. No pregnancy resulted. In this case sexual activity was at least greatly retarded.

8. GENERAL DISCUSSION

The literature on this subject is so extensive that it is practicable to discuss here only that bearing directly on special phases of this work.

In many particulars the oestrous changes in the mouse are similar to those described by investigators of other rodents. Important among the recent workers in this field are Heape ('05), on the rabbit; Konigstein ('07), on the rabbit, rat, and guinea-pig; Rubaschin ('05), Bouin and Ancel ('10), L. Loeb ('11), Lams ('13), Stockard and Papanicolaou ('17), on the guinea-pig, and Long and Evans ('20), on the rat.

Many of my observations merely establish for the mouse processes previously described in other rodents. It has been a pleasure to confirm in many instances the observations of earlier investigators, but in some respects conditions in the mouse throw a new light on certain of these sexual problems and a few new interpretations seem warranted.

In discussing the duration of the cycle, Heape ('00) says: "The differences in sexual periodicity in polyoestrous mammals in allied forms and even within the limits of the same species is due to variation in the quiescent period." In the mouse another possibility presents itself. The mode of the curve for the cycle duration in brown mice is six days—an interval of two days longer than that for black and white and one day longer than that for yellow and gray. Long 'heat' periods are more common to browns than to the other strains. Therefore, although the variation in the dioestrous interval accounts for much of the cycle variation, a good deal of it is also due to the variation in the duration of the oestrous stage itself. The variation in cycle length is great sometimes in the same animal and among different individuals from one litter. In the latter, however, it is less than in different strains of mice.

As stated earlier in the paper, albinos in our stock are browns minus the color factor. The mode of the curve for whites being two days shorter than that for browns suggests that with the loss of the determiner for color a shorter cycle has been effected. Again the modes of the curves of cycle duration of yellow and gray mice coincide, and they are both derived from the same original pair of animals. These facts point to a genetic factor as partly responsible for variation in the oestrous cycle and direct attention to the ovum itself as the ultimate cause.

The present findings of a much shorter oestrous cycle in the mouse than had previously been suspected explains the apparent exceptions reported by Kirkham and Smith to an oestrous cycle of twenty or seventeen and one-half days' duration.

Kirkham ('16) reports a case in which "a set of normal eggs in the 2-cell stage was found six days post partem." Smith ('17) reports ovulation in a mouse six and one-half days after parturition. These may be explained by a failure to ovulate or of the ova to be fertilized at the oestrus following parturition, and the recurrence of another oestrous period five days later.

1. Oestrous changes in the genital tract

a. The vagina and cervix uteri. 1. There is in the mouse little discharge from the uterus into the vagina such as occurs in menstruating animals. Uterine epithelial cells are not often found in the smears. Also, although the uterine cornua during the P and O stages are greatly distended with fluid, the vaginal mucosa, especially during oestrus, is usually dry. Apparently the musculature of the cervix functions as an efficient sphincter.

2. In the mouse the massing of the cornified cells of the vaginal epithelium in clumps (when a standardized smear technique is used) indicates that ovulation has already occurred when that is to be spontaneous. It is therefore of importance in diagnosing ovarian conditions.

As to the fate of the leucocytes so abundant in the M_2 stage, many degenerate in the lumen. It was stated in the descriptive section that during the D interval the vaginal contents were viscous and stringy. These stringy masses when spread on slides show a fine web structure with polymorphs at the interstices entangling varying numbers of epithelial cells. The fine web processes seem to be made of the greatly attenuated protoplasm of the leucocytes.

3. Long and Evans ('20) have again called attention to the formation of the cornified and granular layers of the vaginal epithelium in the rat and guinea-pig as a remarkable histogenetic process because they do not form superficially. However, it should be observed that the overlying layers, although nucleated, early lose their affinity for stain even before the appearance of granular layer which precedes cornification (described under animal 8, fig. 7).

b. The uterus. 1. Gross changes. The attention of investigators has been directed towards the uterus from the very beginnings of anatomical study, primarily because of its importance to the embryo during pregnancy. In the absence of pregnancy in the primates, its striking changes during the menstrual cycle have led to its designation as 'the organ of menstruation.' In the mouse the vagina, and also the oviducts to a less extent, share with it the typical oestrous changes.

2. Destructive histological changes. The amount of epithelial and connective-tissue destruction during the metoestrum and menstruation has been under continual controversy which seems to have compromised on "great variability even within the same species." In menstruating animals extensive removal of epithelium has been reported and denied, but nearly always bleeding occurs. Bleeding has also been reported in many of the lower mammals which periodically exhibit typical oestrus. Lataste ('87) has recorded bleeding during 'heat' in several European rodents. Stockard and Papanicolaou ('17) have reported an occasional slight bleeding in the guinea-pig. There is little denudation of the epithelium and only rarely bleeding during the metoestrum in the mouse. The process is restricted to the degeneration of the epithelium in situ (it seldom breaks free from the stroma) and heavy subsequent leucocytosis. This lack of severe destruction may be accounted for in the mouse by the rapidly ensuing prooestrum, or period of growth, which may set in three days after the metoestrum begins.

3. Is leucocytosis primary or secondary? Loeb ('11), in discussing this problem in the guinea-pig, says: "It is not very probable that the changes in the epithelium (of the uterus)

following ovulation are brought about by a disintegration of some of the epithelial cells." Stockard and Papanicolaou ('17), also working with the guinea-pig, say: "Large vacuoles are to be seen between the epithelial cells, and these are probably produced by the dissolving power of the leucocytes." Long and Evans ('20) state that destruction in the uterine epithelium in the rat is due to 'vacuolar' degeneration. A closely timed series of material in the mouse shows distinct degenerative changes evident in the substitution for the basement membrane of a light staining zone before leucocytosis begins. It seems more probable that these vacuoles in the uterine epithelium are comparable to those in the oviducts (where no leucocytosis occurs) and are evidences of degeneration which may be the cause of the leucocytosis. The evidence from the mouse indicates that epithelial degeneration is a primary, and the leucocytosis a secondary, phenomenon.

4. Regenerative changes. Many regions of the surface uterine epithelium as well as the glands escape destruction during a single metoestrum in the mouse. Also when active mitosis begins it does not appear to be restricted to regions adjacent to the openings of the gland ducts.

5. The distribution of glands. The distribution of glands throughout the uterine mucosa seems possibly to be of some significance in the consideration of the implantation of the blastocysts. Huber ('15), in his contribution to the embryology of the albino rat, calls attention to the even spacing of the implantation sites which are ranged along the sides of the cornua adjacent to the attachment of the uterine ligaments. In the mouse, in all the animals studied, glands were distributed everywhere throughout the mucosa except along this line. That this is the future site of placentae is interesting.

c. *Cyclic changes in the oviducts.* 1. The oviducts seem to have been overlooked by most investigators in considering degenerative changes during the oestrous cycle. So far as I have been able to find, no cyclic degenerative changes have been reported in them in the lower mammals. In summarizing the discussion of the question in man, Novak ('21) disposes of the cases of tubal menstruation reported in the literature as for the most part occurring after hysterectomy from the stump of the oviduct. These are obviously not normal. Czyzewicz ('08) (quoted by Novak) reported after the study of six normal oviducts at different stages of the menstrual cycle that they did not share menstrual phenomena with the uterus.

The oviducts, uterus, and vagina have a common origin from the mullerian ducts, so possibly factors causing cyclic changes in the uterus and vagina may be expressed in some way in the oviducts. They are not, however, subjected to the periodic leucocytosis of the rest of the genital tract. The extrusion of nuclei in the ciliated portion, beginning in the early metoestrum and continuing in a marked degree sometimes to the middle of the following prooestrum, may be interpreted as of degenerative significance, paralleling as it does the stages of "degeneration and removal by leucocytosis" in the uterus and vagina.

2. The importance of certain structural features of the oviducts of the mouse. The careful work of Sobotta ('95), Huber ('15), and H. P. Smith ('17) had made the differentiation between the segments of the oviduct easy. The time of passage of the ova through the different segments as worked out by Smith, ovulation being calculated in the light of the observations of Long and Mark ('11), has been used in placing the time of ovulation and consequently the estimation of the age of corpora lutea.

3. The passage of the ova down the uterine tubes. The mechanism of the passage of the ova down the greater extent of the oviducts is still not understood in the mouse. Ciliary action accounts only for their entry into and passage through the segment proximal to the ovary. Peristaltic action may possibly furnish motive power for the rest of their passage. Waves of peristalsis were not, however, apparent in my material, but the oviducts were always somewhat distended. Valve-like folds of the mucosa at the entrance of the tubes into the cornua have already been mentioned as a possible check to backflow of fluid from the uterus. The arrangement of the muscle layers of the oviduct as they merge into those of the uterine cornu is

identical with that in the 'bile duct sphincter' recently emphasized by Mann ('20). It is possible that they serve such a function here. Surely, a back flow of fluid from a distended uterus would be fatal to the passage of ova down the tubes, if that be by peristalsis.

d. The ovaries. Many interesting ovarian problems have arisen in the course of this work.

a. The follicles and ovulation. 1. Spontaneous ovulation. Certain species of animals (the rabbit and ferret are examples) usually do not ovulate spontaneously at oestrus. There has been much discussion concerning this question in the mouse. Tafani ('89), Sobotta ('95), and several later investigators claim an ovulation without added sexual stimulation. Garlach ('06) and Bouin and Ancel ('09) state that in the mouse ovulation is dependent upon coition. That it usually occurs spontaneously the first day after parturition has been emphasized by many investigators, and made use of by Long and Mark ('11) in artificial insemination.

Possibly the size of the ovarian vessels immediately following parturition may be the deciding factor at this ovulation in some mice, but it is certain that in many mice not lately pregnant ovulation is not spontaneous at every oestrus and in some virgin mice it need not have occurred at all, although several oestrous cycles have been recorded, which indicates the presence of ripe follicles. It is obvious, therefore, that the classification of the mouse as a species ovulating spontaneously is not accurate. While it is not possible to diagnose the absence of spontaneous ovulation by actual cell content of the smear, unusually long oestrous periods in certain animals may indicate a failure or at least a difficulty in ovulation, for the presence of ripe follicles seems universal in all animals in the oestrous condition.

2. Is ovulation in litter-bearing animals synchronous? Long and Mark ('11), in considering the maturation of the ovum of the mouse, state that ovulation is a synchronous process, although imperfectly so. Sobotta and Burchard report similarly for the rat. Huber finds fertilized ova usually bunched in their passage down the oviduct in the rat, which would indicate a synchronous

ovulation. In three mice of this series a distinct difference in the position of the ova in the tubes and a difference in degree of development of the corresponding corpora lutea may indicate that the synchronism is less perfect where mating or artificial insemination do not occur. In these three mice the ova ovulated at one oestrus were plainly separated into two groups with a day's difference between their ovulation time.

3. The number of ova produced at one ovulation. The number of ova produced at an ovulation as an indication of the degree of fecundity of an animal is always of interest. When approached from the number in new-born litters, the failure of insemination, faulty implantation, intra-uterine death, and mortality at parturition introduce an extensive error. Therefore, the problem is most accurately solved through ovarian studies.

Counts of recent corpora lutea checked in many cases by comparison with the number of corresponding ova in the tubes, the number of the next older set of red corpora lutea, and also the number of largest-sized follicles in prooestrous and oestrous ovaries give a total of 449 ova produced at forty-nine oestrous periods.[4] This makes an average of nine ova to an oestrus. Of these 449, 230 were counted in left ovaries and 219 in right, showing the function almost equally divided between the two ovaries in virgin and non-mated animals. Long and Mark, in a large series, obtained an average of seven ova from the ovulation following parturition—a 22 per cent difference. It is possible that a supervening pregnancy reduces the number of ova produced at the oestrus following parturition.

4. The possibility of alternation of major function. The finding of a variation in the numbers of embryos in the horns of bicornuate uteri has raised the question as to alternation of major function between the two ovaries. In twenty-one animals in which data were available for the number of ova produced at two, three, and sometimes four oestrous periods, four show marked and two slight alternation of function, while in

---

[4] All large follicles need not rupture, which introduces a slight error into this data.

the others the function is quite evenly divided between the two ovaries.

5. The period required for the growth of follicles from medium size to ovulation size. My material points to an interval of surely not more than two cycles (eight to twelve days) and probably only one (four to six days) between the beginning of the formation of primary liquor folliculi at the poles of the medium-sized follicles and the time of their final distention and rupture (fig. 20).

6. Atretic follicles. An observation which seems almost universal, although it varies greatly in degree in different ovaries, is the presence of atretic follicles of all sizes. Some investigators have assumed that ovulation is always spontaneous and concluded that follicles atrophy without regard to the stage of the oestrous cycle. This conclusion does not necessarily follow the finding of atretic follicles of different sizes in the mouse ovary. There are two possibilities: first, follicles at any stage of development may atrophy or, second, they may attain full size, but fail to rupture at a certain oestrous period, and begin atrophy and resorption. The finding of maturation spindles in the ova in many of these atretic follicles points to their former large and mature state. Continued atrophy may decrease the size until there are few granulosa cells present and most of the liquor folliculi has disappeared. It has been possible to trace sets of atretic follicles corresponding in progressive degree of atresia to previously recorded oestrous periods through a time equivalent to three full cycles. In some of these the ova have completed their intra-ovarian maturation stages, in others the nuclei are in a resting condition. The former points to the follicular apparatus, the latter to the ovum itself as the cause of atresia, which seems to be by cytolysis of the follicular epithelium.

b. Corpora lutea. 1. Differences in those of pregnancy and oestrus. So much importance has been attributed to the corpora lutea that a study of those of oestrus has been followed with great interest. The time relations of the position of the ova in the uterine tubes has been so carefully worked out that an estimation of the age of these corpora is quite accurate.

Sobotta ('95) has standardized the development of the corpora lutea of pregnancy in the mouse, and carefully timed material has been available in the Washington University Anatomical Collection with which to check this work. Therefore, a comparison of the corpora lutea of oestrus with those of pregnancy has been possible. Sobotta states that their persistence and ultimate size is not altered by conception. Surely, there is no difference discernible histologically during the first four days, and if their later size be any criterion as to the degree of their development, they do not reach a maximum until two new sets have been added, i.e., eight to twelve days after that ovulation which resulted in their formation.

2. Do they inhibit ovulation? Beard ('98) has been followed by many investigators in advancing the idea that corpora lutea prevent ovulation. The fact that they are present in greatly hypertrophied form during pregnancy and that ovulation does not occur at this time seems conclusive enough proof.

Loeb ('18) first proved experimentally, by the removal of the corpora lutea of pregnancy in the guinea-pig after they had ceased to be essential to the tenure of the fetus, that a new ovulation could be induced earlier than would have occurred otherwise. He then extended the work to the corpora lutea of oestrus in non-pregnant animals and found the same effect of inhibition on ovulation. Papanicolaou ('20) has confirmed these experiments. The conclusion drawn was "the corpus luteum, itself the result of an ovulation, provides a mechanism preventing ovulation." However this may be in the guinea-pig, it is surely not normally applicable to the mouse during oestrus for (because of the shorter cycle) ovulation occurs when two or three sets of recent large corpora lutea are present in the ovary. In litter-bearing animals the corpora lutea constitute no inconsiderable part of the ovaries. Might not ovulation following their excision be merely an expression of the compensatory hypertrophy of the remaining ovarian tissue? Corpora lutea of oestrus most surely do not normally prevent ovulation in the mouse.

3. Do they exert a destructive influence on the mucosa of the genital tract? Frankel ('03) attempted to prove that the corpus luteum by means of an internal secretion exerted a destructive influence on the uterus. Although newly forming corpora lutea are present during the metoestrum in the ovaries of mice which ovulate spontaneously, in those which do not, none need be present. And yet typical metoestrous degenerative changes in the genital tract occur. Consequently, the corpora lutea can hardly be considered as the cause of cyclical degeneration.

4. Do the corpora lutea furnish a growth stimulus? Stockard and Papanicolaou think it probable that the corpora lutea of oestrus exert a protective influence on the uterine and vaginal mucosae, because they find well-developed corpora present during the stages in which no degenerative changes are apparent in these organs, and because corpora of oestrus begin to retrogress before the next metoestrum sets in. They write: "The facts obtained in the present investigation might not fully warrant the position that the corpus luteum really exerted an active protective influence over the uterine mucosa, but they certainly in no sense suggest, and actually speak against, any injurious action on the mucosa by the secretion of the corpus luteum." This may be taken as a constructive suggestion to combat Frankel's views. That the corpora lutea are not the principal factors in the growth phase of the genital tract during the prooestrum in the mouse is plain for two reasons: 1) In those animals where spontaneous ovulation is the rule and where two or three sets of large corpora are always present, these degenerative changes occur just the same. 2) In animals that never have ovulated spontaneously, and where consequently no corpora are present in the ovaries, the same regenerative growth processes occur. Consequently, Stockard and Papanicolaou's explanation of growth under the protective influence of a secretion from the corpora lutea is hardly applicable to the mouse.

Therefore, evidence from the mouse brings strongly into question the value of corpora lutea of oestrus, 1) as inhibitors of ovulation, 2) as sources of destructive hormonal influence, or, 3) of endocrine growth stimulus exerted on the uterus.

c. The cause of cyclic oestrous changes. Many investigators have postulated theories from histological evidence. Since 1900 the problems have been approached also by experimental methods and a complicated mechanism built up on experimental evidence to account for oestrous changes. A short review of these results seems advisable.

1. *Is the cause of oestrous phenomena inherent in the genital tract itself?* Heape believed that oestrus and menstruation might occur after the removal of the ovaries. Consequently, he postulated an extra-ovarian cause of these phenomena. Since 1900, however, accumulated evidence from spayed animals has proved that without the ovaries the oestrous or menstrual cycle slowly disappears and the uterus and vagina atrophy. Halban and others, and recently Stockard and Papanicolaou ('17), have called attention to a cyclic atrophy following ovariotomy. This is also evident in the mouse in a periodic appearance at intervals equivalent to the oestrous cycle of cornified cells in the smear, always in the presence of leucocytes, however. From this, cyclic changes inherent in the uterus itself might be implied. Without the presence of the ovaries, however, no growth or regenerative processes occur. Might not cyclic degeneration in the uterus and vagina after spaying be merely secondarily induced by ovarian influences?

Temporarily let us exclude from consideration as improbable any inherent cyclic nature of the uterus itself and see if the ovaries may supply the cause. Three structures are present there that might be responsible: the follicles, the corpora lutea formed after their rupture, and possibly interstitial cells.

2. *The follicles or the interstitial tissue.* Large follicles distended with liquor folliculi have almost invariably been reported present during the prooestrous and oestrous periods in mammals, although the work of Heape ('98) and Leopold ('94) throws some doubt on their being always present in the primates. Consequently, many investigators believe the ripening follicles to be the cause of these phenomena. They are present in all mice studied during the prooestrous and oestrous stages.

Marshall ('14) believes he has experimentally disproved the ripening follicles as a factor in the mechanism of cyclic sexual changes. For criticisms of this work the reader is referred to Stockard and Papanicolaou ('17) and Robinson ('20). It seems to the writer that Marshall's conclusions are not justified. Marshall falls back on the ovarian interstitial tissue as the cause of oestrus. Interstitial tissue in the ovary is a very ill-defined, intangible substance. Any cells not connective-tissue cells appearing between the follicles may be called interstitial. If they bear a resemblance to secreting cells they may be called typical interstitial cells. They are believed by most investigators to be epithelial in nature and to have an origin similar to that of the ova and the follicle cells. The interstitial cells in the mouse ovary are chiefly peripheral in distribution and not to be confused with those appearing in many forms in the theca of atretic follicles or originating from old corpora lutea after connective-tissue ingrowth. There seems to be no periodic hypertrophy of these cells such as would be expected if they were the cause of cyclic oestrous changes in the genital tract.

3. The corpora lutea. As has already been pointed out, the corpora lutea, have had attributed to them the following functions, the inhibition of ovulation even in non-pregnant animals and the cause of degenerative menstrual changes in the uterus of primates, and metoestrous changes in the uterus and vagina of other mammals (by analogy), and, finally, the source of a protective influence on the vaginal and uterine mucosae.

In mice in which ovulation does not occur spontaneously, and consequently in whose ovaries there are no corpora lutea, the normal oestrous cycle goes on regularly. In mice that ovulate spontaneously several healthy sets of corpora lutea are present during the whole cycle. There seems to be no escape from the conclusion that in the oestrous cycle in the mouse (unaffected by pregnancy) the corpus luteum has no primary causative function.

Since the evidence seems to discredit both interstitial tissue and corpora lutea as causative factors in the phenomena of the normal oestrous cycle, the follicles are the only remaining ovarian possibility.

4. Evidence for the follicles. In the mouse and in most mammals studied very large follicles are present in the ovaries during the prooestrous and oestrous stages and absent during the metoestrum and dioestrous interval. The histological evidence from many sources in all mammals studied excepting certain primates overwhelmingly points to the presence of large follicles as the cause of oestrous changes. However, no direct experimental proof has yet been produced.

The cause of long oestrous periods. Animal #20 was diagnosed by the smear method to be in stage M_1 when killed. If this diagnosis was correct, degenerative changes should have begun in the genital tract and ovulation should have occurred one or two days previously. On histological examination, however, the epithelium of the vagina and uterus was found to be in an actively growing condition and a set of large normal follicles were present in the ovaries. Four normal oestrous periods had been recorded for the animal and yet the ovaries contain no corpora lutea. Consequently, ovulation had not been spontaneous and it is probable that the large follicles present at death would not have ruptured had the animal lived. Her present oestrus was first apparent four days before her death. Would it not seem probable, then, that the continued growth phase in the genital tract might (in the absence of ovulation) be directly dependent upon the retention of mature ova in large normal follicles? If so, this is a strong suggestion as to the cause of oestrus. In mice that do not ovulate spontaneously (therefore the simplest condition), they are always present during the pro-oestrous and oestrous stages and absent or atretic during the met- and dioestrum. As the possibility of the corpora lutea and the interstitial tissue actively sharing as causes seems slight in the mouse, all the evidence points to the presence of large normal follicles as the cause of the growth and congestion of the anabolic periods and the absence of normal follicles as the primary cause of the catabolic periods in the genital tract.

In animals that ovulate spontaneously, the rupture of the follicles is the dividing line between these two phases, while in animals that require an added stimulus for ovulation, atresia of the follicles is coincident with the beginning of the metoestrum. This indicates that the growth and congestion of the pro-oestrous and oestrous stages are caused by maturing ova in large normal follicles, and the degeneration and removal by leucocytosis of much of the uterine and vaginal epithelium results after the extrusion of the ovum from, or its atrophy in the follicle.

That ovulation is the dividing line and that mice of different strains have distinct modes of cycle length seems to point to the maturing ova themselves as the ultimate cause of growth changes and the absence of them from the follicle as the cause of degenerative phenomena of the oestrous cycle.

9. SUMMARY

1. External signs are unreliable criteria of oestrus in mice. The presence of cornified cells in the vaginal smear is a much more accurate indication. When these cells appear in masses, ovulation has usually occurred.
2. The chief changes in the vaginal epithelium are its rapid growth, the formation of a stratum corneum, and (after ovulation) its degeneration and removal by leucocytosis. The stratum germinativum is also partly destroyed. It may grow from four to six to twelve or thirteen layers in thickness in one day.
3. There is considerable degeneration and leucocytosis in the uterine epithelium which is, however, seldom removed from the stroma. Bleeding rarely occurs in the mouse, but a heavy leucocytic infiltration takes place during the metoestrum.
4. Periodic degenerative changes in the oviduct parallel those in the rest of the genital tract. They are evidenced in extrusion of nuclei from the ciliated epithelium.
5. Ovulation is the dividing line between the anabolic and catabolic phases of the oestrous cycle, maturing ova in large follicles always being present during the pro-oestrum and oestrum, while newly forming corpora lutea or large atretic follicles replace them in the metoestrum.

6. Ovulation is not always spontaneous in virgin or unmated mice. In some it is regularly spontaneous, in others sporadically so, and in a few it seldom occurs without an added stimulus.

7. The average duration of the cycle is four to six days. The mode of the curve for brown mice is six days; for yellows and grays, five days; for blacks and albinos, four days. The yellows and grays were descendants from one pair of mice, the albinos are browns minus the color factor. Therefore, a genetic factor seems partly responsible for variation in cycle duration, and this may be tied up with the determiner for coat color.

8. Ovulation need not necessarily be synchronous in unmated mice.

9. Pregnancy may reduce the number of ova produced at the ovulation following parturition.

10. There is no difference discernible histologically between corpora lutea of oestrus and pregnancy during the first four days of their development.

11. Corpora lutea of oestrus do not normally inhibit ovulation in the mouse.

12. In mice that ovulate spontaneously two or three sets of corpora lutea of oestrus may be present at all times; in mice that do not ovulate spontaneously corpora lutea may be entirely absent, and yet normal oestrous cycles are experienced in both types of animals. Therefore, the writer concludes that they have no primary causative relation to oestrous changes in the genital tract.

13. As ovulation or the beginning of atresia of follicles is the dividing line between the anabolic and catabolic phases, and as the genetic factor summarized in no. 8 points to the ova themselves, the conclusion is drawn that the presence of maturing ova in large follicles is the cause of the prooestrum and oestrus, and that the removal of the ova at ovulation (or their atresia if this fails to occur) is the primary cause of the degenerative changes of the metoestrum.

LITERATURE CITED

Arai, Hayato 1920 On the cause of the hypertrophy of the surviving ovary after semispaying (albino rat) and on the number of ova in it. Am. Jour. Anat., vol. 28.

Beard, J. 1897 (Quoted from Stockard and Papanicolaou, 1917.) The span of gestation and the cause of birth. Fischer, Jena.

Bouin, P., et Ancel, P. 1908 Sur la differenciation d'une membrane propre d'origine epitheliale pendant le developpement du corps jaune chez le chienne. C. R. de la Soc. Biol., T. 65.

1909 Sur les homologies et la signification des glandes a secretion interne de l'ovaire. C. R. Soc. Biol. Paris, T. 67.

1910 Recherches sur les fonctions du corps jaune gestatif. Jour. de Physl. et de path. gener.

Daniel, J. F. 1910 Observations on the period of gestation in white mice. Jour. Exp. Zoöl., vol. 9.

Danforth, C. H. 1916 Use of early developmental stages in the mouse. Anat. Rec., vol. 10, p. 355.

Frankel, L. 1903 Die Function des Corpus luteum. Arch. für Gynaekol., Bd. 68.

Halban, J. 1911 Zur Lehre von der Menstruation—Protective Wirkung der Keimdrüsen auf Brunst und Menstruation. Zentralbl. f. Gynaekol., Bd. 35.

Halban, J., und Kohler, R. 1914 Die Beziehungen zwischen Corpus luteum und Menstruation. Arch. of Gynäkol., Bd. 103.

Heape, W. 1900 The sexual season. Quart. Jour. Mic. Sc., vol. 44.

1905 Ovulation and degeneration of ova in the rabbit. Proc. of Roy. Soc., vol. B. 76.

Hill, J. P., and O'Donoghue, C. H. 1914 The reproductive cycle in the marsupial Dasyurus viverrinus. Q. J. M. S., vol. 59.

Huber, G. Carl 1915 Development of the albino rat. Jour. Morph., vol. 26.

King, H. D. 1913 Some anomalies in the gestation of the albino rat (Mus norvegicus albinus). Biol. Bull., vol. 24.

Kingery, H. M. 1914 So-called parthenogenesis in the white mouse. Biol. Bull., vol. 27.

1917 Oogenesis in the white mouse. Jour. Morph., vol. 30.

Kirkham, W. B. 1907 a The maturation of the mouse egg. Biol. Bull., vol. 12, no. 4, pp. 259–265.

1910 Ovulation in mammals with special reference to the mouse and rat. Biol. Bull., vol. 18.

1916 The germ cell cycle in the mouse. Abstract in Anat. Rec., vol. 10, p. 217.

1916–17 Prolonged gestation period in suckling mice. Anat. Rec., vol. 11.

Kirkham, W. B., and Burr, H. S. 1913 The breeding habits, maturation of the eggs and ovulation of the albino rat. Am. Jour. Anat., vol. 15.

Lane-Claypon, J. E. 1907 On ovogenesis and the formation of the interstitial cells of the ovary. Jour. of Obstetrics and Gynecology, vol. 11.

LATASTE, F. 1887 Motiere du bouchon vaginal des rouqeurs. Soc. Biol. Dec. 8, pp. 817–821.

LOEB, L. 1911 b The cyclic changes in the ovary of the guinea-pig. Jour. Morph., vol. 22.

1911 c The cyclic changes in the mammalian ovary. Proc. Am. Phil. Soc., vol. 50.

1917 The relation of the ovary to the uterus and mammary gland from the experimental aspect. Trans. Am. Gyn. Soc., 1917.

1918 Corpus luteum and the periodicity in the sexual cycle. Science, vol. 48, no. 1237.

LONG, J. A., AND MARK, E. L. 1911 The maturation of the egg of the mouse. Carnegie Institute of Washington, Publication no. 142.

LONG, J. A. 1912 Studies on early stages of development in rats and mice, No. 3, by Mark and Long. The living eggs of rats and mice with a description of apparatus for obtaining and observing them. University of California Publications in Zoology, vol. 9.

LONG, J. A., AND QUISNO, J. E. 1916 The ovulation period in rats. Abstract in Anat. Rec.

LONG, J. A., AND EVANS, H. M. 1920–21 Oestrous cycle in rats. Proc. Am. Soc. Zool.

MACKENZIE AND MARSHALL 1913 Recurrence of heat in certain cases in sows after removal of ovaries due to remaining pieces of ovary. Jour. Agri. Sci., v. p. 418.

MANN, F. C. 1920 A comparative study of the anatomy of the sphincter at the duodenal end of the common bile duct. Anat. Rec., vol. 18, no. 4.

MARSHALL, F. H. A. 1903 The oestrous cycle and the formation of the corpus luteum in the sheep. Phil. Trans. B., vol. 196.

1904 The oestrous cycle in the common ferret. Q. J. M. S., vol. 48.

1904 The oestrous cycle and the formation of the corpus luteum of the sheep. Phil. Trans. Roy. Soc., vol. B. 196.

1910 The physiology of reproduction. London, 1910.

1911–12 On the ovarian factor concerned in the recurrence of oestrus. Proc. of the Phy. Soc. Jour. of Phys., vol. 43.

1914 The functional correlation between the ovaries, uterus, and mammary glands in the rabbit; with observations on the oestrous cycle. Proc. Roy. Soc., vol. B. 87.

MARSHALL, F. H. A., AND RUNCIMAN, J. G. 1914 On the ovarian factor concerned in the recurrence of the oestrous cycle. Jour. Physiol., vol. 49.

MARSHALL, F. H. A., AND HALMAN, E. T. 1918 Post-oestrous changes in the dog. Jour. Roy. Mic. Soc., p. 36.

O'DONOGHUE, C. H. 1916 On the corpora lutea, and the interstitial tissue of ovary in Marsupialia. Q. J. M. S., vol. 61.

PAPANICOLAOU, G. N. 1920 Influence of removal of corpora lutea and ripe follicles on the oestrous periodicity in guinea-pigs. Abstract 53, Anat. Rec., vol. 18, no. 3.

PEARL, R., AND SURFACE, F. M. 1914 On the effect of corpus luteum substance upon ovulation in the fowl. Jour. Biol. Chem., vol. 19.

ROBINSON, A. 1920 Formation, rupture and closure of ovarian follicles in ferrets and ferret pole cat hybrids. Trans. Roy. Soc., vcl. 52, pt. 2, no. 13.

SMITH, H. P. 1917 Ovarian cycle in mice. Jcur. Royal Mic. Soc., p. 252.

SOBOTTA, J. 1895 Die Befruchtung und Furchung des Eies der Maus. Arch. f. mik. Anat., Bd. 45.

1896 Ueber die Bildung des Corpus luteum bei der Maus. Arch. fur Mikr. Anat., Bd. 47.

1903 Die Entwicklung des Fies der Maus vom Schlusse der Furchungsperiode bis zum Auftreten der Amniosfalten. Arch. f. mik. Anat., Bd. 61.

STOCKARD, C. R., AND PAPANICOLAOU, G. N. 1917 Existence of a typical oestrous cycle in the guinea-pig with a study of its histological and physiological changes. Am. Jour. Anat., vol. 22.

1919 The vaginal closure membrane, copulation, and the vaginal plug in the guinea pig with further considerations of the oestrous rythm. Biol. Bull., vol. 37, no. 3.

WALSH, L. S. N. 1917 Growth of ovarian follicle—guinea-pig under normal and pathological conditions. Jour. Exp. Med., vol. 26, p. 245.

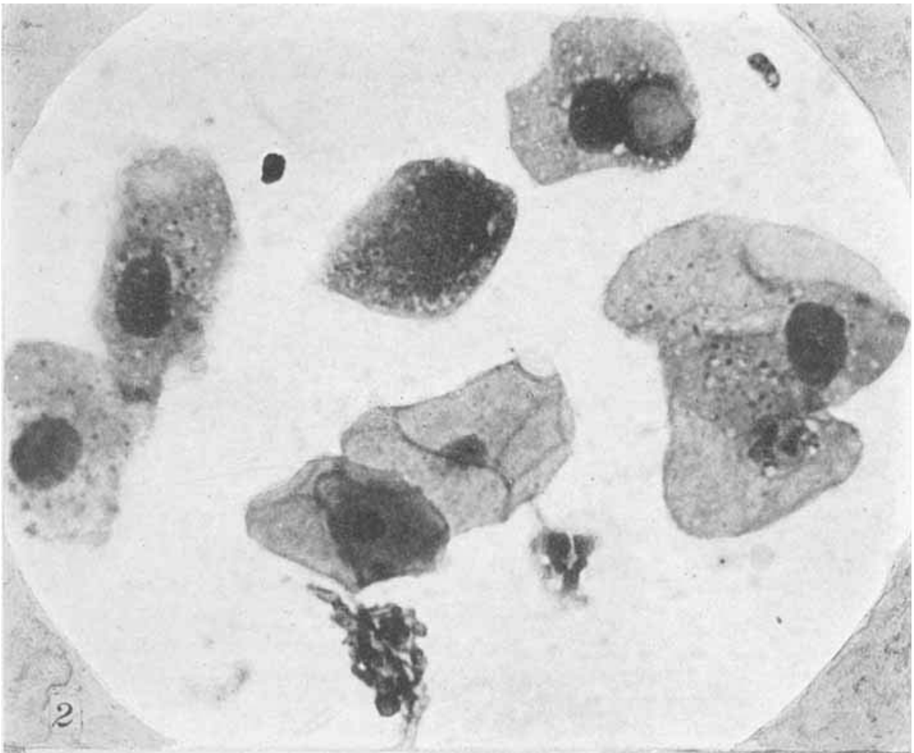
PLATES

PLATE 1

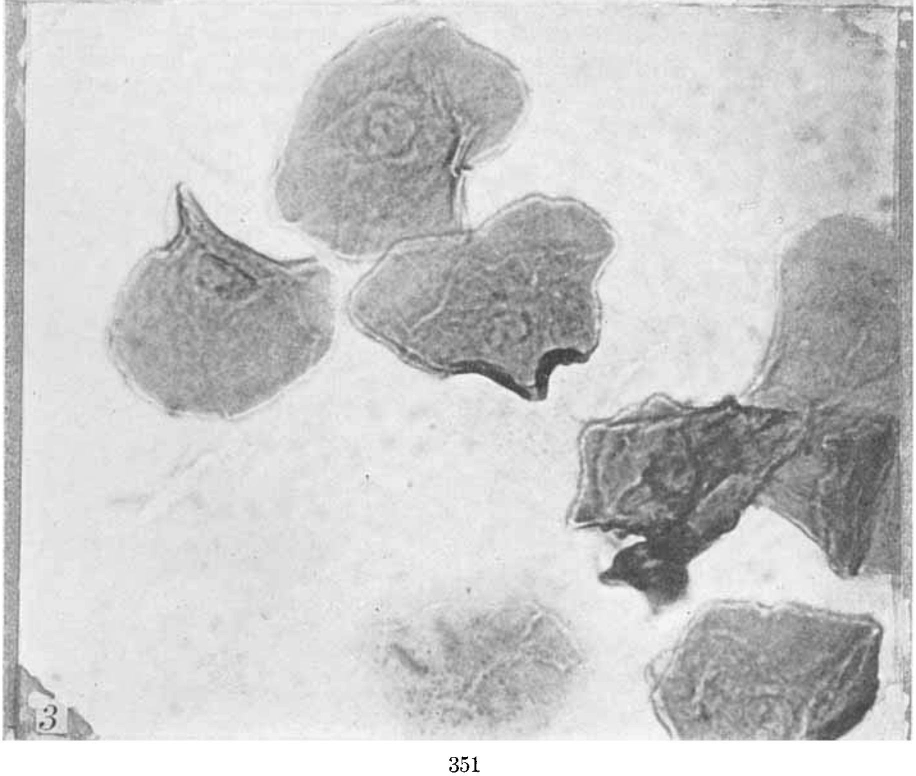
EXPLANATION OF FIGURES

2 Epithelial cells of the prooestrous vaginal smear. × 800. Cytoplasm takes a blue or purple tinge when stained with haematoxylin and eosin.

3 Epithelial cells of oestrous vaginal smear. × 800. The nuclei have lost their affinity for basic dye; the cells are cornified and stain a bright red with eosin.



2



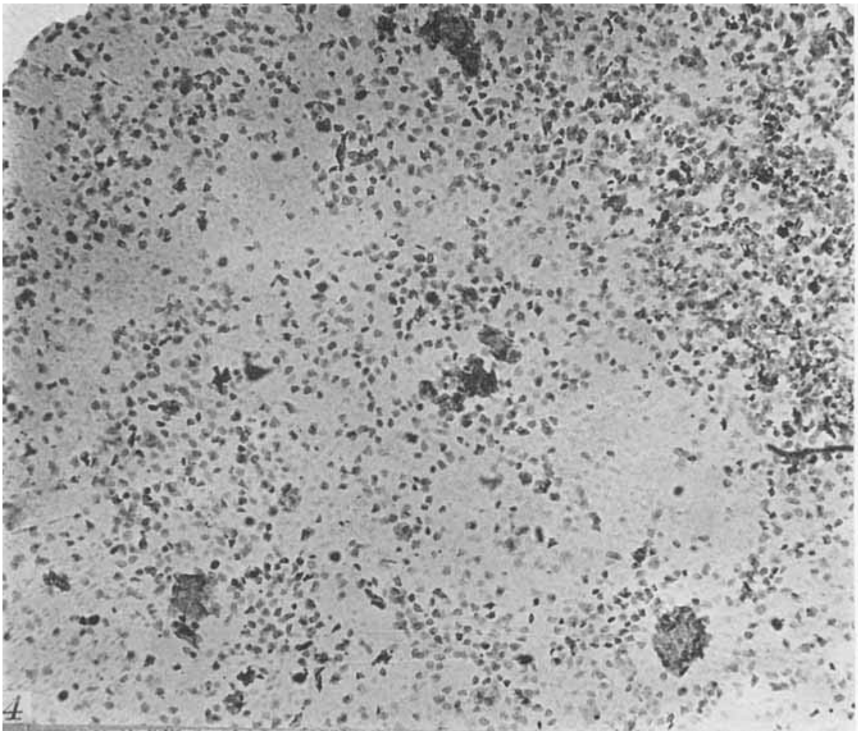
3

PLATE 2

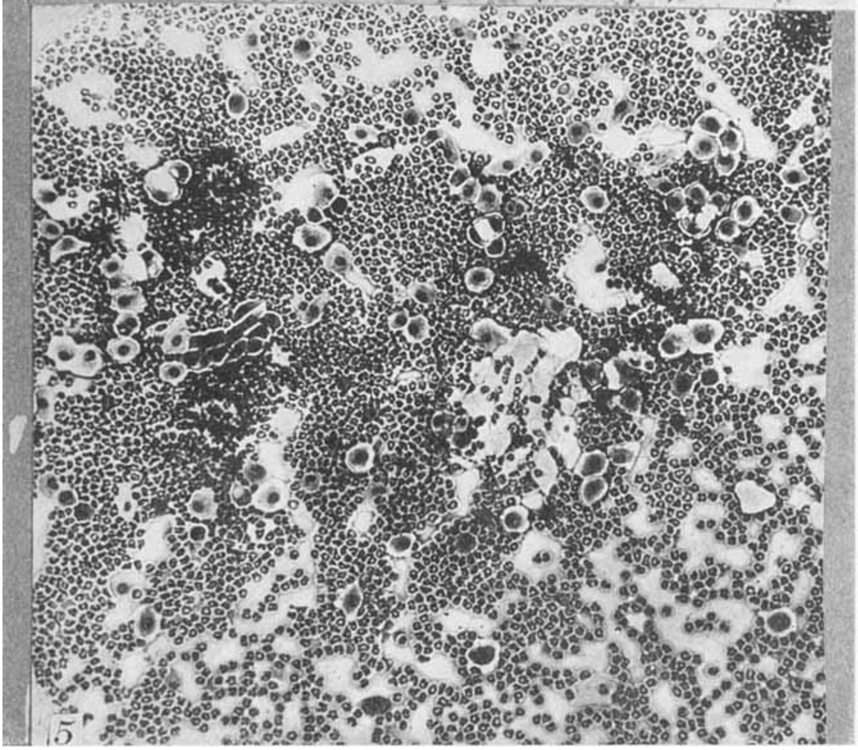
EXPLANATION OF FIGURES

4 Stage M_1 smear. × 35. Cells are similar to those in figure 3, but all degrees of cornification are represented by variable staining affinity, and clumps or masses of cells are frequent.

5 A late stage M_2 smear. × 125. Non-nucleated cornified cells of the previous stage (massed in center) and nucleated epithelial cells of the deeper epithelium surrounded by great numbers of polymorphonuclear leucocytes. Some of the nucleated cells show clear exoplasmic zones indicating the extraction of eosin staining cytoplasm without the entrance of the leucocytes into the cells.



4



5

PLATE 3

EXPLANATION OF FIGURES

6 Vaginal epithelium of the D interval. × 275. Leucocytes are still present, but chiefly in the superficial layers. There is no clear-cut basement membrane.

7 Section of half of the vagina of the early P stage. × 60. Two zones are clearly defined by staining reaction before either granular or horny layers appear (animal no. 8).


6

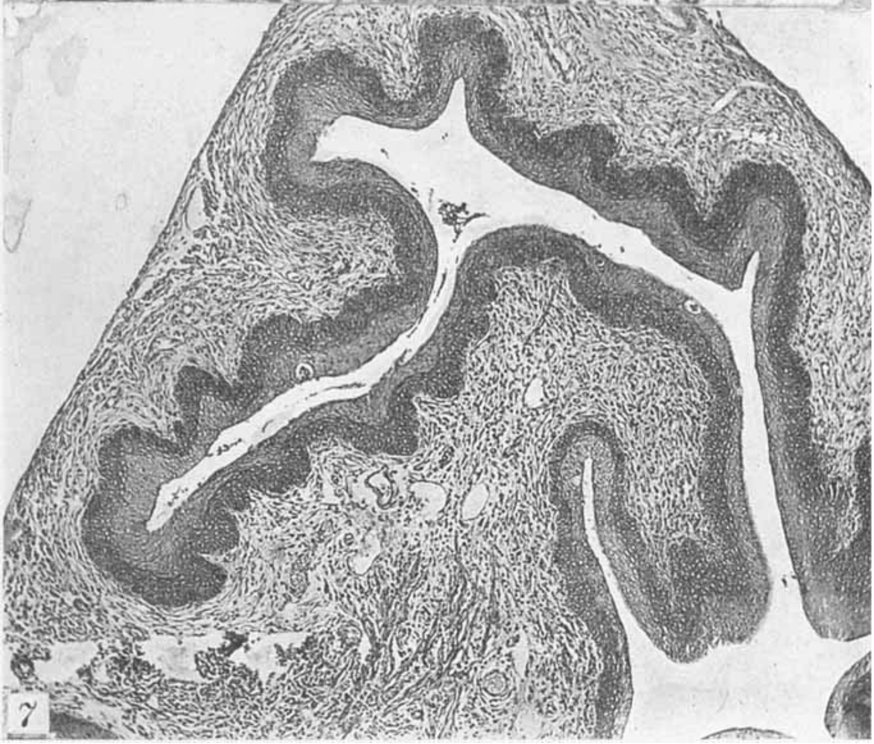

7

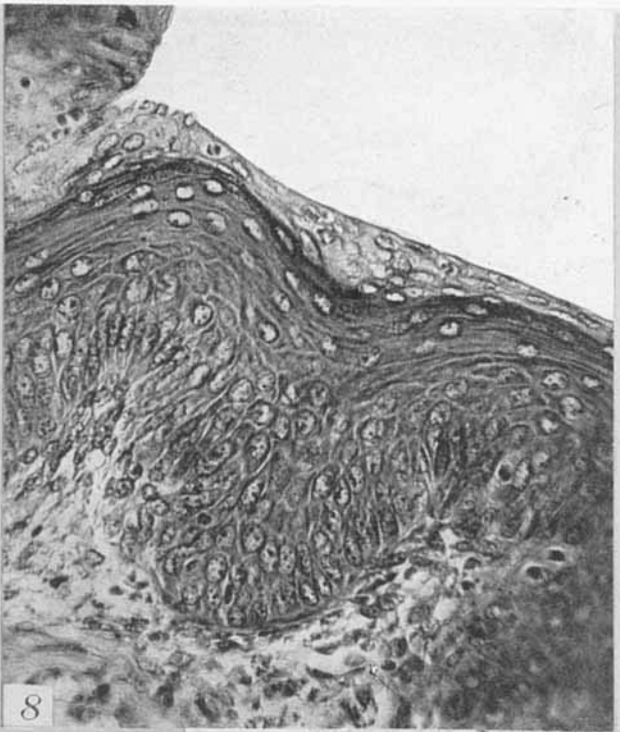
PLATE 4

EXPLANATION OF FIGURES

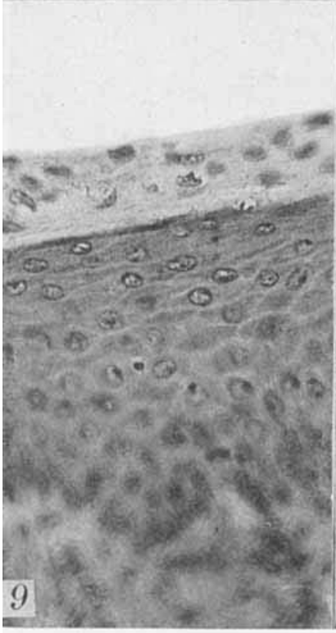
8 Vaginal epithelium of a later phase of stage P. × 275. The granular layer now clearly separates the two zones figured in 7 (animal no. 9).

9 The stratum lucidum of the corneum is forming. × 265. (Animal no. 10.) Sloughed-off nucleated cells make up the stage P smear.

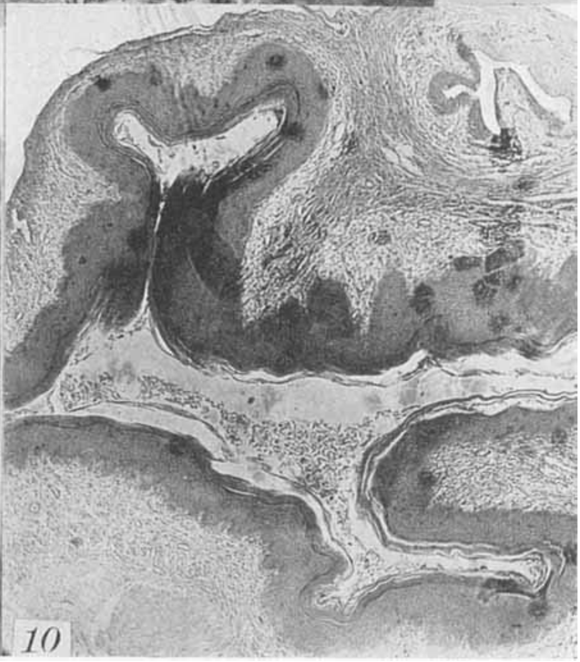
10 Section of the vagina in stage O. × 45. Corneum is well formed, superficial, and still intact. Free cells form the O smear.



8



9



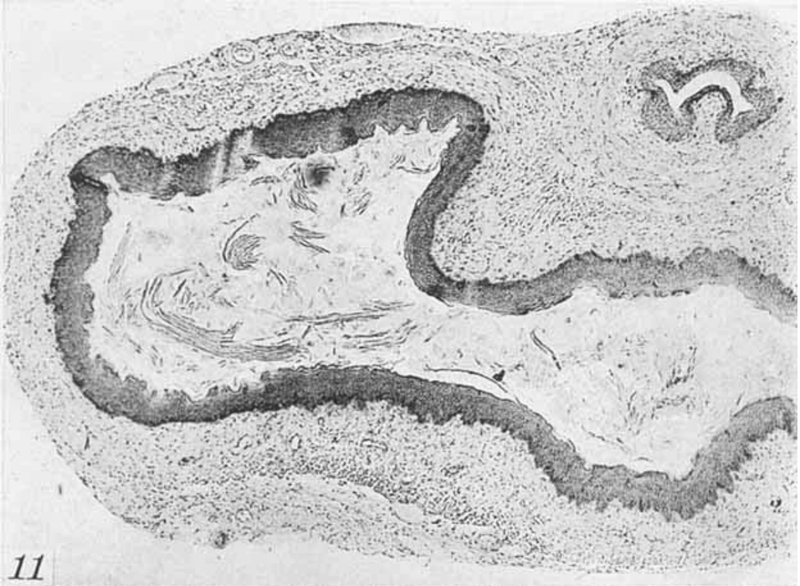
10

PLATE 5

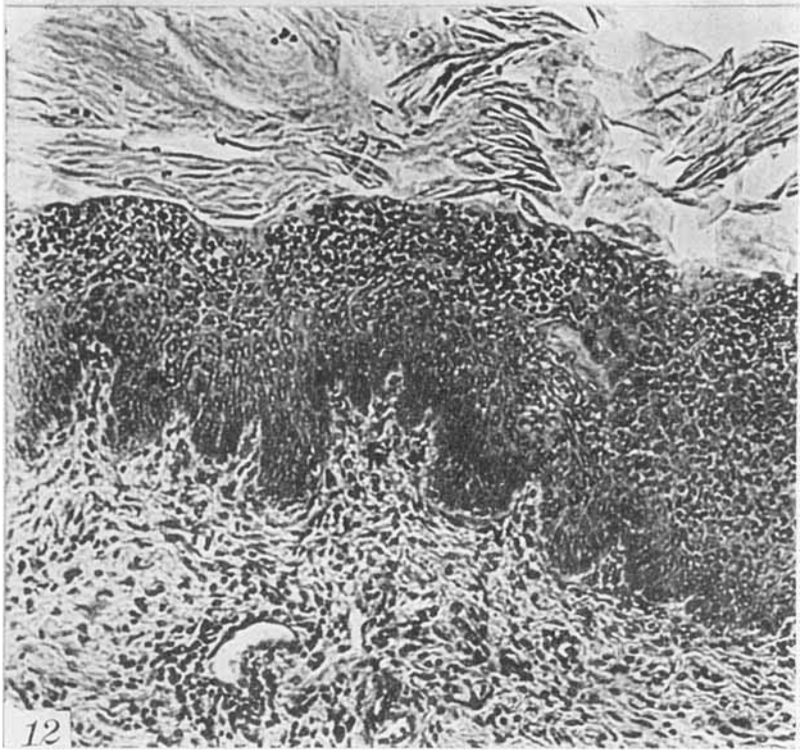
EXPLANATION OF FIGURES

11 Vagina in stage M_1. × 65. Corneum is completely delaminated into the lumen. Leucocytosis has not yet begun.

12 An early M_2 stage of the vaginal epithelium heavily infiltrated with leucocytes. × 180. Few have as yet entered the masses of cornified cells in the lumen.



11



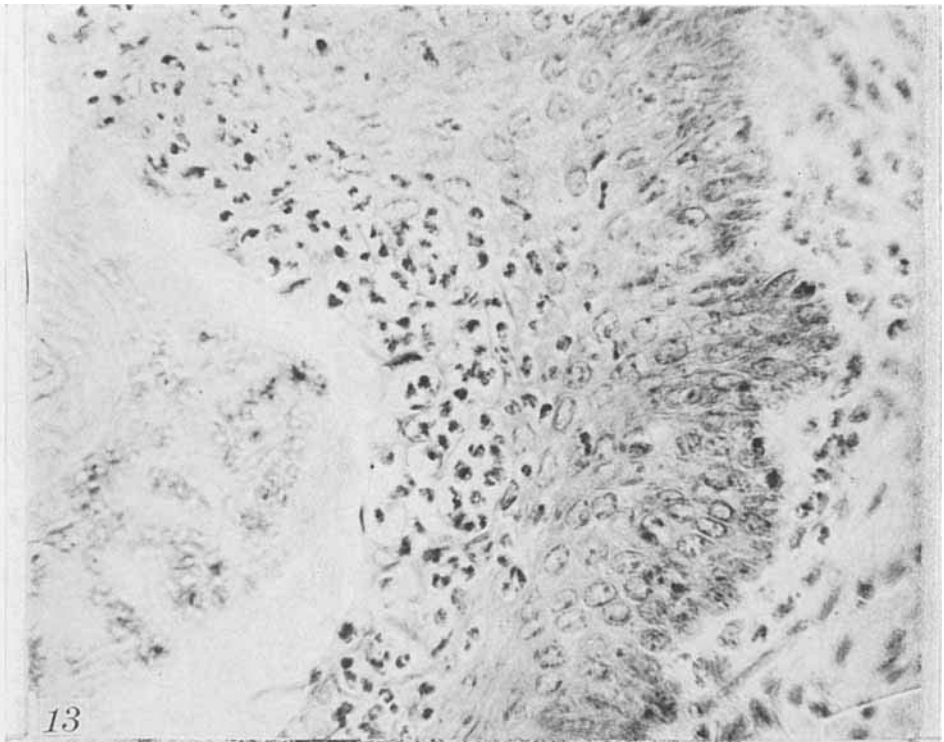
12

PLATE 6

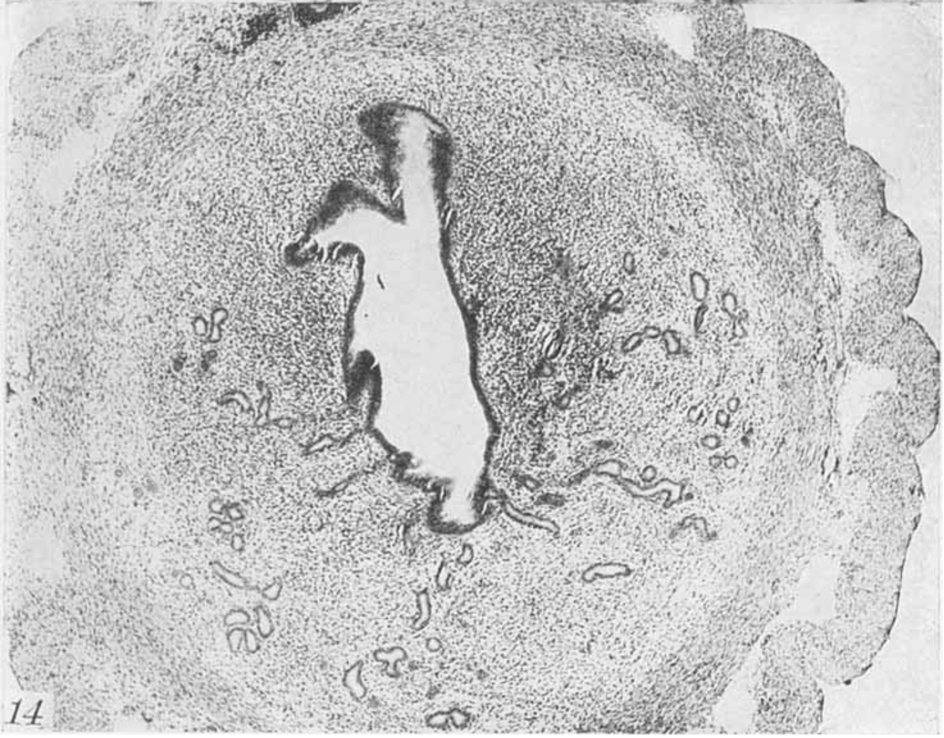
EXPLANATION OF FIGURES

13 Vaginal epithelium of a late M_2 stage. × 850. Groups of leucocytes have dissolved out lacunae in the superficial germinativum and enormous numbers have invaded the cornified masses in the lumen.

14 Section of the uterine cornu during stage P. × 55. The section does not show the distention apparent before fixation. Glands are moderately distended.



13



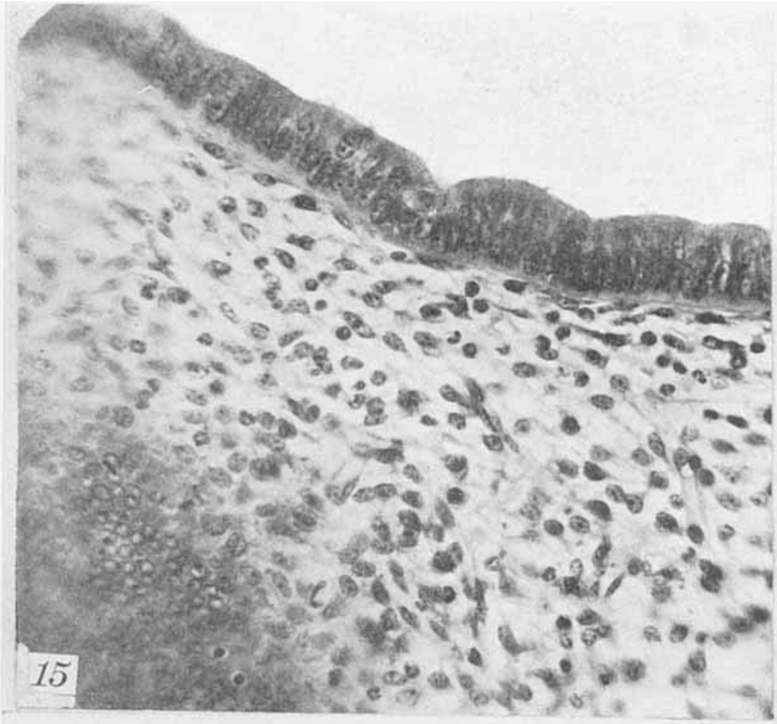
14

PLATE 7

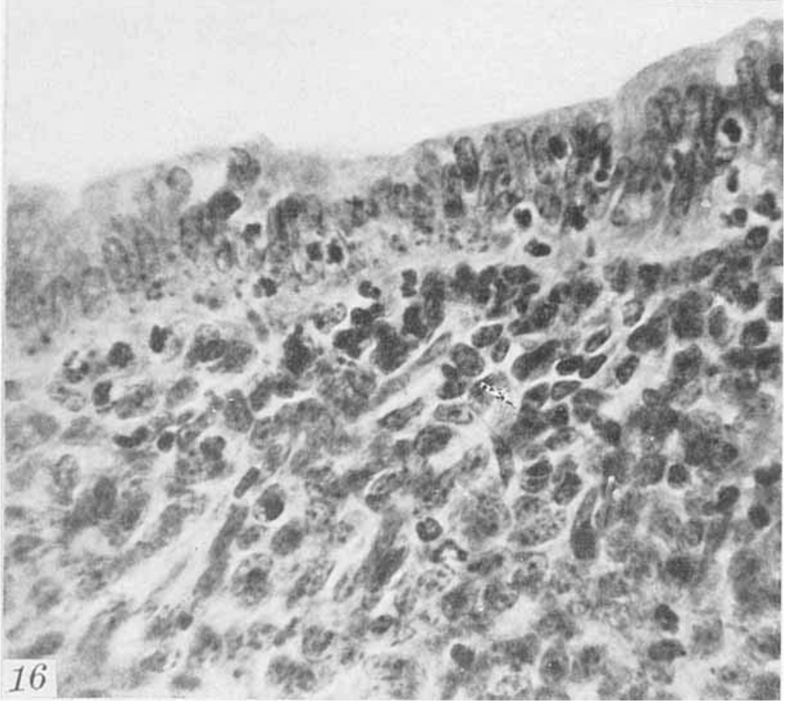
EXPLANATION OF FIGURES

15 Mucosa of the uterine cornu during stage O. × 365. Note the clear-cut basement membrane and high columnar cells.

16 Uterine mucosa during early stage M_2. × 550. The distinct basement membrane figured in 15 has been replaced by a light pink staining zone containing leucocytes.



15



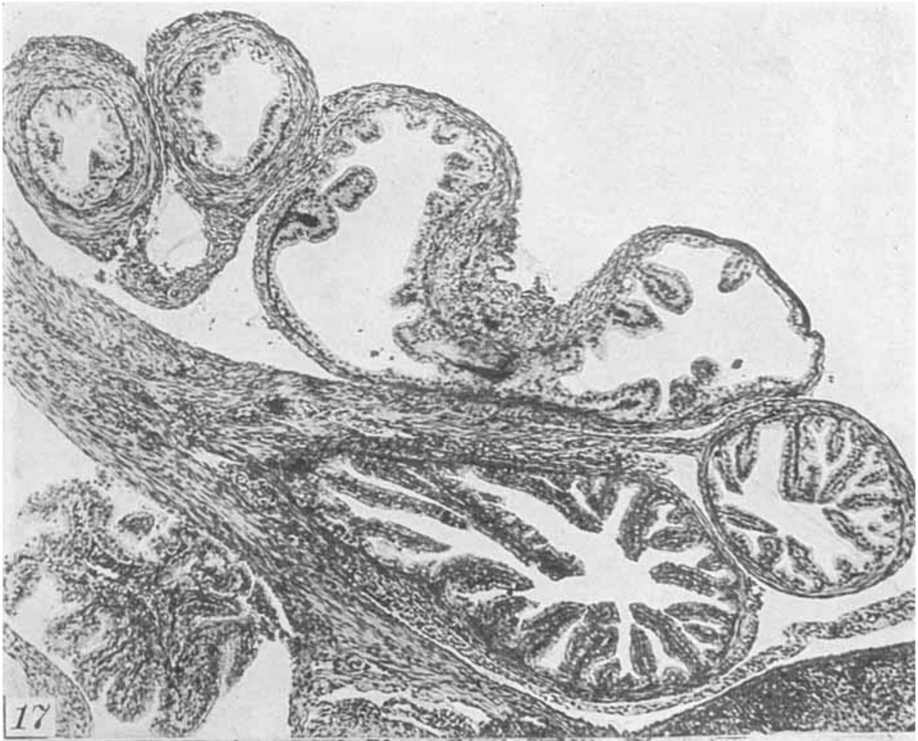
16

PLATE 8

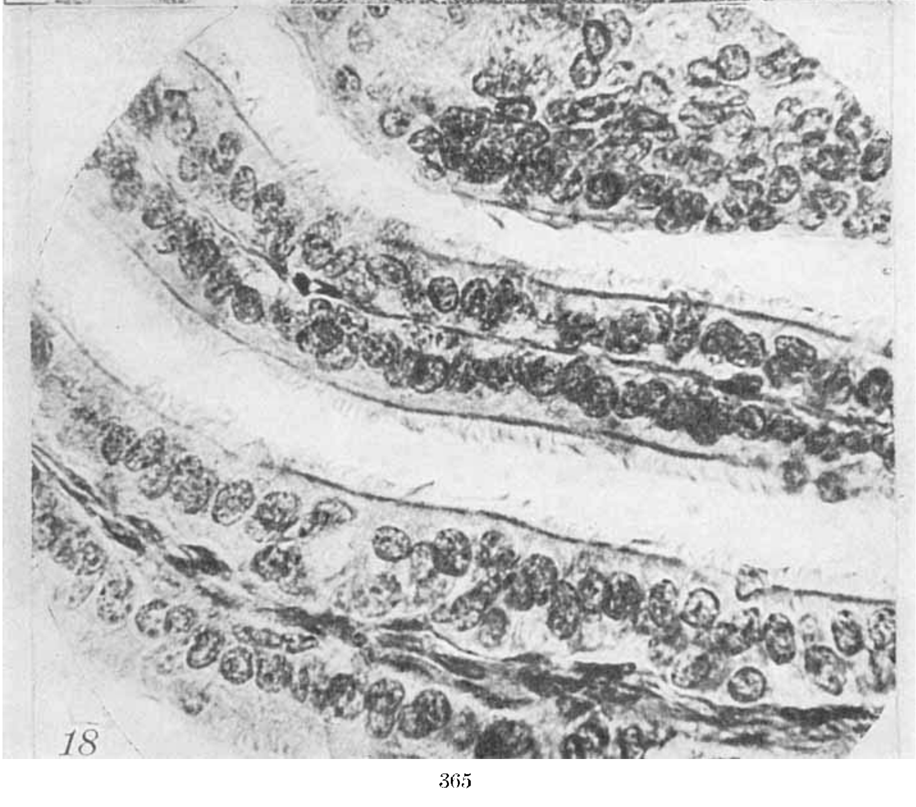
EXPLANATION OF FIGURES

17 Sections of several loops of the oviducts. × 55. Only that in the lower center is ciliated. Segments are distinguishable by ciliation, degree of folding of the mucosa, and thickness of the muscle walls.

18 Ciliated epithelium of the late stage P oviduct. × 550.



17



18

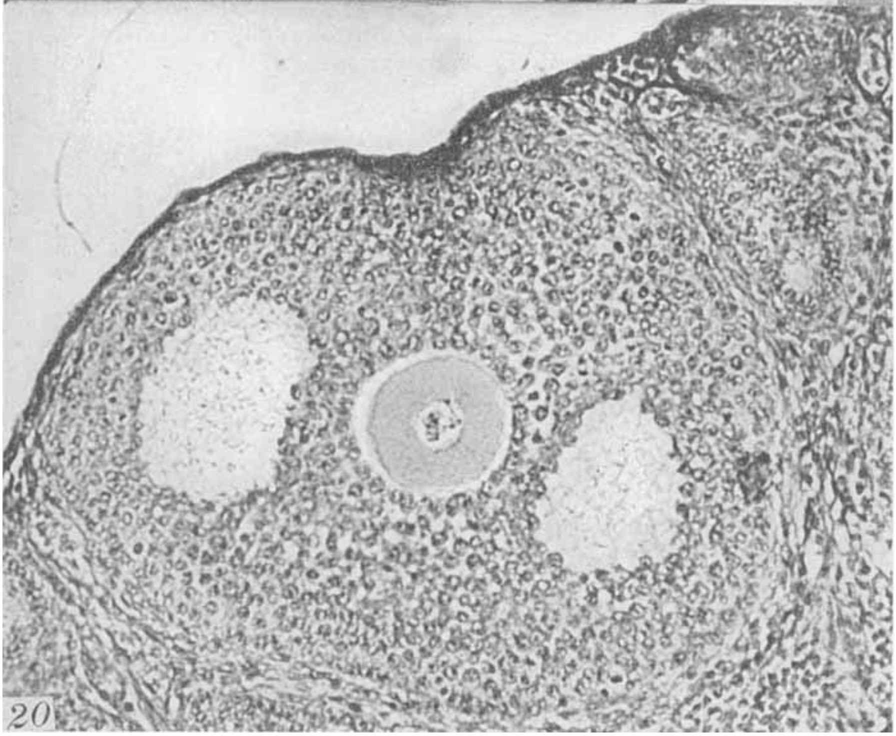
PLATE 9

EXPLANATION OF FIGURES

19 Ciliated epithelium of the oviduct in a late M stage. × 550. The process of extrusion of nuclei is quite general.

20 Largest-sized follicle usually found after ovulation, stage M_1. × 170. Primary liquor folliculi is restricted to two pools. Granulosa contains many mitoses. Note interstitial tissue above to the right.

19



20

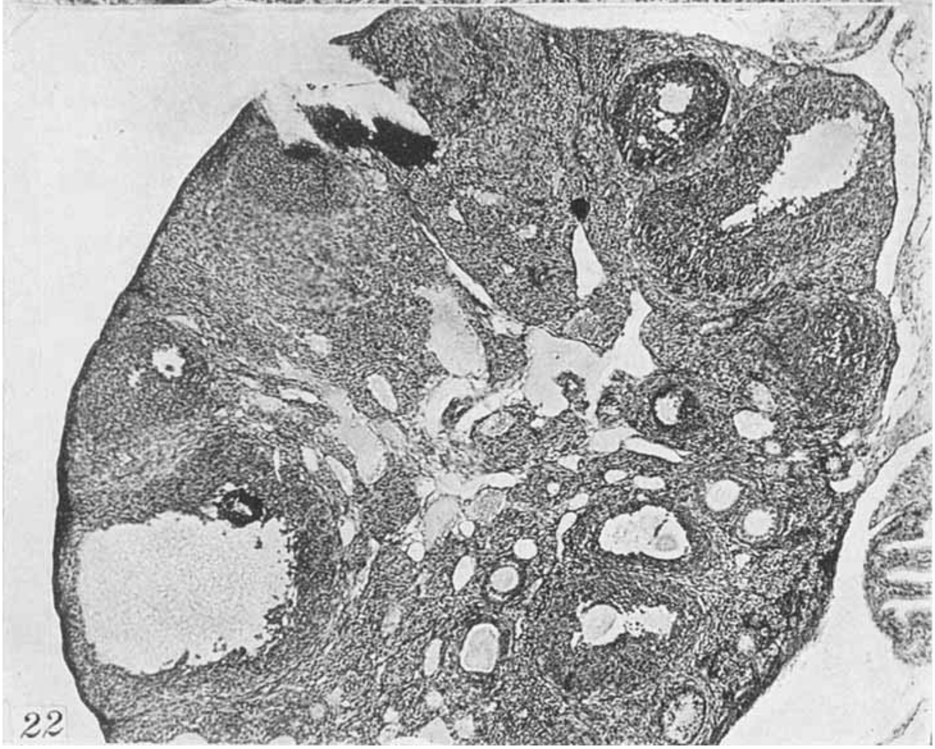
PLATE 10

EXPLANATION OF FIGURES

21 Large follicle (nearly rupture size) in an early stage of atresia. × 125. Several follicles in this set are apparently normal. At right, a medium-sized follicle containing two ova.

22 Ovary of an early M_1 stage. × 58. In upper right field is newly forming corpus luteum, not yet redistended. At lower left is one fully redistended. The age of these corpora is estimated at less than seven hours.

21



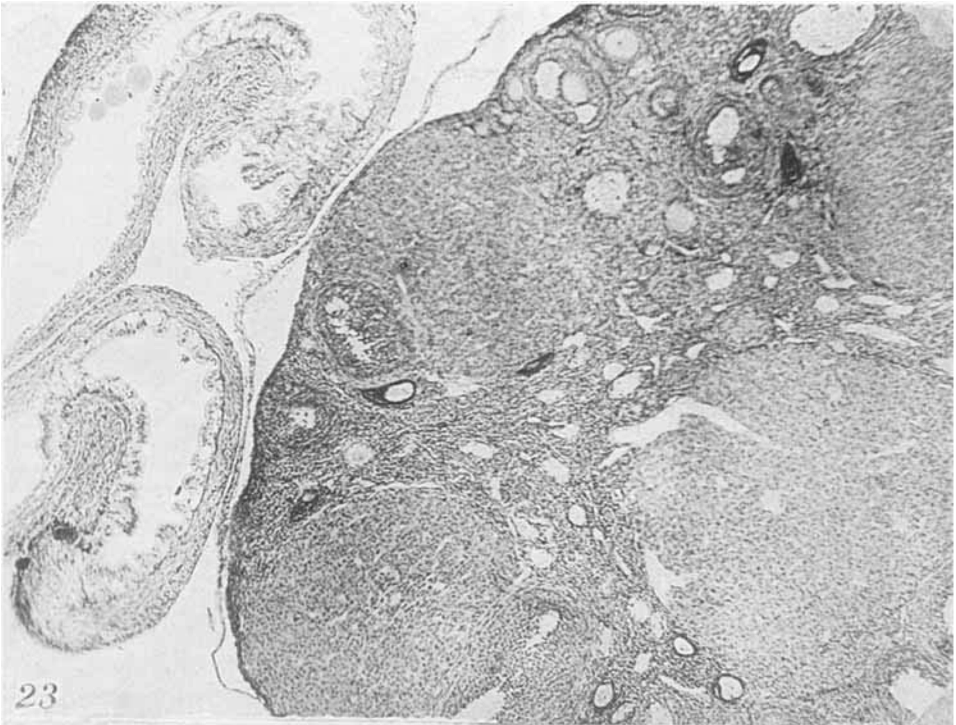
22

PLATE 11

EXPLANATION OF FIGURES

23 Ovary and third segment of oviduct of late stage M_2 animal. × 501. In upper left field are three ova bunched in the last segment of the oviduct. Of the three corpora lutea included in this field, the two at the left surface of the ovary correspond to the ova in the tubes, the one in the lower right field to the second oestrus recorded before death. They are easily distinguished by staining reaction not brought out in the photograph. The ages of these were estimated at three and nine to ten days, respectively.

24 Two corpora lutea representing follicles which ruptured at the first and third Operiods before death. × 58. That to left stains blue and shows "bleeding into the central cavity." That to the right has a pink tinge, is 'corded,' and deeply placed. Ages are estimated at three and fourteen days, respectively.



23

24