

## THE OESTROUS CYCLE IN THE OPOSSUM

CARL HARTMAN

*Department of Zoology, The University of Texas<sup>1</sup>*

### THIRTEEN PLATES

### CONTENTS

Introduction and literature.....	353
Material and method.....	357
Gross changes in the reproductive organs.....	360
<i>a.</i> Gross anatomy of the female genital tract.....	360
<i>b.</i> Changes in the ovaries.....	361
<i>c.</i> In the vagina and the lateral vaginal canals.....	362
<i>d.</i> In the uteri.....	363
<i>e.</i> In the pouch and the mammary glands.....	364
The oestrous cycle.....	365
<i>a.</i> The period of gestation.....	365
<i>b.</i> The relation of oestrus and ovulation: postoestrum.....	366
<i>c.</i> The tubal journey.....	368
<i>d.</i> The length of the oestrous cycle.....	369
<i>e.</i> The phases of the cycle.....	372
<i>f.</i> Cyclic changes in the uteri: pseudopregnancy.....	372
<i>g.</i> Cyclic changes in the vagina and its contents.....	376
<i>h.</i> Cyclic changes in the lateral vaginal canals.....	380
<i>i.</i> Cyclic changes in the mammary glands: pseudopregnancy.....	383
Discussion.....	385
Summary.....	391
Literature cited.....	393

### INTRODUCTION AND LITERATURE

In the September, 1917, number of this Journal Stockard and Papanicolaou published a study on the oestrous cycle in the guinea-pig, announcing in this article their discovery of cyclic changes in the vaginal mucosa recognizable by the simple method of microscopic examination of the vaginal contents. This

<sup>1</sup> Contribution no. 163 from the Department of Zoology, the University of Texas.

method, because of its usefulness, has proved to be an epoch-making one. The work of these authors, coupled with that of L. Loeb and his students, has made the cycle in the guinea-pig very well known.

Using the vaginal-smear method of diagnosis and other experimental methods Long and Evans<sup>2</sup> ('22) completed an exhaustive study of the reproductive cycle in that most generally useful laboratory mammal, the albino rat. Because of the painstaking accuracy of this work, experimental biology has been given another instrument for advances in various lines of research in which the events of the normal reproductive cycle must be known.

With the completion of Allen's excellent study ('23) on the white mouse, the cycle of three laboratory mammals has become known. To this list has been added the domestic pig through the energetic and intelligent efforts of Corner ('17, '21, '22, '23).

From these studies it has become apparent that the domestic mammals exhibit comparable phenomena during the oestrous cycle, and from scattered studies on the physiology of reproduction in other Eutheria it seems probable that all conform fundamentally to one type. To anticipate the results of the present paper, it may be stated at the outset, that the opossum, a 'wild' animal and a 'marsupial,' is no exception to the rule.

Since the monographs mentioned above have adequately reviewed the literature concerning reproductive phenomena in the Eutheria, the reader's especial attention will be directed to the present status of the subject only as it concerns marsupials.

The marsupials have been little studied. Selenka ('87) secured a hundred Virginia opossums, bred them in his laboratory, and made a few observations on the breeding habits. Aside from this and minor scattered notes the work of Prof. J. P. Hill and his students, notably Sandes and O'Donoghue, stands alone. While at Sydney, Australia, Hill collected an excellent series of marsupial eggs and embryos, chiefly of *Dasyurus*, on which

<sup>2</sup> The writer wishes to acknowledge his indebtedness to Dr. H. M. Evans for both wise counsel and sympathetic stimulus in this work.

he published his beautiful monograph ('11). His collection yielded as by-products the ovaries, uteri, and mammary glands in various stages besides detailed notes on the breeding habits of this form.

Hill ('11) regarded *Dasyurus* and indeed all other marsupials studied by him as monoestrous, supporting Selenka's observations in the opossum, who says: "Die Brunst des Weibchen tritt normaler Weise nur ein Mal in Jahre ein." But Selenka also noted that if pouch young are removed the females come into heat again after four to six weeks—a fact which I can corroborate, for I have performed this experiment many times.

In my paper of 1916 I already recognized the fact that in the opossum there are successive oestri; but at that time I also believed that only one litter is borne a year, for I stated ('16, p. 16) "that the opossum has only one sexual season a year and normally (i.e., in the wild state) only a single oestrous period," for in the wild state almost every female becomes pregnant at the breeding season. As a matter of fact, I now know that the opossum has a continuous breeding season, bearing at least two litters (three is very questionable) and closing the year with an anoestrous period of about two months (Hartman, '23 c).

In his study of *Didelphys aurita*, the Brazilian gambá, Hill found that this species bears two litters, one in July and another in October. This discovery raised doubts in his mind as to the accuracy of his statement for *Dasyurus* (Hill, '18, p. 101); but the author still found no facts to conflict with his former view. To my mind it is quite probable that on more prolonged observation, which must exceed a single month, *Dasyurus* will also be found to be polyoestrous.<sup>3</sup>

The suspicion that other marsupials may be polyoestrous was also expressed by O'Donoghue ('16), for he found two sets of corpora side by side in the ovaries of a number of species.

<sup>3</sup> Hill and O'Donoghue's figure 1, plate 6, page 174, of their study on The Reproductive Cycle in *Dasyurus*, 1913, is taken from an animal killed June 17, 1899. This uterus looks like an opossum uterus reconstructed after the dioestrous interval, not after the longer anoestrous period. If this is correct the animal was entering the second prooestrous period of the season (cf. my fig. 28, pl. 6).

A fresh active corpus beside an old degenerating one would indicate successive ovulations if not successive pregnancies.

Also working on Hill's material, Sandes ('03) was able to demonstrate for the first time the complete development attained by the marsupial corpus luteum. On this point the marsupials were thus brought into line with the higher mammals. Sandes made another observation of theoretical interest, namely, the identity of the corpora lutea spuria (c. l. ovulationis of Long and Evans) and the corpora lutea vera (c. l. gravidatis). In this particular the opossum fully agrees. But in another point, namely, the long life of the corpora lutea of *Dasyurus*, the opossum is in striking contrast. However, the reader of Sandes' paper is at a loss to note the number of observations upon which his generalization is based. O'Donoghue ('12) found perfectly preserved corpora with "no signs of beginning to disappear" in an animal twenty days after copulation.

Correlated with the maximal development of the corpora lutea vera as well as c. l. spuria, O'Donoghue ('11) found a maximal hyperplasia of the mammary gland tissue whether pregnancy ensued or not. Later Hill and O'Donoghue ('13) found a similar phenomenon in connection with the uterus and they coined the term 'pseudopregnancy' to designate the excessive development of uterus and mammary glands following a sterile ovulation. That these authors have in no wise exaggerated the case my opossum material amply proves.

For certain experimental purposes marsupials would seem to be excellent material (Ancel and Bouin, '11). This is especially true of the uniparous forms, for here the ovary containing the corpus luteum could be extirpated, leaving the other, thus avoiding the avalanche of criticism cast upon the work of Frankel, Loeb, and others who caused excessive trauma in extirpating the corpora lutea.

Hill and O'Donoghue further noted in certain cases extravasation of blood accompanying degeneration of the uterine epithelium during the involution of the uterus after pseudopregnancy. They thus homologized this process with menstruation in *Eutheria* and this in turn with prooestrus in

Eutheria. Menstruation is supposed, therefore, to have been shifted forward in the cycle. In the opossum there is degeneration of the uterine epithelium following pseudopregnancy; but extravasations of blood are as rare as hemorrhagic corpora, which, of course, occur now and then.

Hill and O'Donoghue further looked upon *Dasyurus* as unique in the long delay of ovulation after oestrus (their 'postoestrum' or 'postoestrous period'). Ovarian eggs were found 4 to 8 days post coitum; unsegmented ova, 5 to 8 days; two-celled eggs, 5 to 7 days; four-celled, 5, 11, and 18 days; and birth occurred 8, 16, and 22 days post coitum. These discrepancies the authors explain by postulating a variable 'postoestrum' or delay in ovulation. But the small number of cases coupled with the deleterious effect of caged conditions (Hartman, '23 a) makes Hill's explanation doubtful. The opossum is equally erratic; indeed I do not feel that my data are quite conclusive on this point. However, recent observations have convinced me that ovulation normally comes very soon after oestrus. Moreover, since Eutheria also have a postoestrum albeit a short one (rat, Long and Evans, '22), the marsupials would no longer be considered unique in this respect.

These are the important papers on the oestrous cycle in marsupials, and I acknowledge my indebtedness to them. With this good beginning, it seemed desirable to extend the observations; to study the entire reproductive cycle, if possible, in one marsupial, and to correlate them with the oestrous changes in guinea-pig, rat, mouse and pig. To anticipate the conclusions reached: Marsupials and Eutheria are in the closest agreement in all steps of the oestrous cycle. The slight differences are highly instructive, but not fundamental.

#### MATERIAL AND METHOD

The writer's first interest in the opossum centered about its early development, hence every effort was made at first to secure the requisite number of stages (Hartman, '16). With the generous financial and moral support of The Wistar Institute, through its director, Dr. M. J. Greenman, it was possible to secure material

for a more complete monograph (Hartman, '19), part 6 of which, on the origin of the mesoderm, is in preparation.

The collection of embryological material, however, soon led to a study of the oestrous cycle and an interest in the physiological anatomy of the organs of generation.<sup>4</sup>

In the first place, it soon became apparent that a promiscuous slaughter of animals even during the breeding season resulted in waste of material and effort. The attempt was made, therefore, to discover reliable outward signs of pregnancy. Such signs as tumidity of pouch and of cloacal margin, moistening of pouch, etc., (Hill, '00, '11), proved so variable in the opossum as to be practically worthless. As to the employment of the mammary gland for diagnostic purposes, the following statement of Selenka ('87) did not seem encouraging:

Ohne operative Eingriffe ist über die Trächtigkeit eines Weibchens keine Gewissheit zu erlangen, da man weder durch Tasten mit dem Finger die weichen Uterushörner auffinden kann, noch auch an den Milchdrüsen eine Veränderung wahrnimmt, bevor nicht die Embryonen nahezu ausgetragen sind.

But in spite of this opinion the mammary apparatus proved to be the best indicator of approaching sexual activity and of pregnancy or pseudopregnancy. In 1919, my first collecting season after the appearance of the paper by Stockard and Papanicolaou ('17, l. c.), their method of examining vaginal smears supplemented in my laboratory but did not supersede the method of mammary palpation. Just at oestrus the vaginal smear offers the only absolute criterion; at other periods sometimes one, sometimes the other method has proved the safer guide.

Over one thousand female opossums have passed through my hands. Many, of course, were worthless; but my notes concern 904 animals and range in detail from mere date and a record

<sup>4</sup> Thanks are due to Mr. H. A. Wroe, President of the American National Bank, Austin, Texas and President of the Board of Regents of the University for a fund of \$500.00 for the continuance of the work in 1921; likewise to the Bache Fund of the National Academy of Science for a similar fund for the completion of the collection in 1922.

of the findings to almost daily entries for one to five months. A hundred selected females were followed through two or more fairly clean-cut oestrous cycles.

Records of observed copulation unfortunately number only about a dozen; for the animal is nocturnal, sleeping through the entire day. But considerable data have accumulated on the rate of development by virtue of the 'didelphic' anatomy of the animal, that is, the possession of two uteri. Thus two stages may be secured through operative procedure from the same animal, and the rate of development recorded (Hartman, '16, '19).

For another reason the opossum, like *Dasyurus* (O'Donoghue, '11), is adapted for experimental purposes. The idea was expressed by O'Donoghue who uses the following words, referring to the virgin state of the organs during anoestrus:

Anoestrus permits the whole of the genital organs, mammary glands and associated structures to return to a condition of complete rest between the breeding periods, whereas in some of the higher animals, for example, in the rabbit, a frequent subject of investigation, they are practically in a constant state of activity.

All material which has been retained for this and other studies has been carefully checked as to stage of pregnancy or the dioestrous cycle. Either the time of oestrus was exactly or approximately known, or the ovaries revealed the stage by the size of the graafian follicles. In the pregnant animal the eggs or embryos were secured; in the pseudopregnant animals, the unfertilized eggs<sup>5</sup> were recovered, the two sets often being removed at various intervals. In many cases the ovaries,<sup>6</sup> mammary glands, and entire genital tract of a given animal were preserved; in others only one or several organs were taken. But almost every conceivable stage is represented in the collection by several to a dozen or more specimens, mostly admirably fixed.

<sup>5</sup> 300 of these eggs have recently been studied by Miss Septima Smith. They will be discussed in a forthcoming publication.

<sup>6</sup> The corpus luteum is being studied by Miss Lillian Janoch who will soon publish the results of examination of 200 ovaries.

Fixation was accomplished by Bouin's fluid, picro-corrosive-acetic, Zenker's fluid, formol-zenker, neutral formalin 10 per cent, arranged in decreasing order of quality of fixation. Halves of ovaries were fixed in several hundred cases in Meves' fluid with the after-treatment suggested by Long and Evans ('22). This method is convenient and satisfactory for studying the lipid granules.

Paraffin imbedding offered no difficulties to even the largest organs, provided these were first cut into slabs about 5 mm. in thickness. The mammary glands cut with difficulty but excellent preparations were often secured. Delafield's hematoxylin and iron-alum hematoxylin, counterstained with eosin or orange G were the usual stains employed. Any combination of them in any tint seemed to photograph well on Cramer's medium iso plates with the aid of Cramer's micro ray filters (Petrunkevitch, '20).

Vaginal smears were studied fresh in salt solution or stained with a drop of aceto-carmin. Others were dried, stained with Delafield's hematoxylin and eosin, and mounted in balsam or damar.

#### GROSS CHANGES IN THE REPRODUCTIVE ORGANS

##### *a. Gross anatomy of the female genital tract*

A word needs to be said in explanation of the female genital tract of the opossum (plates 2 and 3). The vulva may be seen just within the rim of the cloacal aperture. In making vaginal smears the vaginal opening may be found by gentle pulling downward of the ventral border of the cloaca. The median vaginal canal<sup>7</sup> leads forwards several inches to a blind anterior end; behind this it receives the urethra ventrally, and dorso-laterally the two lateral vaginal canals, which lie like interrogation points on either side, for the most part within the body cavity. There are two uteri, each with a separate cervix

<sup>7</sup> The median vaginal canal will henceforth in this paper be termed vagina for the sake of brevity. The lateral vaginal canals will be termed canals where the meaning will be unambiguous.



emptying into a vaginal canal. It is through the lateral vaginal canal that spermatozoa must reach the os uteri; but birth is through a new passage way, the 'pseudovaginal canal,' discovered by Hill ('00). This is forced de novo straight to the median vagina.

*b. Changes in the ovaries*

The cyclic changes in the opossum ovaries parallel the changes in the ovaries of other mammals in every essential. It is therefore unnecessary to recite familiar processes in detail, particularly since the genesis of the opossum corpus agrees essentially with that of *Dasyurus* (Sandes, '03). However, since the ovaries determine, directly or indirectly, the cyclic changes in the other reproductive organs, several important points, relating especially to the corpus luteum, must be mentioned.

During December and for some time before and after, all females are in anoestrus. The ovaries are small, weighing 0.05 to less than 0.1 gram. It is wrong, however, to designate the ovary as 'resting' during this time, for the organ consists of an almost solid mass of follicles, mostly undergoing chromatolytic and lipolytic atresia. The follicles never attain any considerable size at this time, always degenerating before very much liquor folliculi has accumulated (fig. 10, pl. 3). I have never seen an opossum ovary in December that offers an exception to this rule. Usually the ovaries contain no trace of corpora lutea; only occasionally does one find scarcely recognizable remnants of them.

In January ovulation usually takes place. It is spontaneous, normally; but cage conditions may interfere with ovulation, upsetting the cycle. An experimental analysis of the influence of cage conditions was attempted but cannot be discussed here (Hartman, '23 a).

At oestrus numerous clear, large follicles bulge from the surface of the ovary (fig. 6, pl. 2). In section they seem to occupy most of the organ. Upon rupture the ovary shrinks suddenly to nearly the size and weight of the resting organ. The corpora lutea now grow rapidly, fill out and reestablish and even exceed the size and weight of the ovary at oestrus (fig. 13, pl. 3). In

three days the corpora have reached full size which is maintained till the seventh or eighth day, after which they assume a dirty yellow color due to lessened vascularity, and sink deeper beneath the surface of the ovary while they gradually shrink. At the twentieth day all that is visible of corpora are small yellow specks seen on the surface and in the depth of the organ. Such bodies also result from the degeneration of corpora atretica; but in section these are distinguishable from corpora albicantia by the fact that the cells of the corpora atretica alone retain their power of reducing osmic tetroxide.

The opossum corpus luteum declines much more rapidly than that of *Dasyurus*, as noted in the Introduction. At parturition or about twelve days after ovulation, the corpus is already infiltrated with swarms of leucocytes and numerous connective tissue cells. At the corresponding period after pseudopregnancy the degeneration is slightly more advanced.

During the dioestrous interval the ovary is reduced again to a minimal size, almost as in anoestrus, but remains of corpora may still be found by the time a normal oestrous cycle is at an end.

*c. Gross changes in the vagina and the lateral vaginal canals*

The most striking of the gross changes affecting the female genital tract during the oestrous cycle is the sudden swelling and the equally sudden subsidence of the lateral vaginal canals. This holds true, though to a lesser degree, for the vagina itself. When the ovarian follicles are of medium size, only slightly protruding from the ovary, the canals begin swelling; this continues until oestrus, when they are very vascular and enormously distended with a slightly viscous fluid. The vagina itself enlarges through a thickening of its walls (fig. 51, pl. 10).

These organs retrogress as quickly as they enlarge. By the time the corpora lutea are fully formed (three to four days) they have returned practically to the state in which they will remain until the next oestrus.

The size changes in vagina and lateral vaginal canals, particularly the distention of the latter by its fluid contents, strictly

parallels the growth and dehiscence of the ovarian follicles. This is precisely comparable to the behavior of the rat uterus as described by Long and Evans ('22); and the conclusion of these authors with reference to the rat uterus, namely, that the accumulated fluid serves to facilitate the locomotion of the sperms, seems altogether reasonable also with reference to the lateral vaginal canals of the opossum.

*d. Gross changes in the uteri*

Progressive changes begin in the uteri in early prooestrus. The organs become large, more vascular and increase in turgidity. The freshly opened uterus presents a smooth, pink mucosa which becomes softer and fluffier as liquid gathers among the glands (fig. 13, pl. 3).

After ovulation, whether pregnancy ensues or not, the uteri continue to swell until almost the maximum is reached on the fifth or sixth day, when normally 2 mm. vesicles in the primitive streak stage are found in the uterus, or in the non-pregnant animal there are found degenerated eggs having slightly opaque encrusted shell membrane. In the latter case the condition of pseudopregnancy is manifested, for the uteri from outside appearances do not disclose their contents. If fertilized eggs are within, the uterus increases only slightly in size up to the time of parturition, but the internal pressure increases greatly. However, one often finds enormous uteri with shiny tight walls as though they contained large embryos; but on inspection only old, fragmenting, unfertilized eggs are found. Such a uterus, however, soon becomes flaccid, and may almost be said to collapse (fig. 7, pl. 2). The mucosa of such a shrunken uterus has a dirty brownish tinge indicative of degenerative changes in the cells of the epithelium (fig. 14, pl. 3). In thirteen to fifteen days the uteri are much reduced (fig. 8, pl. 2), by the eighteenth or twentieth they have usually become about the size of the anoestrous organ (fig. 11, pl. 3). The twentieth day sees the involutionary changes completed and the reconstruction well under way.

*e. Gross changes in the pouch and the mammary glands*

O'Donoghue has described some prooestrous changes in the pouch of *Dasyurus*: increase in size, tumidity of the margin and increased moisture due to growing activity of the sweat glands in the skin. During pregnancy these changes continue, together with a great hypertrophy of the sebaceous glands, which soon secrete a reddish, greasy, substance, designed to "facilitate the cleaning of the pouch" (O'Donoghue, '11) for the reception of the young.

Similar changes take place in the opossum. But there is so great a variation that the adaptive significance of the secretions must be doubted. Some pouches, notably those of old *multi-parae*, are roomy, very sweaty, and so coated with the red oily secretion that they stain the fingers in palpation. Young animals have small pouches, dry and very hairy, and yet the pouch young are found safely attached to teats amid such a forest of hair.

Swelling of the mammary glands begins before oestrous or ovulation. This is sometimes so considerable that a case may be diagnosed as pregnant when ovulation had not yet taken place. The reciprocal mistake is not often made by the observer. An example of the employment of this method in following an animal through an oestrous cycle is the following:

No. 507. Received Jan. 20, glands thin; Feb. 5, rather thick, flabby; Feb. 9, very thick and firm (indicates pseudopregnancy, as no male was present); Feb. 20, glands thin; Feb. 24, thicker; Mar. 5, distinctly thicker; Mar. 7, thick; killed; eggs several days old in uterus.

A favorite pastime of my assistants, chiefly premedical students, has been the request of my diagnosis of the conditions of a given female on the operating table,<sup>8</sup> the diagnosis is recorded in the note book under the heading 'stage predicted.' Several examples are given:

<sup>8</sup> Prof. F. Thomas Flynn of Tasmania writes me: "I have met a bushman who could tell that an animal was pregnant by palpation."

<i>No.</i>	<i>Stage predicted</i>	<i>Stage found</i>
500	Young eggs	4-celled eggs
501	Resting	Resting
503	(Jan. 17, anoestrous) Jan. 29, thicker	In prooestrus
516	Late prooestrus	Early prooestrus
519	Embryos or old degenerated eggs	Atretic follicles
520	Eggs in late cleavage	Early bilaminar blastocysts
521	Young eggs	Ripe follicles

A sample of great miscalculation (of which many more could be given) is as follows:

No. 530. Jan. 25, signs of sexual activity; Jan. 28, doubtful whether in late prooestrus or in late pseudopregnancy; Feb. 2, newly born pouch young!

The thickening of the mammary glands is one of the most striking phenomena of the entire cycle. The growth is extremely rapid during the first seven or eight days after ovulation and it is as pronounced when the uterus contains only fragmented eggs as when it contains embryos. No better example of pseudopregnancy can be found than the mammary gland of a non-pregnant opossum eight to ten days after oestrus.

In order that the reader may readily visualize the gross changes which the reproductive organs undergo in correlation with one another the curves presented in the accompanying chart 1 have been prepared. The rhythm in each organ is apparent at a glance (page 367).

#### THE OESTROUS CYCLE

##### *a. The period of gestation*<sup>9</sup>

There is little accurate data upon the period of gestation in any marsupial; we only know that it is extremely short, to be measured in days and hours, rather than weeks and days.

<sup>9</sup> It is impossible within the limits of this paper to go into detail on this topic. It is the writer's intention to present his data for publication in a future number of the Journal of Mammalogy (see also Hartman, '23 c).

Hill made two widely divergent observations on the time between copulation and parturition in *Dasyurus*: 8 and 16 days respectively. The latter he considers the more reliable. If the post-oestrous period be taken as 5 days, the period of gestation in *Dasyurus* would be 11 days.

Selenka ('87) made a single important observation on the opossum. He says: "nicht ganz 13 Tage, wahrscheinlich 12 Tage 20 Stunden nach der Begattung erfolgt die Geburt." With this my own single case corresponds almost exactly:

No. 190. Jan. 22, laparotomy showed animal to be in the dioestrous interval; Feb. 1, stitches taken out, mammary gland thin; Feb. 10, 10:00 a.m., copulation observed, sperms recovered from vagina; Feb. 11, removed from male; Feb. 22, 10:00 a.m., mammary glands very thick; Feb. 23, 8:00 a.m., newly born young, some attached, others unattached, and these a few hours dead.

If in this case from 12 days, 22 hours we deduct 8 hours, the probable age of the pouch young, we have 12 days, 14 hours for the interval between copulation and birth. I have accumulated considerable data on the rate of development that leads me to believe that 12 to 13 days is very nearly correct for this period.

#### *b. The relation of oestrus to ovulation*

It is evident that this relation is an important factor in determining the exact period of gestation; for if there is a variable delay in ovulation after copulation (the 'post-oestrous period' of Hill and O'Donoghue, '11) it follows that the total period from copulation to ovulation is also variable (Hartman, '23 c). Hill found a very great variability in the stage of the eggs of *Dasyurus* at given periods after copulation, as noted above; and I have data that point to the same conclusion. For example, nos. 298 and 314 yielded eggs in nearly the same stage of development 6 and 3½ days after copulation.

Recent data have however, convinced me that the post-oestrous period in the opossum is not five days but much less, possibly not usually much more than that of some Eutherian mammals. Long and Evans ('22) have shown that in the rat ovulation comes some hours after oestrus. A half dozen cases

may be cited from my protocols as illustrative of the evidence that has been accumulated on this point. 'Oestrus' has been diagnosed first by the observed copulation and second by the character of the vaginal smear.

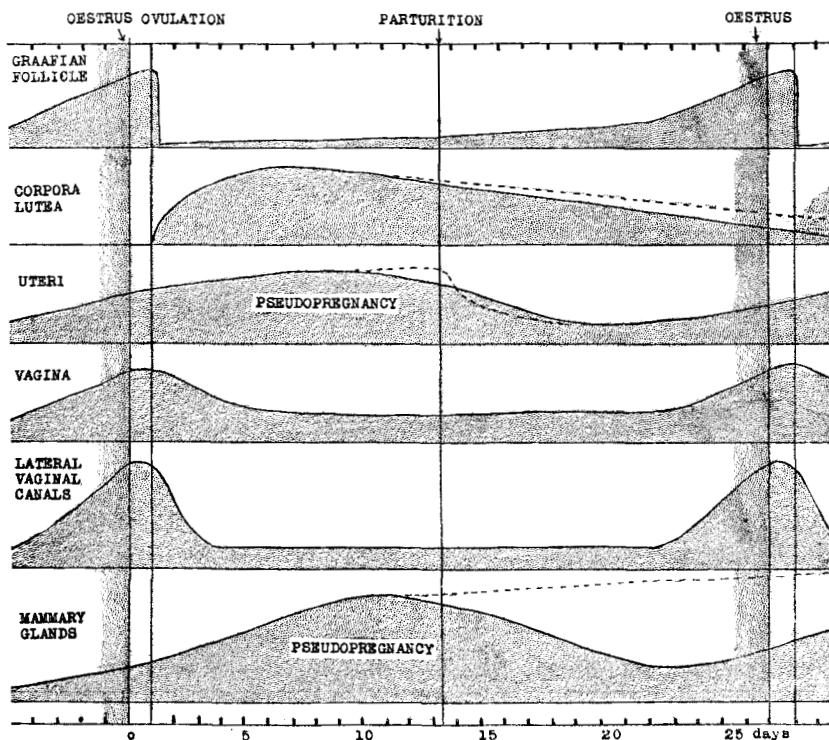


Chart 1

No. 821. Mar. 4, leucocytes and vacuolated epithelial cells; Mar. 6, cornified cells only; Mar. 7, slightly clumped cornified cells, unsegmented eggs in uterus.

No. 762. Jan. 22, smear typical of anoestrus, few cells, L. & E.; Jan. 25, almost pure culture of cornified cells; Jan. 26, ditto, uterine eggs unsegmented, slightly flattened.

No. 620. Apr. 5, mammary glands thickish, nucleated flat cells (nearly cornified) in sheets, indicating late prooestrus; Apr. 8, pure culture of cornified cells; mammary glands thick; young eggs, mostly four-celled.

No. 521. Received Jan. 21. Palpation of mammary glands indicated young eggs. 9:00 p.m., laparotomy: ripe follicles; killed forty-two hours later; smear showed a few leucocytes; young eggs in early stage of fragmentation.

No. 838. Pouch young born Jan. 10; these were lost and animal again in heat Feb. 17; Mar. 15, slight indication of oestrus. Mar. 28, vacuolated epithelial cells and leucocytes in smear. Apr. 1, pure culture of cornified cells, oestrus unmistakable; Apr. 8, 56 eggs (26 + 30) recovered, mostly normal bilaminar blastocysts.

No. 737. Jan. 24, copulation attempted; smear shows pure culture of cornified cells; Jan. 27, C, L and few E in smear; eggs with slightly opaque shell membrane.

No. 837. Jan. 22, 4:00 a.m., copulation attempted; Jan. 23, 3:00 p.m. young eggs found in uterus.

No. 842. Smear made at 9:30 p.m., Jan 21, showed pure culture of cornified cells (fig. 36, pl. 8); placed with male; copulation took place at 4:00 a.m., Jan. 22; sperms recovered. Killed 5:00 p.m. same day; still almost pure culture of cornified cells in smear, only a few leucocytes appearing. Tubal ova found (fig. 50, pl. 9).

These examples suffice to show that the postoestrous period is often very short, indeed at times certainly less than a day. Intimately connected with this problem is the next.

### *c. The tubal journey*

To determine this point an attempt was made to 'mark' one or more eggs. This was successfully accomplished by the injection of a suspension of *Ascaris* ova under the fimbriated end of the fallopian tube of an animal at oestrus. *Ascaris lumbricoides* worms from pigs were used; and to free the eggs from the tough gelatinous matrix in which they are imbedded, the worms were killed and allowed to decay. The eggs were then washed in frequent changes of Ringer's solution. Exactly 24 hours after the injection the animal was operated, the left uterus being removed. It contained uterine eggs. This tubal journey was therefore accomplished in 24 hours or less. After another interval of 16 hours the other uterus yielded eggs one of which had an *Ascaris* egg entangled in the meshes of the albumen. Since the eggs are always discharged from both ovaries at the same time and are always at the same stage, it is certain that the egg containing the *Ascaris* ovum made the journey in 24 hours or perhaps somewhat less.



The opossum therefore seems to hold the record on this point, for three days is the time required for the tubal journey in the rat (Long and Evans, '22), in the mouse (Allen, '23), and in the pig (Corner, '21).

*d. The length of the oestrous cycle*

Since 1915 the writer has followed many female opossums through successive oestrous cycles by the method of mammary palpation supplemented by direct observations on operated animals; and the impression was early gained that the cycle was very near a lunar month. More careful observation, especially by Stockard and Papanicolaou's method of diagnosis by examination of vaginal smears, has not demanded any revision of these figures—28 days is very near the average length of the cycle in the opossum.

As is to be expected, the length of the cycle varies greatly as it does in other mammals, for example the rat and the guinea-pig. It would indeed be surprising if a wild animal, subjected to the deleterious conditions of crowded cages, would show uniform regularity in its reproductive behavior. The gonads have been shown to be highly sensitive, notably by the careful work of Evans and Bishop ('22).

One of the commonest defects in the reproductive processes in the opossum is that of follicular atresia. Follicles may become nearly ripe before being attacked by degenerative changes or they may degenerate when half grown; in the former case, the vagina would show oestrous changes and oestrus would be recorded in the notes. Atresia may be continuous for some time and the animal then exhibit the dioestrous smear for many weeks, suddenly to surprise the experimenter by a sudden burst of activity resulting in ovulation. It is readily seen that such an animal would get a record for a long dioestrous period. Thus animal no. 578 had two normal cycles of 28 and 26 days respectively, followed by one of 45 days (March 18 to May 2). Animal no. 838 showed a pure culture of cornified cells in the vaginal smear February 17 and again April 1; on April 8, fifty-six eggs, mostly bilaminar blastocysts were removed. But between Feb.

17 and April 1, namely on March 16, the vaginal smear showed a great many cornified cells, indicating an abortive or abnormal oestrus.

The shortest cycles noted were several of 22 days; apparently normal cycles run up to 34 days or more. A half dozen examples must suffice:

No. 572. Jan. 24 and Feb. 20, ovulation was diagnosed by mammary palpation (27 day cycle); on Mar. 18 there was a preponderance of cornified cells in the smear (26 day cycle); Apr. 23 again; animal killed; ripe follicles (fig. 6, pl. 2); cycle 34 days.

No. 617. Feb. 18 (second oestrus of year) and Mar. 16, pure cultures of cornified cells—cycle 28 days. This animal was killed Apr. 23 in typical dioestrus 38 days after the preceding oestrus.

No. 662. Pouch young lost between Mar. 14 and 23. Mar. 29, pure culture of cornified cells; again Apr. 20 (cycle 22 days).

No. 781. Oestrus about Jan. 17. Feb. 13, almost pure culture of cornified cells (cycle about 27 days); Mar. 14, ditto (cycle 29 days).

Finally, the accompanying table (table 1), abbreviated from the record, is presented.

The foregoing cases establish the fact that the opossum is polyoestrous and that the cycle is about 28 days. Corroborative evidence is furnished by the following classes of observation:

1. Through operative procedure the left uterus is removed and the age of the unfertilized eggs which it contains is estimated. After a period of time the animal is killed and examined.
2. Oestrus is noted after pregnancy and abortion.
3. Oestrus is observed after removal of pouch young (Selenka, '87).

It is possible here to cite only a few cases:

No. 132. Mar. 9, eggs about 6 days old; Mar. 30, the surviving uterus contained 44 eggs perhaps 3 days old. Cycle about 25 days.

No. 112. Feb. 11 and Mar. 19, two batches of eggs, the latter the older by 3 or 4 days. Cycle 29 or 30 days.

No. 194. Jan. 24 eggs about 5 days old. Feb. 13, copulation (cycle about 25 days).

No. 611. Large embryos Feb. 2; Feb. 22, animal in prooestrus.

No. 644. Pouch young, born night of Feb. 9 to 10, removed next day; Feb. 20, mammary gland rather thin; Feb. 28, thicker; Mar. 7, abortive oestrus, perhaps case of atresia. Apr. 2, pure culture cornified cells. Apr. 5, eggs in early fragmentation stage.

No. 442. Feb. 1, pouch young about a day old removed; Feb. 16, pure culture cornified cells in vagina.

TABLE 1

NUMBER	DATE	MAMMARY GLAND	C	L	E <sup>10</sup>	REMARKS	
438	Jan. 27	Thickening	+	0	0	Oestrus (cf. fig. 40, pl. 8)	
	Jan. 27	Thicker	+	+	0		
	Feb. 1	Thick, firm	+	+	0	(30 days)	
	Feb. 8	Thick, firm	+	++	++		
	Feb. 14	Very thick	+	++	+vac.		
	Feb. 19	Flaccid	+	0	++vac.		
	Feb. 22	Flaccid	+	0	++vac.		
	Feb. 26	Firmer	++	0	0		Oestrus
439	Feb. 28	'Resting'	+	++	+	(27 days)	
	Feb. 29	Rather thicker	++	+	+		
	Feb. 30	Rather thicker	+	0	0		Oestrus
	Feb. 1	Thicker	++	0	0		
	Feb. 5	Thicker	+	+	+		
	Feb. 14	Very thick	+	+	++		
	Feb. 16	Thinner	+	+	++		
	Feb. 22	Thin	few	few	++vac.		Oestrus
Feb. 26	Thicker	++	0	0	Oestrus		
440	Jan. 26	Begins to thicken	+	few	0	(31 days)	
	Jan. 28	Distinctly thicker	++	0	0		Oestrus (cf. fig. 37, pl. 8)
	Jan. 30	Thin (?)	++	++	0		
	Feb. 2	Very thick	++	+	+		
	Feb. 4	Flabby	+lip.	+	+sheets		
	Feb. 13	Thinner	+	+	++		
	Feb. 22		few	few	0		
	Feb. 26		++	0	0		Oestrus
Mch. 5					Old eggs at least 7 days old		
441	Jan. 16		++	0	0	Oestrus	
	Jan. 25	Thick as in pregnancy	+lip.	0	0	Prolonged dioestrum	
	Jan. 27		+	+	+		
	Feb. 2	Thick	+	++	+		
	Feb. 10	Rather thin	+	+	+		
	Feb. 14	Thin	+	+	+vac.		
	Mch. 12	Resting	+	+	+vac.		No further change Feb. 14 to March 12

<sup>10</sup> C, cornified cells of vaginal smear; L, leucocytes; E, epithelial cells; Vac., vacuolated; Lip., lipid granules in cornified cells.

No. 453. Feb. 12, 24 mm. pouch young removed; Feb. 28, pure culture cornified cells; Mar. 2, eggs in early fragmentation stage, perhaps 2 or 3 days after ovulation.

No. 525a. Jan. 22 received. Feb. 4 pouch young born (6 attached); Feb. 20, one dead; Mar. 14, 5 pouch young in healthy condition; Mar. 16, pouch young lost, female in poor condition; Apr. 2, mammary gland again firm; Apr. 4, cornified cells, ripe follicles.

No. 265. Copulation Feb. 21, sperms recovered but eggs unfertilized (oestrus probably passed); Mar. 7, killed; organs including mammary glands in dioestrus.

No. 609. Feb. 2, eggs about 7 days old; Feb. 8, killed; animal in dioestrus.

No. 190. Jan. 22, animal in dioestrus; 19 days later, copulation observed (adding the periods indicated by Nos. 609 and 190, gives a total of 31 days for the approximate cycle).

#### *e. The phases of the cycle*

The terms prooestrus, oestrus, posteoestrus, pseudopregnancy, dioestrous interval are used in their generally accepted meanings (Heape, Hill, Long and Evans). The term metoestrus I have avoided using because of the phenomenon of pseudopregnancy which is so prominent in the opossum uterus and mammary gland after ovulation. The term might well be applied however, to the degeneration and phagocytosis of the opossum vagina (cf. Allen on the mouse).

In the next sections the microscopic changes in the uterus, the vagina, the lateral vaginal canals, and the mammary glands will be briefly described (chart 1).

#### *f. Cyclic changes in the uteri and the phenomena of pseudopregnancy*

*Anoestrus.* During anoestrus the uteri return practically to the virgin state (fig. 15, pl. 4). Both mucosa and muscularis are reduced, lymph spaces are as elsewhere in the body, the tissue is close-packed. The uterine glands are straight or little coiled (fig. 16, pl. 4) and their walls are made up of low columnar or cuboidal cells with large nuclei that nearly fill the cells. The lumen is very small and my preparations of this least interesting stage are inadequate to determine whether ciliation is present or not. The uterine epithelium is one-layered but the large nuclei are alternately placed.

*Prooestrus.* By the time the ovarian follicles have attained one-fourth their maximum size the uteri have undergone distinct changes. Their larger size is to be accounted for by the increase of elements, by increased vascularity and above all by infiltration of lymph into the mucosa. All these processes are progressive and continuous, beginning in early prooestrus, culminating some days after oestrus (plate 2).

The nuclei of the glands early manifest a tendency to migrate to the basal end of the cell opposite the lumen of the glands and to become denser and less vesicular. A few nuclei, however, can always be seen here and there nearer the lumen of the gland (fig. 24, pl. 5). These are probably the products of recent divisions. Cilia abound in the glands but there seems to be none on the uterine epithelium. As far as the preliminary study has shown, this holds throughout the series; but the matter will have to be submitted to experimental test. The opossum thus appears to agree with the pig as determined by Corner ('21). The epithelium also contains an increasing number of mitoses, thickens somewhat, and the nuclei begin to arrange themselves in three levels.

As seen in fig. 17, pl. 4, the mucosa begins to differentiate into a basal and a central layer. In the former the glands are more closely packed; in the central portion more lymph gathers, thus separating the glands from each other.

*Oestrus and ovulation.* The opossum uteri are considerably swollen (fig. 6, pl. 2) by the time oestrus sets in. The glands are already greatly coiled (fig. 17, pl. 4), especially at their basal portion. The lumen is somewhat enlarged and mitoses are very numerous. Many nuclei are to be seen scattered in the glands at levels below the peripheral row. Cilia are well developed (fig. 18, pl. 4). The epithelial lining has the nuclei in three levels. Large, thin walled blood vessels pervade the loose connective tissue and more capillaries are to be found under the epithelium.

*Early pregnancy or pseudopregnancy.* The first few days after ovulation witness a still greater increase in size and turgidity of the uterus. Sections show that this is due largely to the accumu-

lation of the fluid in the hypertrophied mucosa and observations on the living organ indicates that the fluid is under considerable pressure. The glands have also increased. Mitosis reaches its maximum rate when the eggs have reached about the cleavage stage (fig. 19, pl. 4). But the presence of fertilized eggs is without the slightest effect upon the uterus at this stage.

*Mid-pregnancy and pseudopregnancy.* We may tentatively place the early primitive streak stage at the end of the sixth day after ovulation. The uteri at this time are nearly maximal in size whether they contain vesicles with embryos or opaque, encrusted eggs. The two uteri are almost indistinguishable both macroscopically and microscopically. Fig. 13, pl. 3, may well be allowed to represent either as it appears everted in physiological saline solution; it is the usual picture at this stage. If care is taken to fix a fold of the mucosa spread out in its natural position the sections will give an excellent idea of the wide separation of the glands by lymph spaces, a condition already made clear through the artistic ability of Selenka ('87, plate XXVIII). Figure 20, plate 5, is from a pregnant, figure 21 from a pseudopregnant, uterus.

Mitoses still occur though in greatly reduced numbers. The small dense nuclei are basally placed (plate 5) in which position they will remain until involution sets in. In the epithelium there is a tendency for the nuclei also to assume a basal position (though this is not invariable at this stage) and to arrange themselves in two rows, leaving a free space next to the lumen of the uterus. This arrangement may be initiated at an earlier stage. It marks a transition towards the simple epithelium of late pregnancy.

*Late pregnancy and pseudopregnancy.* The condition of the glands and the uterine epithelium at this stage is well illustrated by the photographs shown on plates 5, 6, and 7.

The epithelium of the pregnant uterus is definitely one-layered, the nuclei are again large and vascular and occupy the middle of the cuboidal cells (fig. 22, pl. 5; fig. 27, pl. 6). These have rounded processes much like those described by Corner ('21) for the sow. Normally the chorionic ectoderm (fig. 26, pl. 6)

lies against the epithelium; fetal blood vessels with their nucleated erythrocytes are frequently seen in section and beneath the uterine epithelium are numerous maternal capillaries, small and large. A striking feature at this stage is the enormous size of some of the 'capillaries,' as large as veins but with almost purely endothelial walls.<sup>11</sup> In pseudopregnancy similar conditions are found although the vascularity and edema are less pronounced and collapse of the uterus soon follows.

By the twelfth day of pregnancy, or at parturition, the sub-epithelial region becomes infiltrated with clouds of leucocytes. One pseudopregnant specimen, no. 119, fifteen days after sterile coitus, also shows a considerable number of extravascular leucocytes. Towards the end of term the glands become greatly dilated, more especially at their central ends, i.e., towards the lumen of the organ and the cells tend to become nearly cuboidal in shape. It is difficult to demonstrate cilia in the glands at this stage.

*Involution after parturition.* After parturition the uterus rapidly returns to the resting condition. Transitional stages of the involuting uterus have not yet been prepared.

*Involution after pseudopregnancy.* Pseudopregnancy consists of changes simulating pregnancy, hence of changes in preparation for the nidation of the embryo. With a failure of fertilization degenerative changes or a kind of menstruation might be expected to set in. Hill noted degeneration of the mucosa in *Dasyurus* together with extravasation of blood.

In the opossum the mucosa is greatly collapsed by the withdrawal of lymph, a process which is hastened by ovariectomy. Both the epithelium and the glands undergo degeneration.

The epithelium seems to thicken with the first collapse, containing several layers of cells. Vacuolated cells and cells with pycnotic nuclei occur, also normal cells and even cells in mitosis-regenerative and degenerative processes side by side.

The glands of the dioestrous uterus consist definitely of low columnar to cuboidal cells and the lumen is filled with cellular

<sup>11</sup> This observation is of interest in view of Krogh's discovery of the capillario-dilator action of pituitrin and its possible relation to the mechanism of parturition.

material which is often made up of normal or only slightly degenerating cells (figs. 30 and 31, pl. 6).

What is the origin of these cells?

In a somewhat earlier stage, e.g., no. 119 (fig. 29, pl. 6), many nuclei may be seen occupying the central region of the tall columnar cells, whereas the great majority are normally placed at the basal end. Later, namely after the collapse of the uterus, one finds the gland wall consisting of cuboidal cells, which contain the former basal nuclei, while within the dilated lumen are seen the cell masses. One gains the impression that the central ends of the cells next to the lumen are cut off and thus extruded. Since some cytoplasm is also cut off, the process differs from the extrusion of nuclei from the oviduct of the mouse as described by Allen. Both processes are degenerative.

The included cells seem to disappear in a few days. In no. 265, fifteen days after copulation they have already disappeared and the glands have attained the reconstruction preparatory to the next prooestrus. Notwithstanding this case, however, which was one of sterile and therefore probably late coitus, many other cases indicate that twenty or more days is the minimum of time usually required for the involution and regeneration of the pseudopregnant uterus.

*g. Cyclic changes in the vagina and the vaginal contents*

*Anoestrus.* During the resting period (December) the epithelial lining is relatively thin, about five to six cells deep, and there is no cellular debris in the lumen (fig. 44, pl. 9). The cells are low columnar at the base, grading off to a single layer of flat cells lining the lumen.

*Prooestrus.* With the resumption of sexual activity in January and at the end of each dioestrous interval the vagina grows in diameter and in thickness of epithelium until in late prooestrus the mucosa is at least twelve to fifteen cells deep and may be much thicker in places (fig. 46, pl. 9). Mitoses are scattered throughout the stratum germinativum. The basal half of the epithelium is made up of columnar cells which are close-set and regular next to the submucosa. This layer is surmounted by



polygonal cells which grade off into the flat cells of the central half of the epithelium. These become more and more flattened toward the lumen of the organ. The flattening of the cells increases as oestrus advances. There is usually no difference in the staining reaction between the 'stratum corneum,' that is the portion which is destined to slough off, and the underlying layers, nor are these layers separated by a 'stratum granulosum' which is entirely absent except for a slight intimation of such a layer here and there. It is more usual that the limit of the stratum corneum is indicated by a lighter, more vacuolated layer. Usually only toward the end of prooestrus do the cells of the stratum corneum lose their nuclei and hence appear lighter in the sections (fig. 45, pl. 9).

At least during the latter part of prooestrus no leucocytes are seen in the epithelium. The vaginal smear contains large flat nucleated cells in considerable numbers as oestrus approaches.

At each succeeding prooestrous period of the year the vaginal mucosa (unlike that of the lateral vaginal canals, v.i.) behaves as at the first prooestrus.

*Oestrus and ovulation.* At oestrus the nucleated epithelial cells are replaced in the vaginal smear by large flat non-nucleated cells (true cornified cells.) The stage is indicated in my protocols as "pure culture of cornified cells" (figs. 36 to 39, pl. 8).

Sections of the vagina at this stage disclose the source of these cells: they are seen to arise from the stratum corneum (figs. 47 to 49, pl. 9). At first the cells come off singly and appear discrete in the smear (fig. 36, pl. 8); later they are clumped (fig. 39, pl. 8) as Allen found to be the case in the mouse just after oestrus. Later still the lining of the organ undergoes more massive desquamation, indeed at times this process appears as a veritable molt (fig. 53, pl. 10) as it does also in other mammals, e.g., in the guinea-pig.

This picture has been seen in many cases; hence there is not the slightest doubt that the opossum agrees with the Eutheria thus far studied in the absence of epithelial cells and leucocytes at the oestrous period. The fact that in the several opossums furnishing tubal ova (fig. 49, pl. 9) the smear still exhibited the same picture

might be given as further evidence of the shortness of the 'post-oestrous' period.

But the picture is not always as clear-cut as just described.

In the first place, it has been found that in atresia of the ovarian follicles there is an interference in the normal cycle which is reflected in the vaginal smear. If the follicles degenerate while medium sized or smaller, there is a continued dioestrous smear containing vacuolated epithelial cells. But if the follicles reach maturity the vaginal changes are more or less clear-cut. The involution of the vagina proceeds whether the follicles rupture or not. Thus in no. 578 a the vagina was in 'metoestrus,' the mucosa being laden with leucocytes (quite comparable to Allen's fig. 12); but the follicles were intact though slightly cloudy in appearance. They had failed to dehisce at the normal time and were beginning to degenerate, but the vaginal changes went on nevertheless.

A second factor tending to obscure the picture is the secretion of the cloacal glands. These glands, situated at either side of the cloaca, open on papillae near the cloacal orifice (fig. 74, pl. 13). They discharge a greenish, 'gangrenous' secretion which on microscopic examination is seen to be entirely made up of bodies that look like leucocytes. They doubtless are leucocytes and the secretion constitutes a veritable 'physiological pus.' I am convinced that this often finds its way into the vagina. One day the smear of a given animal will contain only a few epithelial cells and leucocytes; the next day the spatula may remove from the organ great masses of the secretion from these glands. Thus the source of the vaginal contents shown in figure 57, plate 11, is almost certainly not the vaginal mucosa, but the cloacal glands.

*Postovulatory changes (metoestrus).* These changes may be illustrated by examples of the pregnant or the non-pregnant animal, for no differences have been noted in the two classes of animals; nor does ovariectomy appear to hasten the normally rapid-deline of the organs.

For a day after ovulation the vaginal smear may remain little changed. Thus the smear shown in figure 36, plate 8, was photographed fresh at 9:30 p.m., January 21st. The animal was then

placed with a male and copulated at 4:00 a.m. the next morning. The smear contained chiefly cornified cells and a very few leucocytes at 3:00 p.m., January 23rd—fewer than those shown in figure 40, plate 8. The corpora lutea had increased considerably in size and the eggs had arrived in the uterus.

Within three or four days, however, the desquamation of the vagina may be completed (fig. 54, pl. 10). In the meantime leucocytes have been filtering through the epithelium and have collected in the superficial layers or penetrated into the lumen (fig. 52, pl. 10). The vaginal smear is now made up of leucocytes and cornified cells (fig. 40, pl. 8). We may adapt the terminology of Long and Evans and call this the C and L stage.

By the fourth or fifth day the nucleated epithelial cells may again have made their appearance in the smear, and in sections of the vagina they are found to be superficial. The cornified cells gradually decrease in number and many of them contain lipid granules of various sizes (fig. 41, pl. 8). These cells are never entirely absent from the smear except in anoestrus and in prolonged dioestrus. The L and E stage is at its height about the eighth day (fig. 55, pl. 10), but a considerable number of leucocytes may be encountered in preparations of animals killed twelve to fifteen days after oestrus.

*The dioestrous interval.* This period is marked by a monotonous smear of a few epithelial cells, many of which are vacuolated, an occasional leucocyte, and still more rarely a remaining cornified cell. The origin of the leucocytes is uncertain, since these are hard to find in the epithelium. Figures 58 and 60, plate 11, represent the vaginal mucosa at twenty days after oestrus. It presents a clean surface epithelium, which is six to seven cells deep, much like the condition in anoestrus (fig. 44, pl. 9).

*Pregnancy.* During pregnancy the vaginal changes are quite comparable to those occurring at corresponding times after oestrus in the non-pregnant animal. Leucocytes may be numerous present until near term (fig. 56, pl. 10); but from birth on the vaginal mucosa assumes a typical dioestrous condition. Lactation usually inhibits the recurrence of oestrus, hence also the return of concomitant changes in the reproductive

organs. Pregnancy cannot be diagnosed by the vaginal smear; this discloses only the approximate time after oestrus and ovulation.

*h. The lateral vaginal canals*

*Prepuberty.* In the rat according to Long and Evans ('22) "in immature animals the lumen of the vagina does not extend to the exterior, but is closed by what appears to be a thick membrane," etc. A similar membrane is present in the guinea-pig, where it regenerates more or less completely at each oestrous cycle (Stockard and Papanicolaou, '19).

Somewhat different is the condition in the lateral vaginal canals of the opossum. No membrane has been discovered in the vagina nor in the lateral vaginal canals: but the latter appear not to communicate with the vagina until puberty. In several preparations they are not patulous posteriorly, the epithelium being solid in that region. Only during the first prooestrus does the lumen become continuous by a slit in the mucosa, the process being much like the opening of the eyelids, for example. This matter will be the subject for a more detailed investigation.

*Anoestrus.* The epithelium of the lateral vaginal canals during anoestrus resembles that of the vagina, though it is somewhat thinner, being three to four cells deep. A layer of flat epithelial cells is superficial. There is no indication of the vast desquamation of material characteristic of later periods. The surface is clean; there is no cellular debris in the lumen (fig. 61, pl. 11).

*Prooestrus.* In discussing prooestrus it is necessary to distinguish between the first prooestrus of the year and that of succeeding cycles. For in January the organ begins its activity from the stage described above, whereas during the dioestrous interval it lacks sufficient time to rid itself of its molted layers before another cycle begins. In other words, throughout the breeding season the lateral vaginal canal appears to be actively sloughing off its cornified layer as a continuous, not as a rhythmic process (fig. 63; pl. 11). At best the dioestrous wave is a shallow one.

As the canals distend in prooestrus the epithelium thickens as in the vagina. The cornified stage is reached earlier than in the vagina, in fact by the time the ovarian follicles are of medium size. By this time there is already established a typical stratum granulosum which is often very pronounced (fig. 64, pl. 11). In this particular, it will be noticed the lateral vaginal canals of the opossum resemble the vagina of higher mammals. The stratum granulosum early limits the stratum corneum, which takes on a light orange stain with orange G (fig. 62, pl. 11). It is usually non-nucleated. In only a single specimen (fig. 65, pl. 11) is there a light layer separating two nucleated layers.

*Oestrus and ovulation.* The desquamation begins in prooestrus and continues progressively until at ovulation it is well under way and there is a variable amount of debris within the lumen of the organ. The cornified layer is well differentiated (fig. 62, pl. 11).

*Postovulatory changes.* In five to six days after oestrus the amount of debris is already considerable, as may be seen in figure 64, plate 11, which is taken during the first oestrous cycle of the year. It is important, as implied above, in all stages to differentiate between the first and succeeding cycles. Thus figure 72, plate 13, is from an animal killed soon after ovulation. But the canals are full of debris since this animal was killed April 5, in the third observed cycle of the year. The first oestrus of this animal had resulted in pregnancy and the young were removed when a day old. But even after the first ovulation of the year one may sometimes find very great desquamation several days after oestrus, as shown by specimen no. 521 (fig. 73, pl. 13). Figure 67, plate 12 is taken from a pregnant animal near term. The epithelium beneath the granular layer is seen to be only three to four cells deep. The lumen is fairly well filled with the sloughed-off cornified cells.

It thus happens that the canals become filled with masses of cheesy material which attain their greatest dimensions some twelve to fifteen or more days after ovulation. In figure 35, plate 7, a section through the canals of no. 609 is shown at low magnification. The lumen is all but filled with the cheesy exfoliation of the cornified layers which lie especially between the

trabeculae of the organ. An enlargement of a portion of this section is shown in figure 70, plate 12, which illustrates another point of considerable interest in connection with the question: What becomes of the cornified debris of the canals?

When first sloughed off the cornified layers are neatly laminated and for some time preserve a semblance of this arrangement. But later the mass behaves as though it were digested, since it becomes more fluid and structureless. The transition is seen in figures 68 and 70, plate 12. The study of a smear made of this mass discloses the fact that motile bacilli are active within it, doubtless serving to disintegrate the cells to facilitate their removal. The odor of the cheesy mass is similar to that found in the rat during stage four where bacteria also abound. Long and Evans attribute the dissolution of the cornified mass to the leucocytes ('22, l. c., p. 21): "The leucocytes cause a softening of the granular masses seen in stage three and convert them into a substance of a cheesy, creamy and increasingly fluid consistency." In the lateral vaginal canals of the opossum there are never many leucocytes and their appearance I have not been able to correlate with any special stage (e.g., fig. 69, pl. 12). It is, therefore, apparent that bacteria play an important rôle in the physiology of the lateral vaginal canals (perhaps also of the vagina of Eutheria).

Desquamation continues after parturition. Figure 71, plate 12, is from an animal with pouch young three weeks old. The granular layer is visible, the epithelium is somewhat thickened and the cornified layer is still present though greatly reduced.

In the pseudopregnant animal, another cycle has begun before much reduction in the contents of the canals has taken place. Nevertheless, the organ swells and becomes gorged with its liquid contents. The presence of the bacteria does not seem to interfere with the progress or viability of the spermatozoa, for pregnancy may ensue at any oestrous period.

It is thus seen that the lateral vaginal canals differ from the vagina in their behavior during the dioestrous cycle. As to the homology of the lateral vaginal canals, I am not informed; from their structure and behavior they belong rather to the vagina than to the uterus or to the cervical canals.

*i. Changes in the mammary glands: pseudopregnancy*

In a previous section a method of diagnosis of cyclic events in the female opossum by palpation of the mammary glands was described. It has been of interest to discover what is the cause of the thickening of the glands in prooestrus: whether this is due merely to edema and increased vascularity, to growth of the sebaceous glands and sweat glands, or to actual hyperplasia of the glandular tissue itself. The pseudopregnant hypertrophy of the mammary glands, referred to by Hill and O'Donoghue ('13) as involving 'the most striking changes' of the pseudopregnant period, has also been investigated.

*Anoestrus.* Sections of the skin containing the mammary glands in anoestrus show a few acini scattered between the fat and the epidermis. The walls of the acini of all adult organs in the resting stage are one-layered, not two-layered, as described by O'Donoghue for *Dasyurus*. The two- and three-layered condition does not set in until about oestrus as a result of active mitosis.

*Prooestrus to oestrus and ovulation.* Mitoses are encountered in considerable numbers in the mammary gland some days before oestrus, when the ovarian follicles are of medium size, that is, somewhat protruding from the surface of the ovary (fig. 77, pl. 14). It is, therefore, certain that in the opossum as in the rat (Sutter, '19) proliferative activity begins before there is a corpus luteum in the ovary. Other changes which result in a swelling of the organ begin even before this, at least a week before oestrus. But the study of microscopic sections offers little help in explaining the growth of turgidity which is so readily palpable in the living animal.

By oestrus and ovulation the gland is already in a very active state. The alveoli are much more numerous and mitoses are seen in almost every section of each alveolus. Figure 81, plate 14, is taken from an animal furnishing tubal ova. Three alveoli in the center of the figure show at least ten mitotic figures in this section. Proliferation is well under way and in some specimens there is a tendency to crowding of the cells so that in places the

alveolar wall is two- and three-layered. But the stratified condition is quite transitory.

Cell proliferation continues (fig. 78, pl. 14) throughout the period of gestation. The maximum growth period by cell division I have not yet determined. In an animal having pouch young five days old cell division has all but ceased. In one with three weeks' old pouch young no mitotic figures could be found. The increasing thickness of the glands after parturition is doubtless due to dilatation of the acini, as O'Donoghue found in *Dasyurus*. In the opossum the dilatation begins before parturition (figs. 79 and 80, pl. 14) and continues as the pouch young grow and make demand upon it. The lactiferous tubules finally become very thin-walled.

As in *Dasyurus* (O'Donoghue, '11) the sweat glands and the sebaceous glands greatly hypertrophy as pregnancy (or pseudopregnancy) advances. The details agree very well with those described by O'Donoghue for the native cat.

The changes just outlined for the pregnant animal hold for the pseudopregnant one as well. For perhaps ten days the glands of the latter are indistinguishable from the former (figs. 79 and 80, pl. 14, and figs. 75 and 76, pl. 13). Mitoses are numerous during this period and the acini dilate considerably. By the thirteenth day, however, the turgidity has definitely lessened and the gland is best described as 'flabby' to the touch. By the twentieth day the tubules have become scattered and greatly reduced in number, and the gland is ready for another prooestrus.

The new prooestrus sets in with the gland better developed than in the first prooestrus following anoestrus; for in the former the new cycle sets in before the large lactiferous tubules have time fully to retrogress. Hence new acini grow by proliferation from the rather large old acini of the previous pseudopregnant growth. In anoestrus the gland is more nearly in the resting stage than in any, except an excessively long, dioestrous interval.

Further details need to be determined in the behavior of the mammary gland of the opossum. For the present it is established that active hyperplasia is well under way by the time



ovulation occurs. The opossum agrees with the Australian native cat in the striking development of the organ out of pregnancy, so striking as to deserve the designation given it by Hill and O'Donoghue, that of 'pseudopregnancy.'

#### DISCUSSION

1. In the present work the opossum is definitely proved to be polyoestrous. This holds true of the animal in the wild state, as shown by observations on females that have accidentally lost their pouch young; and in captivity this wild mammal has been observed to pass through definite if somewhat variable dioestrous cycles, involving all the reproductive organs in rhythmic changes.

The suspicion has been expressed in this paper that the Australian forms, including *Dasyurus* studied by Hill and his students, may likewise be found to be polyoestrous upon detailed observation covering long periods. On this point both O'Donoghue ('13) and Hill ('18) preserved an open mind.

The polyoestrous condition therefore is probably the primitive one, the monoestrous condition, if such exists, having arisen secondarily.

2. Successive pregnancies (probably two only in one year) or successive dioestrous periods of about a lunar month each, are followed by an anoestrous or rest period of some two months' duration. During this time (which always includes all of the month of December at Austin, Texas) the female organs return to the infantile or virgin condition, to be stimulated into renewed activity at the beginning of the new breeding season. This fact renders the animal more useful for certain experimental purposes than the more constantly active breeders like the rabbit, guinea-pig and other laboratory mammals.

3. With this study the opossum has been brought into line with the rat, guinea-pig, pig, mouse, and other domestic mammals; for the cyclic changes in ovary, uterus, vagina, and mammary glands are in every way comparable in the two groups, opossum and the higher mammals. The marsupials are, therefore, brought nearer the Eutheria, and the fact emphasized that fundamentally in their physiological relations the two groups are quite homogeneous.

4. The opossum ovary undergoes the same rhythmic changes as that of other mammals. The follicles increase to a maximum at oestrus, ovulation takes place spontaneously, the corpora lutea form after ovulation, reach their full structural and functional development, and degenerate. In the extent of this degeneration during the dioestrous interval the opossum exceeds all other mammals with which I am acquainted, for the corpora of one ovulation are very nearly obliterated before another batch of follicles develops—a condition which L. Loeb would consider optimal for the growth and dehiscence of a new crop of follicles. But the surprising thing, from Loeb's point of view, is the great length of the cycle in view of the rapid degeneration of the corpora.

5. It is interesting to note the changes which the other reproductive organs undergo *pari passu* with the growth of the graafian follicles. These correlative changes may be taken in at a glance by reference to chart 1. No other mammal exhibits such definite changes in all the organs before ovulation. This is probably an adaptation correlated with the short period of gestation in marsupials—about twelve days as compared with sixty-seven, for example, in the guinea-pig.

In the uterus of the rat Long and Evans have described vascular engorgement together with great swelling of the organ due to accumulated liquid within the lumen. Allen records similar distention and hyperaemia in the mouse. These are indeed striking signs of prooestrus. The prooestral turgescence is also decidedly characteristic of the opossum uterus, where, however, lymph collects and remains within the uterine mucosa (fig. 6, pl. 2; fig. 13, pl. 3). Coupled with this edema there appears long before oestrus a very considerable cellular proliferation of the uterine glands and epithelium.

Macroscopically the most striking of all the changes in any organ during the oestrous cycle is the swelling of the lateral vaginal canals (fig. 6, pl. 2). With ovulation the rapid subsidence begins; hence the organ behaves like the rat uterus and the function of the swelling is doubtless to facilitate the passage of spermatozoa into the uterus. The vagina also swells and its

mucosa performs cyclic ecdyses of the cornified layer much like the other mammals thus far studied.

The mammary glands of the rat undergo proliferation of the tubules, that is, a genuine hyperplasia of the organ, during prooestrus (Sutter, '21). In the guinea-pig Loeb and Hesselberg, ('17) find proliferating glands in all animals at the time of heat; the proliferation decreases to the resting stage for five days after ovulation. Hence there is no semblance of pseudopregnant phenomena in the guinea-pig during its oestrous cycle. The opossum outdoes all Eutheria in the dioestrous swelling and subsidence of the mammary glands.

6. As has been established for all mammals so the prooestrous growth of the organs in the opossum is under the control of the ovary. Ovariectomy completely snuffs out the oestrous cycle, as I have observed many times. What element of the ovary constitutes the source of stimuli that lead to prooestrus and to oestrus?

The opossum affords an unequivocal answer which is in full accord with the clear and succinct statements by both Allen ('22) and Robinson ('18) in which they make out a case for the graafian follicles. Their reasons I consider conclusive. On this point the opossum has the charm of simplicity; the evidence it offers is of the most direct kind.

At the first prooestrus of the breeding season and indeed at each succeeding period the corpora have all but disappeared. For other reasons also the corpora cannot be seriously considered; for else where would the first oestrus of puberty find its explanation? As to the interstitial gland in the opossum it hardly deserves the name. It is of course true that there are large cells filled with lipoid granules within the stroma of the opossum ovary; and Lillie's analysis of the free martin ('23) has indeed heightened one's respect for the power of an incertion elaborated by even a vanishingly minute aggregation of cells. But no cyclic changes have been noted in the interstitial cells, so called, and the same holds true of other stroma cells. But the follicles are prominent structures, strictly correlated in their growth with rhythmic changes in the other organs of generation (chart 1).

Corroborative evidence was pointed out by Loeb and Hesselberg to the effect that hypotypical ovaries are incapable of initiating prooestrous changes, a fact that I have observed in scores of opossums. Nor is the effect due to deciduomata—they do not occur in the opossum; nor to placentae—they too are absent; nor to embryos, for pseudopregnant changes simulate those of pregnancy. Nor is the uterus responsible, for I have removed both uteri and one ovary frequently and still noted a return of oestrus with great proliferation of the mammary glands.

I therefore agree with Loeb and Hesselberg in the following statement (l. c., p. 302):<sup>12</sup>

Another constituent, namely, in all probability the follicular apparatus or part of it, is responsible for the occurrence of heat, ovulation, and the proliferation of the mammary gland in the early period of the sexual cycle.

7. It is highly probable that the corpora lutea continue the work begun by their precursors, the graafian follicles. In the opossum the bulk of the corpus luteum arises out of granulosa cells; they are perhaps simply more actively secreting granulosa cells. Our opossum material gives abundant evidence that in case of failure of ovulation the atretic follicles may cause a profound stimulating effect on uteri and mammary glands so that pseudopregnancy of considerable intensity results as normally with corpora lutea. Full data on this point will be presented later.

8. The phenomena of pseudopregnancy are decidedly manifest in the marsupials. Uteri and mammary glands of pregnant and non-pregnant animals are indistinguishable. Which condition obtains in a given case can only be decided by examination of the contents of the uteri. This phenomenon may be associated with the high development of the corpora lutea, for in marsupials the terms corpora lutea vera and corpora lutea spuria have no significance.

<sup>12</sup> However, the statements of these authors in the summary following this quotation (pp. 303-4) leave me completely mystified. I have been unable to harmonize the apparent discordancies.

The relation of corpora lutea to pseudopregnancy is supported by the experiments of Ancel and Bouin ('10) who produced essentially the condition of pseudopregnancy in the rabbit by means of sterile coitus with a vasectomized male. Recently Long and Evans verified these experiments in the rat and they made the further remarkable discovery that simple mechanical stimulation of the cervix uteri lengthened the oestrous cycle from four to ten or more days and caused persistence of the corpus luteum together with the usual phenomena attending pseudopregnancy (Freyer and Evans, '23).

Pseudopregnancy is thus manifested by both Eutheria and marsupial mammals. Marsupials differ in the spontaneity and in the exaggerated form of the phenomenon.

9. In the Eutheria ovulation may take place within the oestrous period (pig); or there may be a delay in ovulation after copulation, as in the guinea-pig, rat, mouse. Hill, Selenka, and the present writer believed that in marsupials this refractory period was variable and prolonged but recent observations on the opossum seems to indicate that, normally and usually, ovulation is not far removed from oestrus. This new data again brings the opossum a little nearer the Eutheria. But the fact remains that ovulation is an independent phenomenon, bearing no hard and fast time relation to oestrus, much less to the act of copulation.

10. Pseudopregnancy is followed by degeneration of the uterus, involving both glands and epithelium. This degeneration accompanies degeneration of the corpora lutea, is perhaps dependent upon it. This phenomenon is universal among mammals, both Metatheria and Eutheria. The primates doubtless should be included, for menstruation and the metoestrous uterine degeneration of mammals are probably homologous (Evans, '22).

11. Lactation inhibits ovulation as a general rule in the opossum as in the rat (Long and Evans, '22) and other mammals. Ovaries of lactating animals may be described as hypotypical like those of an animal palpably decrepit (fig. 11, pl. 3). Correlated with such ovaries all organs are in the resting stage, save only the mammary glands. It seems to matter little if several

only or if thirteen young occupy the teats. It should be stated incidentally that only the occupied, that is the suckled, glands remain active, all others of the same pouch return to the resting stage. Every female opossum having a vacant teat is nature's own experiment in the effect of nursing on the activity of the mammary gland.

Now the corpora lutea degenerate soon after parturition; in four or five weeks they have all but disappeared. Yet the growth of the graafian follicles is usually most thoroughly inhibited to the end of lactation (fig. 11, pl. 3). But if the pouch young are removed ovulation takes place in vigorous females ten to fifteen days later. The corpora lutea are unequivocally not necessary for the inhibition of ovulation during lactation in the opossum.

12. The lateral vaginal canals are distinctly marsupial structures. Their homology is not clear. In their desquamation of cornified cells they are structurally and physiologically like the vagina; but after the first prooestrus they differ from the latter in that they take little part in the dioestrous rhythm other than the oestrous swelling. They undergo little or no degeneration or leucocytosis of the mucosa in metoestrus. The contents of cornified debris undergoes dissolution through bacterial action rather than through phagocytosis.

Before puberty the lining of the lateral vaginal canals is a solid plug devoid of lumen; hence the uterus and the canals are shut off from communication with the outside. At puberty a slit appears in the epithelium and the organ becomes patulous for the first time.

13. The shell membrane of the marsupial egg has been regarded as a useless vestigial structure. A possible function of the shell membrane in gestation was suggested by the writer ('20 b). In the lower end of the fallopian tube are glands which the writer tentatively homologizes with similar glands of reptiles responsible for the fibrous shell membrane of the reptilian egg. The structure of these glands and the possible oestrous changes which they may undergo have not yet been studied in detail.

## SUMMARY

1. The opossum is polyoestrous, both in its natural habitat and in captivity. Reasons are advanced for considering the polyoestrous condition primitive, the monoestrous condition secondary.

2. As may be expected of a wild animal in captivity the oestrous cycles are variable and at times modified by follicular atresia which prevents the normal spontaneous ovulation.

3. Most females, however, pass through definite dioestrous cycles which average twenty-eight days in length.

4. Broadly speaking the cyclic events are in every way comparable with those described for the guinea-pig, rat, mouse, and pig, hence the Metatheria are brought into line with the Eutheria. Cyclic changes in the reproductive organs are, therefore, of very ancient origin.

5. The ovary, uterus, lateral vaginal canals, median vaginal canal (vagina) and mammary glands and to some extent the oviducts were studied in their cyclic changes.

6. The corpora lutea ovulationis and the corpora lutea gravidatis are indistinguishable except that the latter persist somewhat longer. The corpora of pregnancy begin to degenerate at parturition. If pregnancy and lactation do not follow ovulation the corpora lutea are reduced to a mere scar before the next ovulation. There is never more than one set of corpora lutea in a given animal at the same time.

7. The eggs make the tubal journey in twenty-four hours or a little less, receiving in this time much albumen and the shell membrane.

8. Shell membrane glands, perhaps homologous of those found in the Sauropsida, were discovered in the lower portion of the opossum oviduct.

9. It has been established that the activity of the uteri and the mammary glands begins some days before ovulation, hence without the influence of a corpus luteum. This applies to hyperplasia as well as to hypertrophy of these organs. The initial stimulus is furnished by the graafian follicles and is continued by their derivatives, corpora lutea or corpora atretica.

10. 'Pseudopregnant' growth of the uterus and especially of the mammary glands is described and shown to be as striking in the opossum as in the Australian *Dasyurus*. While it is true that pseudopregnancy may be induced experimentally in rodents and may occur at times in other mammals such as the bitch and the sow, yet it seems probable that the spontaneous and excessive pseudopregnant growth of the uteri and the mammary glands in the Marsupalia constitutes a class character.

11. The pseudopregnant growth of the mammary glands, which begins before ovulation, may be recognized by palpation and hence forms a fairly accurate external sign of internal conditions.

12. Certain advantages of marsupials for experimental purposes are pointed out.

13. Histological changes in the uterine epithelium and glands and the parallelism of such changes in pregnancy and pseudopregnancy are described.

14. Degeneration of the uterine mucosa occurs at the end of pseudopregnancy and is homologous with the metoestrous degeneration of the uterine epithelium in other mammals and therefore to menstruation in the primates.

15. The vagina exhibits cyclic histological changes in every way comparable to similar changes in the rodents thus far studied.

16. The changes in the vaginal epithelium may be followed in the living animal by the microscopic examination of its contents, which varies in strict correlation with changes in the ovaries and other reproductive organs. The vaginal smear method of diagnosis is as applicable to the opossum as it is to the guinea-pig, the rat, and the mouse.

17. Oestrus is characterized by a pure culture of cornified cells in the vaginal content; in late prooestrus there is an unmixed mass of large flat nucleated epithelial cells originating from the vaginal epithelium.

18. During massive desquamation of the vaginal wall excessive leucocytosis develops by infiltration of leucocytes through the vaginal epithelium.

19. The lateral vaginal canals are marsupial organs; their cyclic behavior is here given for the first time.



20. The canals remain closed posteriorly until puberty as does the vagina of rodents. It becomes patent by the appearance of a lumen within the epithelial layer.

21. During anoestrus the lateral vaginal canals like the vagina are lined with a clean epithelium. Cornification begins earlier than in the vagina and continues throughout the breeding season. There is a granular layer beneath the cornified layer.

22. Exfoliation of the mucosa is excessive and a thick cheesy mass soon fills the lumen. This cellular debris is disintegrated by bacterial action. Few leucocytes if any are present in these organs.

23. General conclusions deduced from a study of the reproductive cycle in the opossum are given in the Discussion.

#### LITERATURE CITED

- ALLEN, EDGAR 1922 The oestrous cycle in the mouse. *Am. Jour. Anat.*, vol. 30, pp. 297-324.
- BOULIN, P., AND ANCEL, P. 1910 Recherches sur les fonctions de corps jaune gestatif. I. Sur le déterminisme de la preparation de l'uterus a la fixation de l'oeuf. *J. de la Physiol. et de la Path. gén.*, T. 12, pp. 1-16. 1911 Recherches, etc. II. Sur le déterminisme de la glande mammaire au cours de la gestation. *J. de la Physiol. et de la Path. gén.*, T. 13, pp. 31-41.
- CORNER, G. W. 1921 Cyclic changes in the ovaries and uterus of the sow, and their relation to the mechanism of implantation. Publication no. 276, Carnegie Institution of Washington, pp. 117-146.
- CORNER, G. W., AND AMSBAUGH, A. E. 1917 Oestrus and ovulation in swine. *Anat. Rec.*, vol. 12, pp. 287-291.
- EVANS, HERBERT M. 1922 The rhythm of gonadal function with special reference to the relation between uterus and ovary in Barker, Hoskins and Mosenthal: *Endocrinology and metabolism*, New York, vol. 2, p. 580.
- EVANS, H. M., AND BISHOP, KATHERINE 1922 a On the existence of a hitherto unknown dietary factor essential for reproduction. *Am. Journ. Physiol.*, vol. 63, pp. 396-7; *Science*, vol. 56, pp. 650-1. 1922 b On the relations between fertilization and nutrition. I. The ovulation rhythm in the rat on a nutritional regime. II. The ovulation rhythm in the rat on inadequate nutritional regimes. *Journ. Metabolic Research*, vol. 1, Feb. and March.
- FREYER, M. E. 1921 On the cause of the effects produced by stimulation of the cervical canals in the rat. *Anat. Rec.*, vol. 21, p. 57.
- FREYER, MARY E., AND EVANS, HERBERT M. 1923 Participation of the mammary gland in the changes of pseudopregnancy in the rat. *Anat. Rec.*, vol. 25, p. 108.

- HARTMAN, CARL G. 1916 Studies in the development of the opossum, *Didelphys virginiana* L. I. History of the early cleavage. II. Formation of the blastocyst. *Jour. Morph.*, vol. 27, pp. 1-83.
- 1919 Studies etc., III. Description of new material on maturation, cleavage and entoderm formation. IV. The bilaminar blastocyst. *Jour. Morph.*, vol. 32, pp. 1-142. V. The phenomena of parturition. *Anat. Rec.*, vol. 19, pp. 1-11.
- 1920 The free martin and its reciprocal: opossum, man, dog. *Science*, vol. 52, pp. 469-471.
- 1921 a Dioestrous changes in the mammary gland of the opossum and the diagnosis of pregnancy. *Am. Journ. Physiol.*, vol. 55, pp. 308-309.
- 1921 b Traditional belief concerning the generation of the opossum. *Journ. Am. Folklore*, vol. 34, pp. 321-323.
- 1923 a Sterility of animals under changed conditions. *Anat. Rec.*, vol. 24, p. 394.
- 1923 b Relation of the ovary to the gravid uterus in the aplacental opossum. *Am. Journ. Physiol.*, vol. 63, p. 23.
- 1923 c Breeding habits, development, and birth of the opossum (illustrated). In the appendix to the 1921 Report of the Secretary of the Smithsonian Institution, Washington (in press).
- HEAPE, WALTER 1900 The sexual season of mammals and the relation of pro-oestrus to menstruation. *Quart. Journ. Micr. Sci.*, vol. 44, pp. 1-70.
- HILL, J. P. 1900 On the foetal membranes, placentation and parturition of the native cat (*Dasyurus viverrinus*). *Anat. Anz.*, Bd. 18, s. 364-373.
- 1910 The early development of the Marsupalia with special reference to the native cat (*Dasyurus viverrinus*). *Quart. Journ. Micr. Sci.*, vol. 56, pp. 1-134.
- HILL, J. P., AND O'DONOGHUE, CHARLES H. 1913 The reproductive cycle in the marsupial *Dasyurus viverrinus*. *Quart. Journ. Micr. Sci.*, vol. 59, pp. 133-174.
- LOEB, L. 1911 The cyclic changes in the ovary of the guinea-pig. *Jour. Morph.*, vol. 22, pp. 37-70.
- 1914 The correlation between cyclic changes in the uterus and the ovaries in the guinea-pig. *Biol. Bull.*, vol. 27, pp. 1-44.
- 1917 The relation of the ovary to the uterus and mammary gland from the experimental standpoint. *Trans. Am. Gyneec. Soc.*,
- LOEB, L., AND HESSELBERG, CORA 1917 The cyclic changes in the mammary gland under normal and pathological conditions. I. The changes in the non-pregnant guinea-pig. II. The changes in the pregnant guinea-pig, the effect of lutein injections and the correlation between the cycle of the uterus and ovaries and the cycle of the mammary glands. *Journ. Exp. Med.*, vol. 25, pp. 285-321.
- LOEB, L., AND KURAMITSU, CHOIZU 1921 The influence of lactation on the sexual cycle in the rat and guinea-pig. *Am. Journ. Physiol.*, vol. 55, pp. 443-449.
- LONG, J. A., AND EVANS, H. M. 1922 The oestrous cycle in the rat and its associated phenomena. *Memoirs of the University of California*, vol. 6, pp. 1-148.

- O'DONOGHUE, C. H. 1911 The growth changes in the mammary apparatus of *Dasyurus* and the relation of the corpora lutea thereto. *Quart. Journ. Micr. Sci.*, vol. 57, pp. 187-234.
- 1912 The corpus luteum in the non-pregnant *Dasyurus* and polyovular follicles in *Dasyurus*. *Anat. Anz.*, Bd. 41, S. 353-368.
- 1914 Ueber die corpora lutea bei einigen Beuteltieren. *Arch. f. Mikr. Anat.*, Bd. 84, Abt. II, S. 1-48.
- PETRUNKEVITCH, ALEXANDER 1920 Standardized microphotography. *Anat. Rec.*, vol. 19, pp. 289-307.
- ROBINSON, ARTHUR 1918 The formation, rupture and closure of ovarian follicles in ferrets and ferret-polecat hybrids, and some associated phenomena. *Trans. Roy. Soc. of Edinburgh*, vol. 52, pp. 303-362.
- SANDES, F. P. 1903 The corpus luteum of *Dasyurus viverrinus* with observations on the growth and atrophy of the graafian follicle. *Proc. Linnæan Soc. N. S. Wales*, vol. 28, pp. 364-405.
- SELENKA, EMIL 1887 Studien über die Entwicklungsgeschichte der Thiere. 4. Heft. Das Opossum, Wiesbaden.
- STOCKARD, C. R., AND PAPANICOLAOU, G. N. 1917 The existence of a typical oestrous cycle in the guinea pig with a study of its histological and physiological changes. *Am. Jour. Anat.*, vol. 22, pp. 225-284.
- 1919 The vaginal closure membrane, copulation, and the vaginal plug in the guinea-pig with further considerations of the oestrous rhythm. *Biol. Bull.*, vol. 37, pp. 222-245.
- SUTTER, M. 1919 On the behavior of the mammary epithelial cell toward vital dyes in various functional epochs of its life cycle. *Anat. Rec.*, vol. 16, pp. 164-5.
- 1921 Cyclic changes in the mammary gland of the rat associated with the oestrous cycle. *Anat. Rec.*, vol. 21, p. 59.

PLATE 1

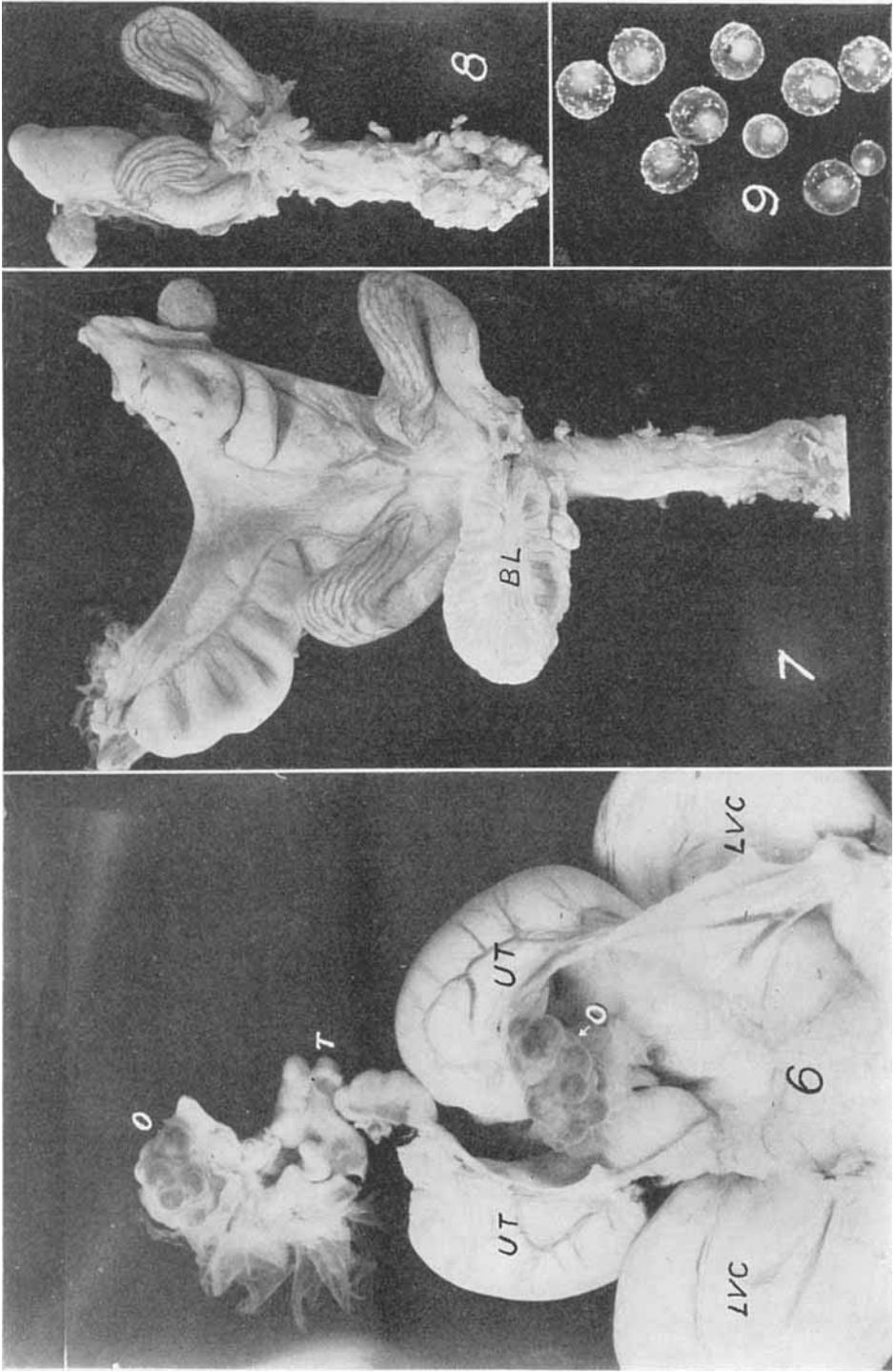
*Figures 1 to 5 have been omitted*

---

PLATE 2

EXPLANATION OF FIGURES

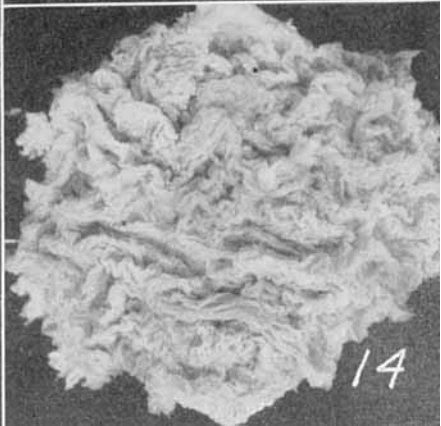
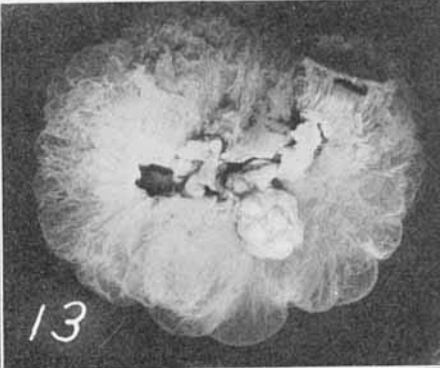
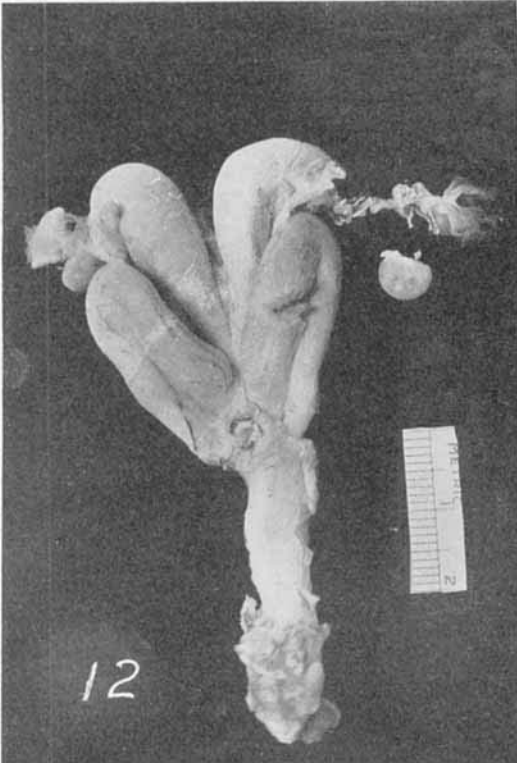
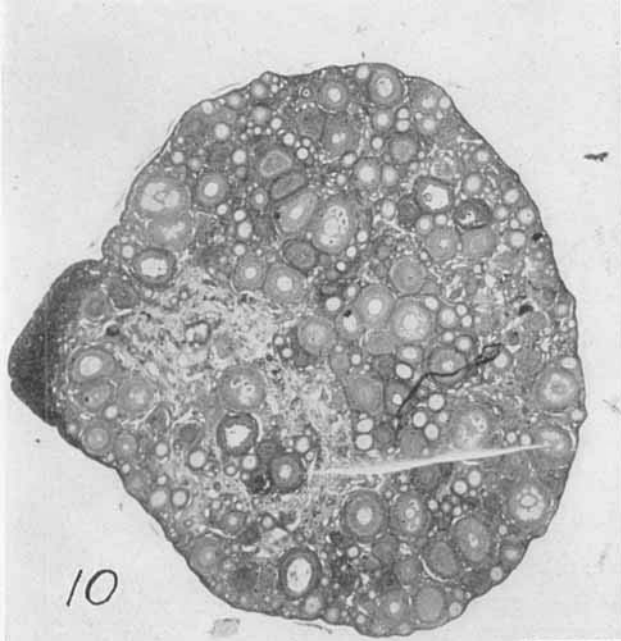
- 6 Genital tract of animal at oestrus; ripe follicles in ovary (*O*), oviduct (*T*) dilated; uterus (*U*) considerably advanced in development; *LVC*, lateral vaginal canals greatly swollen.
- 7 Genital tract and bladder (*BL*) of pseudopregnant animal no. 322, Jan. 25; uteri collapsed; degenerating corpora lutea sunk beneath surface of ovary; lateral vaginal canals shrunken, the longitudinal trabeculae showing through the wall. Photograph by Dr. C. H. Heuser.  $\times 1$ .
- 8 Genital tract of pseudopregnant animal no. 609'; about thirteen days after oestrus. Surviving uterus and ovary much reduced in size. Lateral vaginal canals show corrugations due to trabeculae within (cf. fig. 35, pl. 7).  $\times 1$ .
- 9 Opaque eggs removed from uterus shown above (no. 609').  $\times 8$ .



### PLATE 3

#### EXPLANATION OF FIGURES

- 10 Section through ovary no. 78, Jan. 17; typical condition in anoestrus.  $\times 16$ .
- 11 Genital tract typical of the condition in anoestrus or during lactation; this specimen is from no. 671 which had pouch young weighing 7 grams.  $\times 1$ .
- 12 Genital tract of animal no. 638, Feb. 22, twenty days after oestrus. Sections of vagina are shown in figs. 58 and 60, pl. 11.  $\times 1$ .
- 13 Uterus and ovary of pseudopregnant animal no. 427, Feb. 22. After the uterus was slit open, the musculature contracted, completely everting mucosa, which is seen to be loose and fluffy. The lymph pockets under epithelium are well shown (cf. fig. 21, pl. 5). Photograph by Dr. C. H. Heuser.  $\times 1$ .
- 14 Collapsed mucosa of pregnant animal no. 390' after abortion near term. Photograph by Dr. C. H. Heuser.  $\times 1$ .

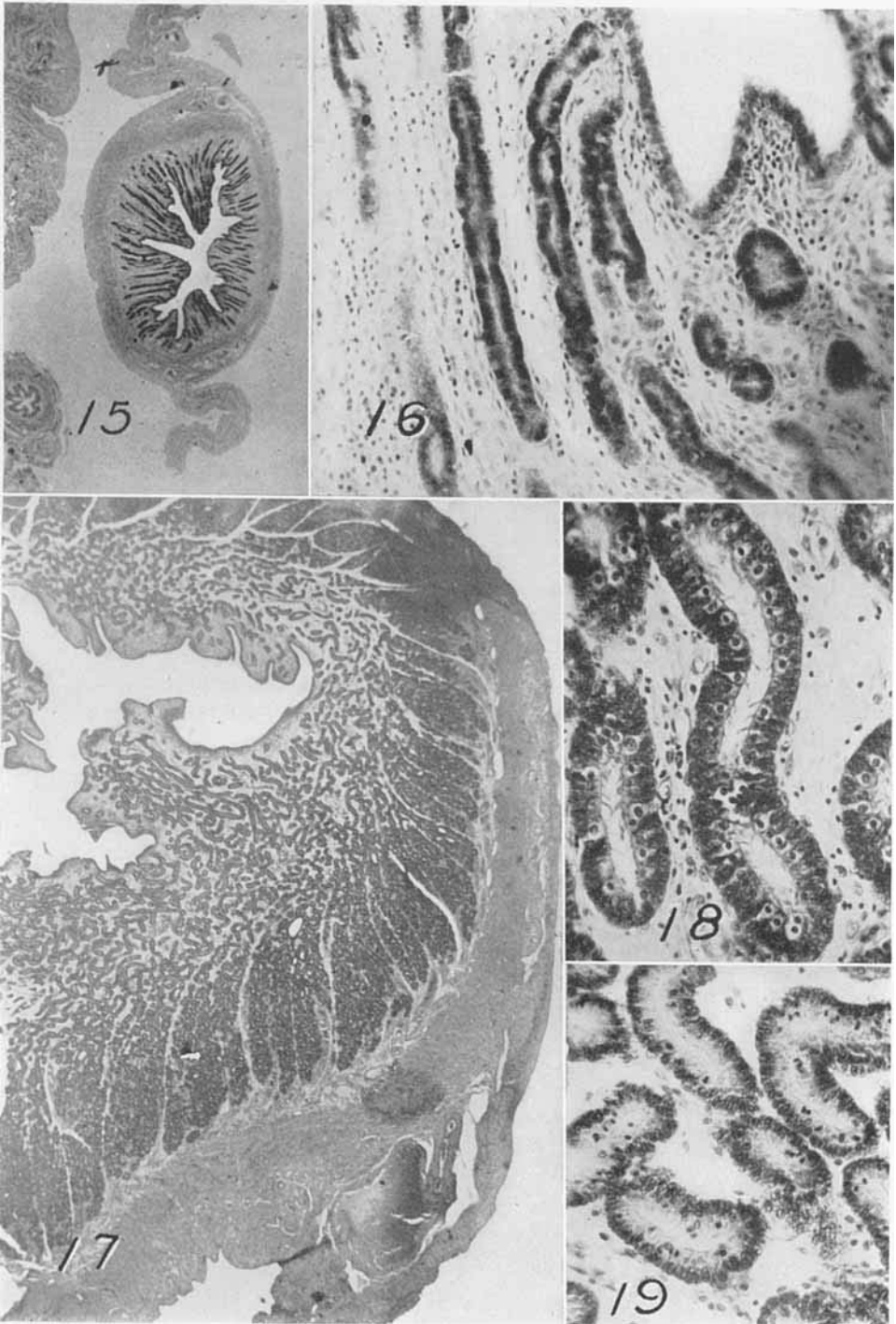


## PLATE 4

### EXPLANATION OF FIGURES

- 15 Section through uterus of no. 275 a, small female, November.  $\times 16$ .  
16 Portion of figure 15, showing uterine glands and epithelium.  $\times 200$ .  
17 Uterus of animal no. 578 a, May 2, ovarian follicles maximum, slightly cloudy. The much coiled glands in basal layer of mucosa are well shown. (Cf. Selenka, 1887, fig. 2, pl. XXVIII.)  $\times 16$ .  
18 Uterine glands of no. 541, showing cilia; oestrus, April 30.  $\times 200$ .  
19 Uterine glands of no. 81, Jan. 19; eggs in cleavage; note great number of mitoses.  $\times 200$ .

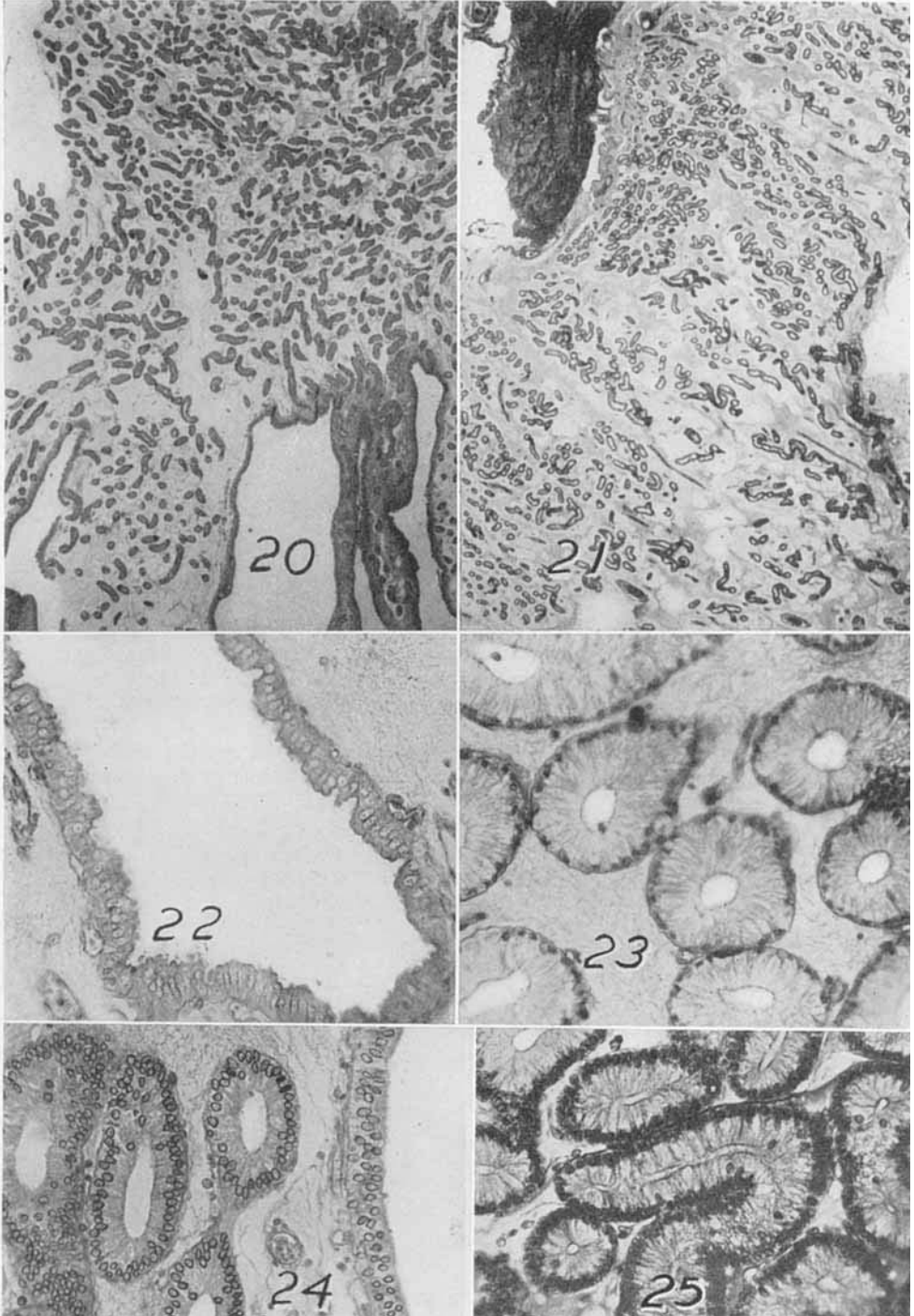




## PLATE 5

### EXPLANATION OF FIGURES

- 20 Section through uterine glands of pregnant animal no. 211', Feb. 2; eggs in early primitive streak stage (cf. fig. 13, pl. 3).  $\times 16$ .
- 21 Similar to figure 20, but from pseudopregnant animal no. 236'; Feb. 16.  $\times 16$ .
- 22 Uterine epithelium of no. 134, ten days after copulation; young embryos with 3 to 4 mm. allantois. The nuclei have just arranged themselves in a single layer.  $\times 100$ .
- 23 Glands from no. 134; the nuclei are nearly all basally situated.  $\times 200$ .
- 24 Glands and epithelium from uterus of no. 114, nine days postcoitum, embryos of about 11 somites, Feb. 15. (Nuclei have been emphasized with ink.)  $\times 200$ .
- 25 Glands from pregnant uterus of no. 544', embryos several days of term (7 days after early vesicular stage like the one shown in fig. 1, pl. 7, Hartman, '19). Cf. uterine epithelium of same uterus, figure 27, plate 6.  $\times 200$ .



## PLATE 6

### EXPLANATION OF FIGURES

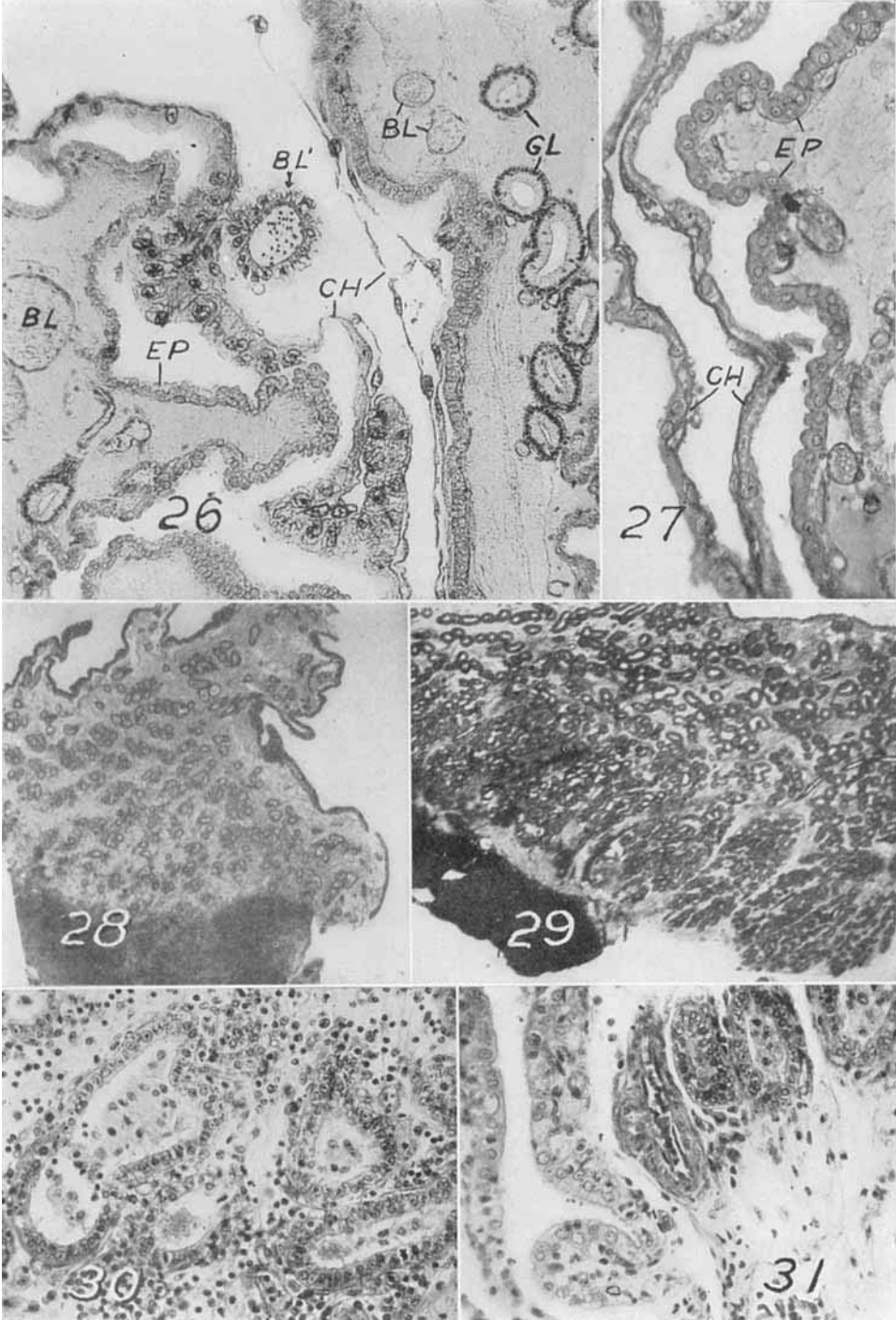
26 Portion of section shown in figure 32, plate 7; *BL*, maternal blood vessels; *BL'*, embryonic blood vessels; *CH*, chorion; *GL*, glands; *EP*, epithelium, better shown in figure 27.  $\times 100$ .

27 Fold of chorion and uterine epithelium of no. 544; embryos several days of term (cf. fig. 25, pl. 5).  $\times 200$ .

28 Portion of uterus no. 117', March 17; glands are much scattered, reconstruction not yet having taken place since the last pseudopregnancy, although the uterus already contained numerous (very small) eggs in cleavage.  $\times 16$ .

29 Portion of uterus no. 119, similar to that shown in figure 7, plate 2; Feb. 22; uterus collapsed, glands undergoing degeneration.  $\times 16$ .

30 and 31 Uterine glands of nos. 117 and 123, respectively, showing cellular debris within glands; in figure 31 the degenerating epithelium is also shown.  $\times 200$ .



## PLATE 7

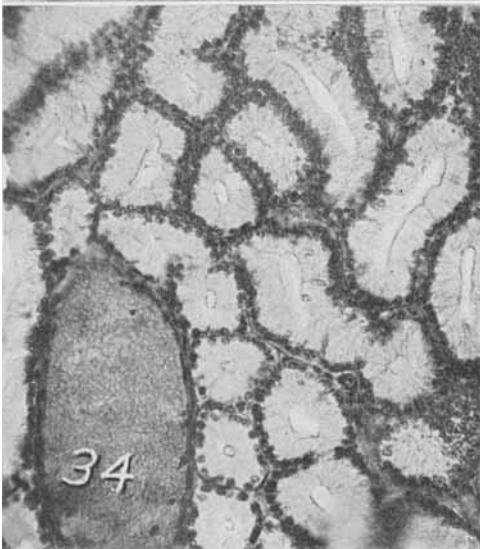
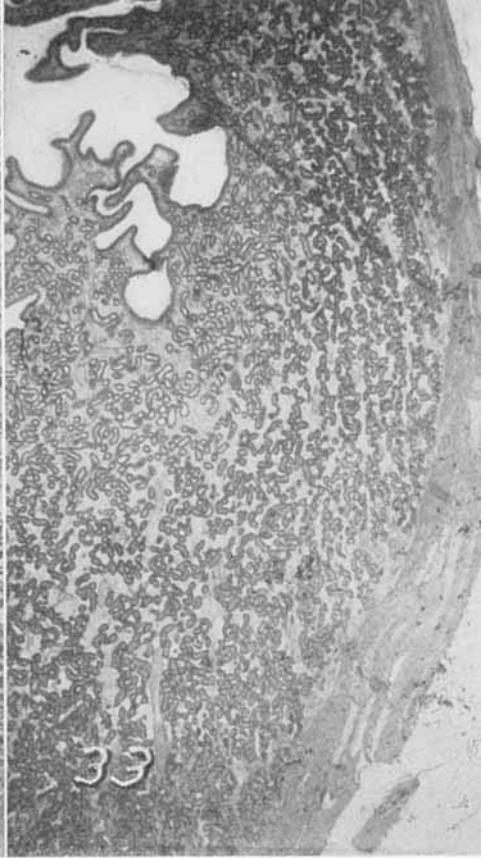
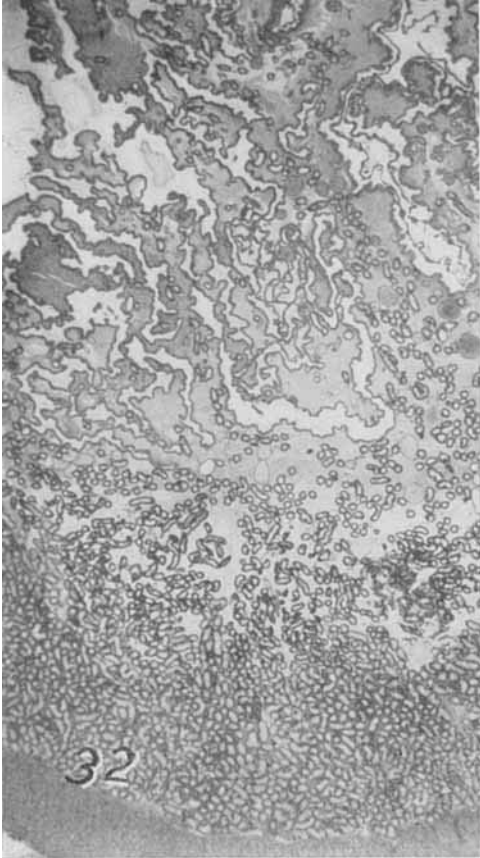
### EXPLANATION OF FIGURES

32 Segment of uterus no. 841', Jan. 29, eight days, fifteen hours after cleavage, when left uterus and right ovary were removed; glands closely packed at base, but more loosely scattered through lymph spaces centrally, where chorion and folds of uterus interdigitate (cf. Selenka, 1887, fig. 1, pl. XXVIII).  $\times 16$ .

33 Uterus no. 644 in early pseudopregnancy, April 5.  $\times 16$ .

34 Glands of no. 554', seven to eight days pseudopregnant; nuclei occupy the extreme base of cells, the two rows of nuclei of abutting glands almost blending into one. *BL*, blood vessel.

35 Section through the lateral vaginal canal no. 609' shown in figure 8, plate 2.  $\times 16$ . For detail see fig. 70, pl. 12.



## PLATE 8

### EXPLANATION OF FIGURES

- 36 Vaginal smear of no. 837, 9:30 p.m., Jan. 21, 6½ hours before copulation; pure culture of cornified cells photographed fresh in Ringer's solution. × 100.
- 37 A similar stage photographed from a stained specimen no. 440, Jan. 28 (table 1).
- 38 Same as figure 36, more highly magnified.
- 39 Cornified cells clumped; photographed from stained specimen no. 500; eggs just arrived in uterus.
- 40 C and L stage, no. 438, Jan. 28, two days after oestrus. × 100.
- 41 Epithelial cells and one cornified cell with lipoid granules (outlines in ink).
- 42 C, L, and E stage, from stained specimen.
- 43 L and E stage; epithelial cells somewhat vacuolated.



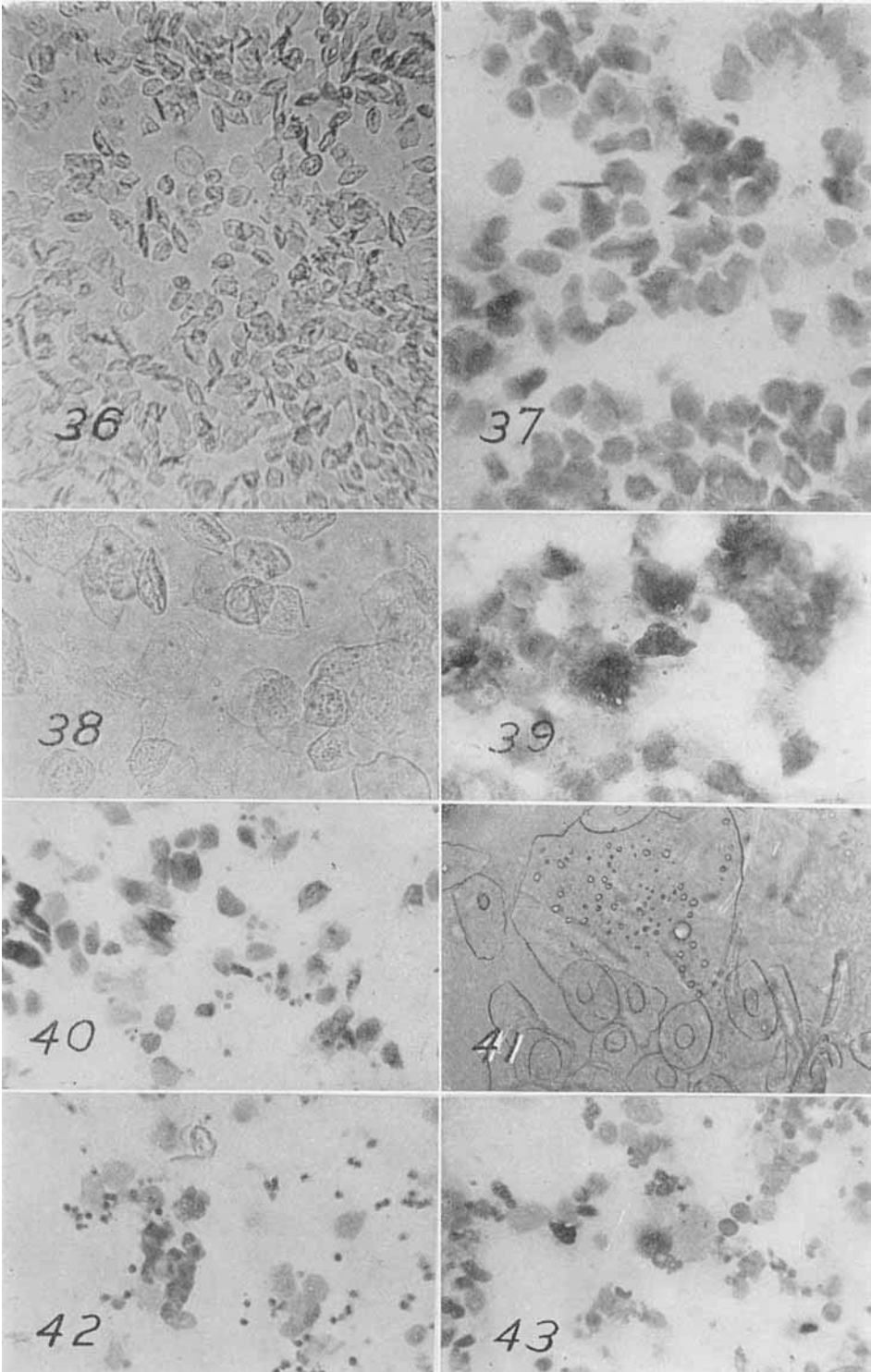


PLATE 9

EXPLANATION OF FIGURES

Sections through the vagina

- 44 No. 516, Jan. 20; earliest prooestrus.  $\times 200$ .  
45 No. 478, March 7; prooestrus; ovarian follicles large but not ripe. The future cornified layer is indicated.  
46 No. 700, Feb. 22; late prooestrus, near oestrus, large, flat nucleated cells at surface.  $\times 200$ .  
47 No. 833, Jan. 22; somewhat tangential section; near oestrus.  $\times 200$ .  
48 No. 541, oestrus, April 30; considerable amount of cornified material lies in lumen of organ.  $\times 200$ .  
49 No. 842, about the same as figure 48, but eggs had arrived in fallopian tube (cf. fig. 50).  $\times 200$ .  
50 Tubal ova from oviduct of no. 842, Jan. 22.  $\times 100$ .

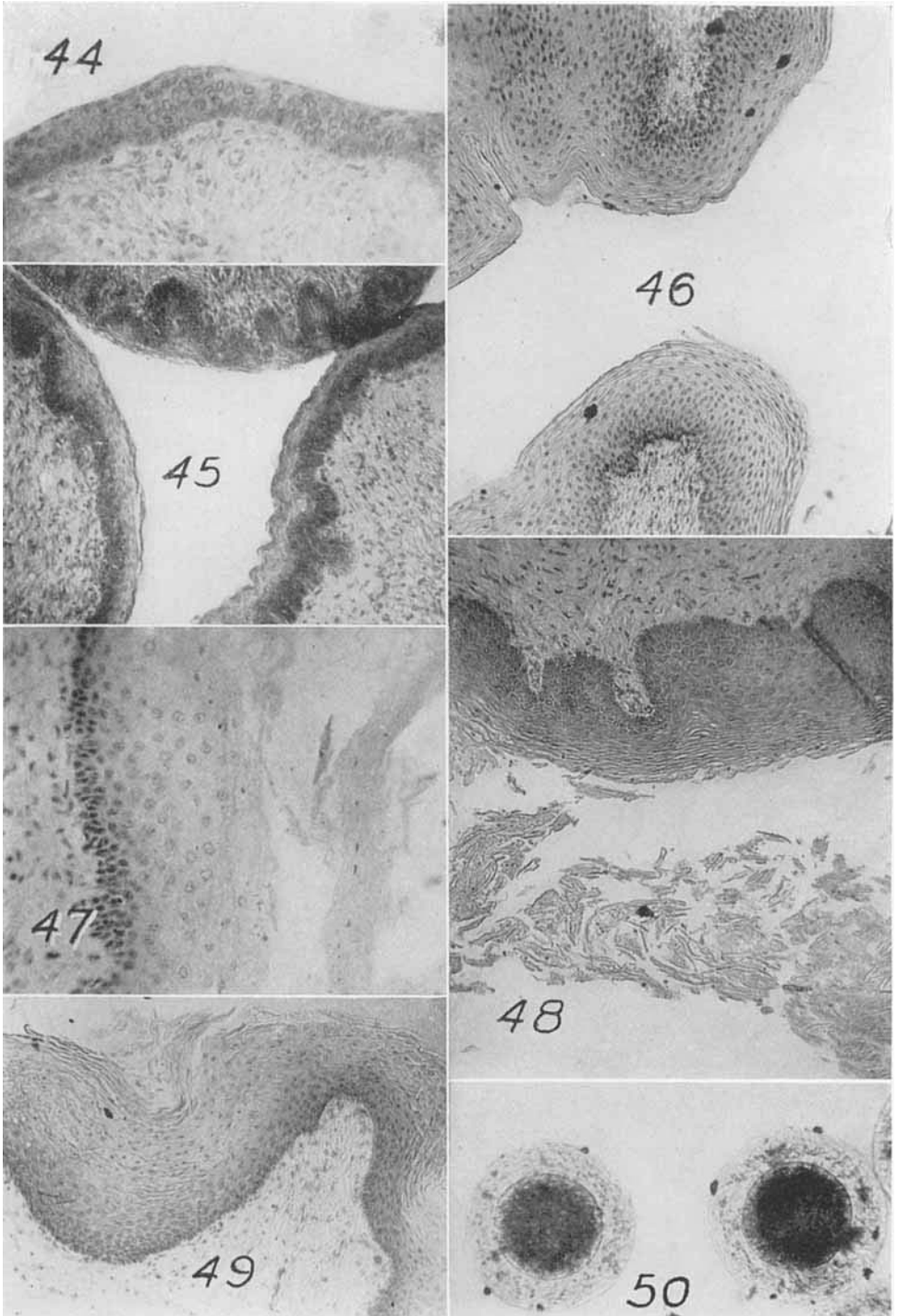
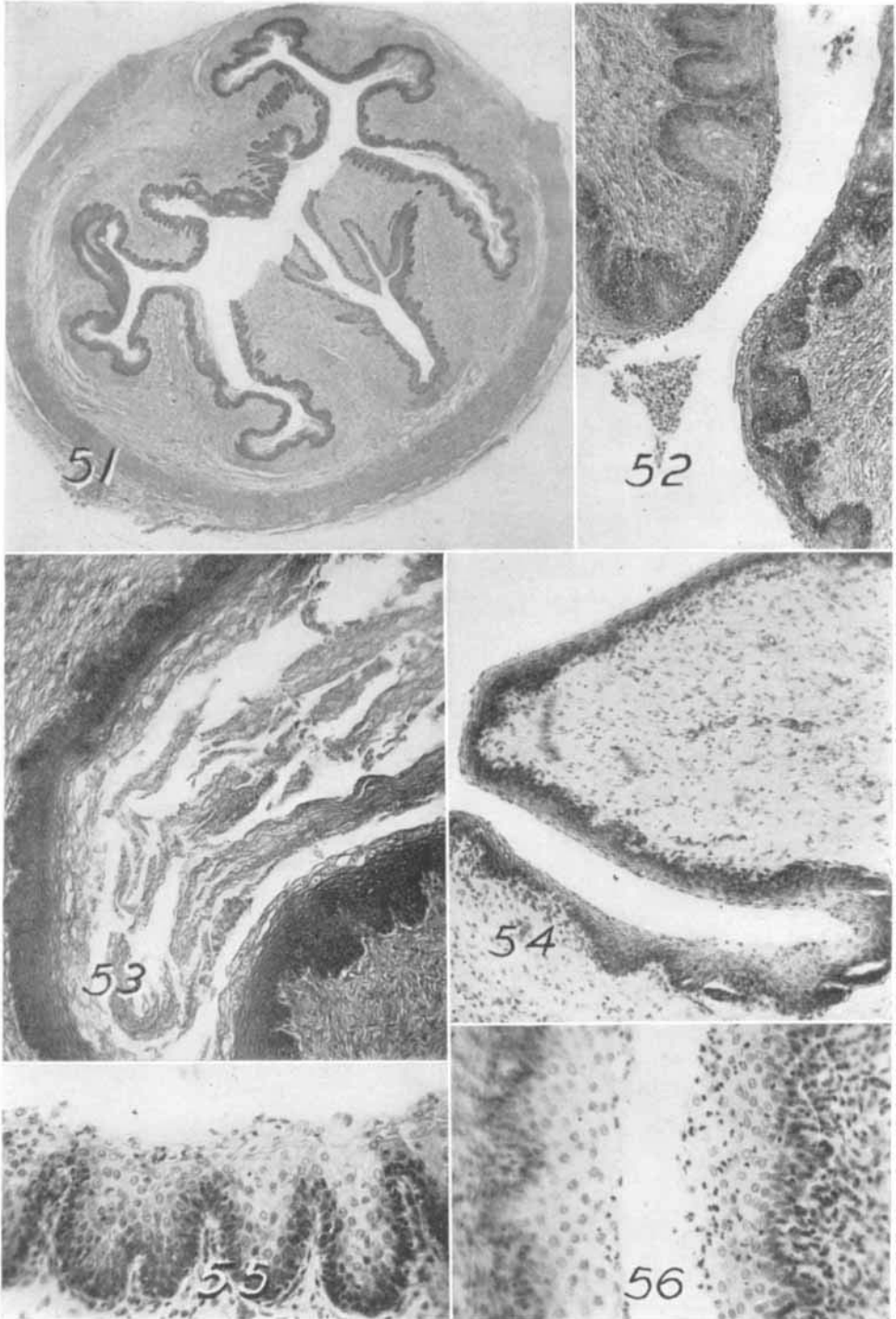


PLATE 10

EXPLANATION OF FIGURES

Sections through the vagina

- 51 Whole section of vagina no. 626; tubal ova, March 9. Cf. figure 1, plate 1, and figure 58, plate 11.  $\times 16$ .
- 52 No. 644, April 3, several days after oestrus, eggs in early stage of fragmentation; leucocytosis of mucosa; cornified layer gone.  $\times 200$ .
- 53 Illustrates great desquamation of cornified layer immediately after oestrus.  $\times 200$ .
- 54 No. 737. about four days after oestrus. History: Jan. 20, epithelial cells in smear; Jan. 24, copulation probable, cornified cells, somewhat clumped; Jan. 27, killed. L and E stage.  $\times 100$ .
- 55 No. 552', March 24; L and E stage. The leucocytes are the black dots near the surface; fragmented eggs about five days old in uterus.  $\times 200$ .
- 56 No. 841, late pregnancy (cf. fig. 32, pl. 7). L and E stage.  $\times 200$ .



## PLATE 11

### EXPLANATION OF FIGURES

Figures 59 to 65 are sections of lateral vaginal canals

57 Section of vagina of no. 791, Jan. 30, twelve days after oestrus. L and E stage. Leucocytes in lumen probably originate mostly from the cloacal glands.  $\times 100$ .

58 Section of vagina of no. 638, shown in figure 12, plate 3; cf. figure 60.  $\times 16$ .

59 Lateral vaginal canal of no. 548, 850 gram animal, Jan. 22, just before reaching puberty. Canal patent in center only (completely closed posteriorly).  $\times 16$ .

60 Portion of figure 58.  $\times 100$ .

61 No. 516, earliest prooestrus; mucosa of canals still in the anoestrous condition (cf. fig. 44, pl. 9).  $\times 200$ .

62 No. 842 stage of tubal ova. Cf. figure 49, plate 9. Cornified layer clearly differentiated.  $\times 200$ .

63 No. 147', prooestrus, April 3. Note cornified layer; the animal was killed in the second prooestrus of the year.

64 No. 564 late prooestrus, second of the year, Feb. 26. Note granular layer staining dark with Delafield's haematoxylin.  $\times 200$ .

65 No. 556, oestrus just passed, March 16.  $\times 100$ .

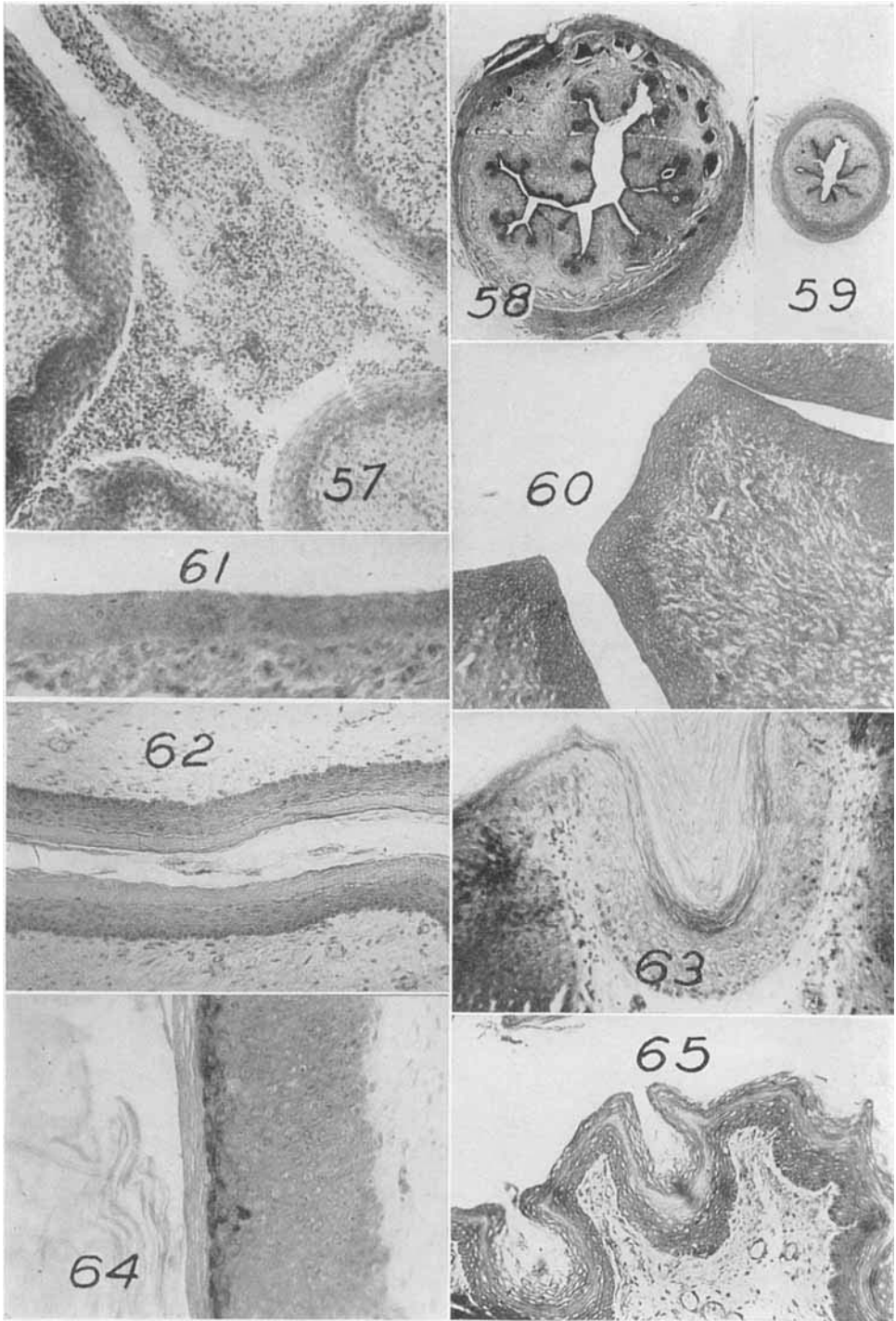


PLATE 12

EXPLANATION OF FIGURES

Sections of the lateral vaginal canals.  $\times 200$

66 No. 816, Jan. 31; pseudopregnant, about five days after oestrus, a few leucocytes are to be seen.

67 No. 841; late pregnancy; cf. figure 32, plate 7.

68 No. 552', early pseudopregnancy; cf. figure 55, plate 10. The cornified mass is being disintegrated by bacterial action.

69 No. 629; nearly resting stage, May 3, small atretic follicles in ovary; leucocytes in lumen of canal.

70 No. 609'; thirteen days after oestrus; cf. figure 8, plate 2. Cornified mass to left of figure is being disintegrated by bacteria.

71 No. 655, pouch young three weeks old, Feb. 16; granular layer prominent.



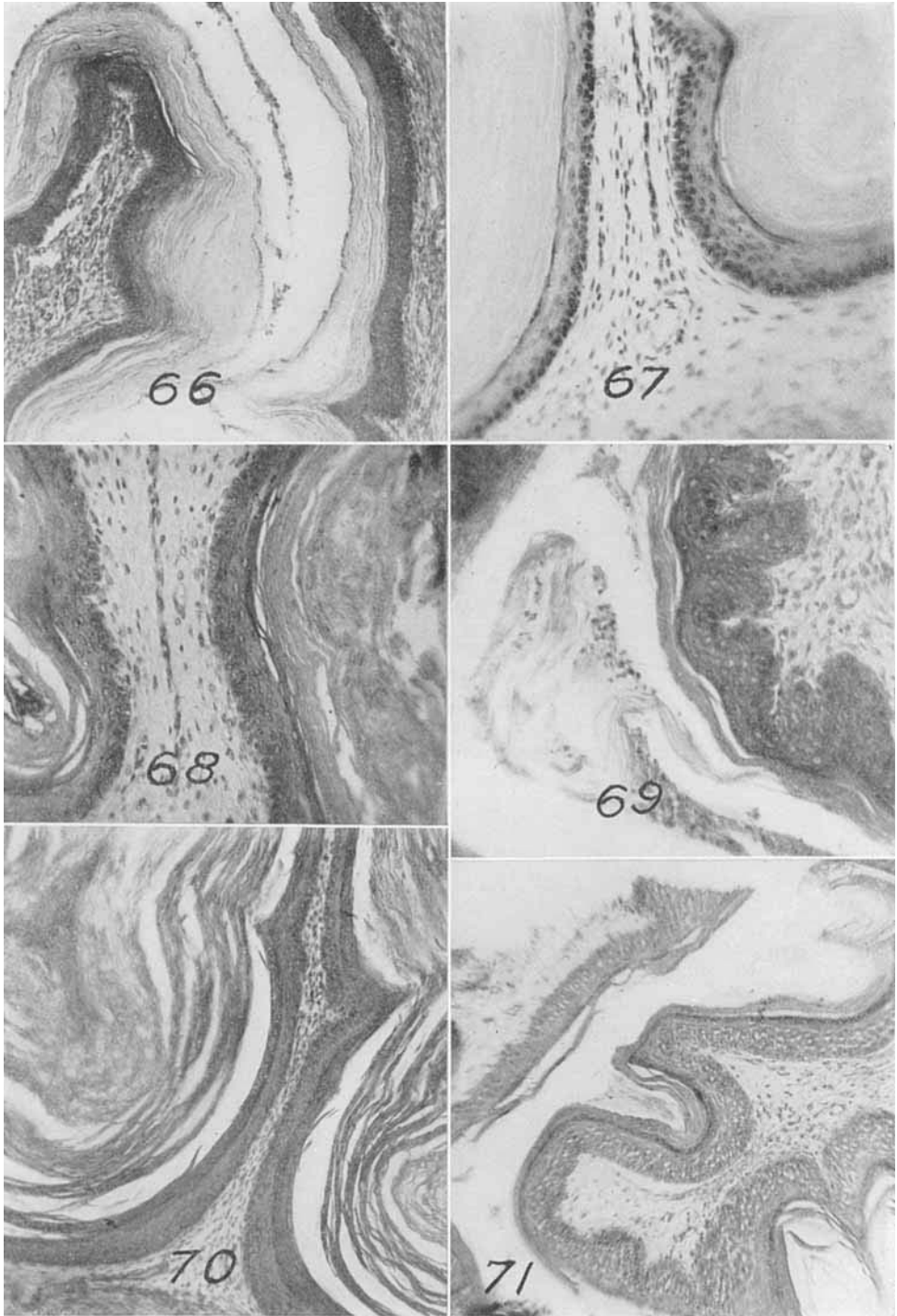


PLATE 13

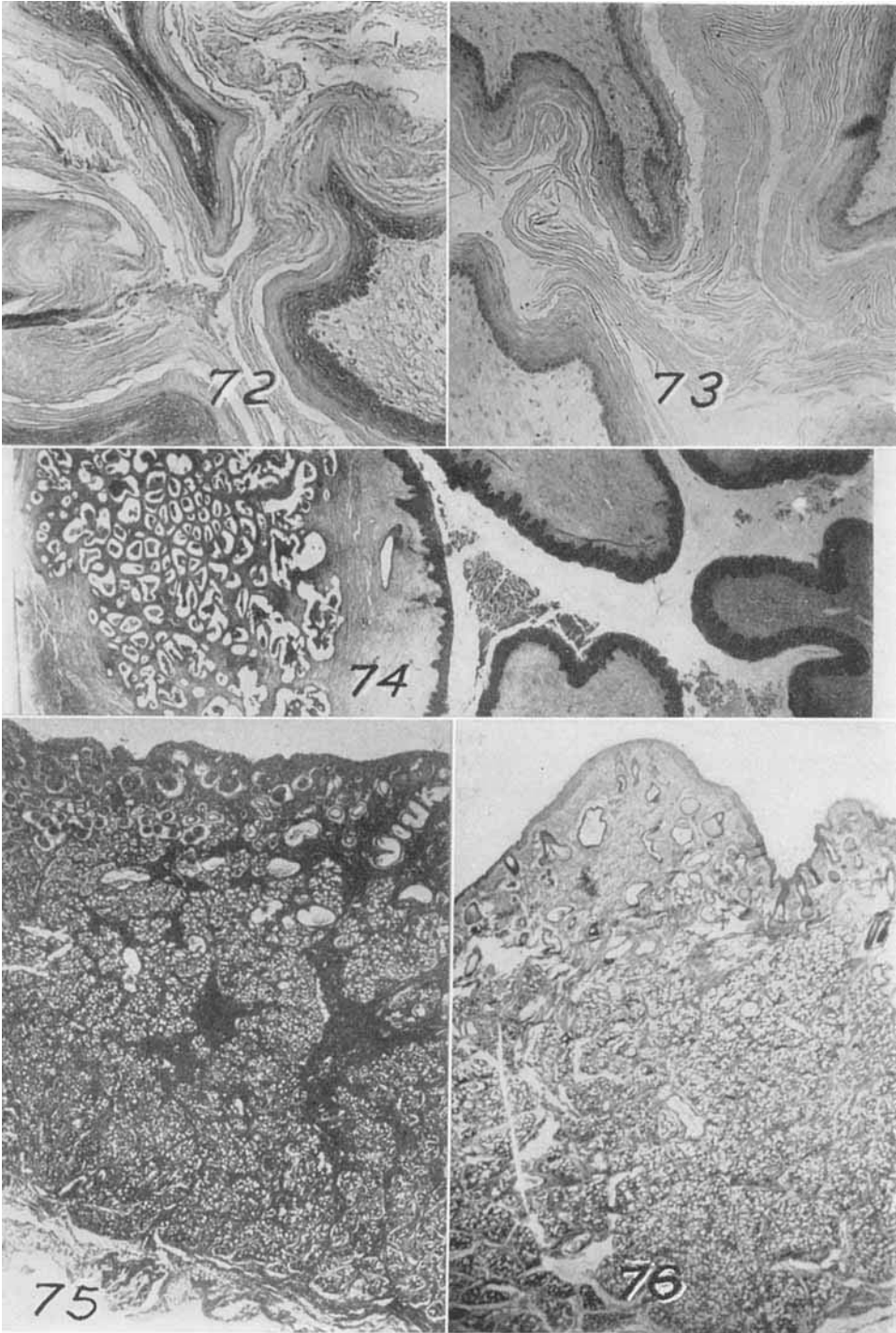
EXPLANATION OF FIGURES

72 Section of lateral vaginal canal of no. 644; April 3; eggs in early fragmentation stage; massive desquamation of the cornified layer, not yet disintegrated by bacterial action (cf. fig. 70, pl. 12).  $\times 200$ .

73 No. 521. Jan. 23; young eggs found forty-two hours after ripe follicles were seen in ovary in an exploratory operation; appearance as in figure 72, though this stage succeeded the first oestrus of the year.

74 No. 667 a. About oestrus; to the left the cloacal gland; to the right the vagina.  $\times 100$ .

75 and 76 Sections of mammary glands of pregnant animal at parturition (no. 190, fig. 75) and of pseudopregnant animal, no. 170.



## PLATE 14

### EXPLANATION OF FIGURES

#### Sections through alveoli of mammary glands

77 Alveoli of mammary gland. No. 831, late prooestrus, Jan. 21. Note mitoses in alveoli to right and to left, and tendency toward the two-layered condition.  $\times 500$ .

78 No. 552'; cf. figure 55, plate 10 and figure 68, plate 12; fragmenting eggs in uterus; mitoses numerous.  $\times 500$ .

79 and 80 Sections through glands of pregnant animal (no. 585', fig. 79) and of pseudopregnant animal no. 609 (cf. fig. 8, pl. 2). Both glands are well developed; alveoli dilated.  $\times 100$ .

81 No. 626 (tubal ova); the glands show very many mitoses; alveoli dilated from preceding oestrous cycle.  $\times 200$ .

82 No. 251. Double hysterectomy Feb. 27; at that time the mammary gland was very thick (pseudopregnancy); March 15, killed; animal in oestrus. Alveoli still dilated and containing coagulum (colostrum?).

