III. *The Reproductive Processes of Certain Mammals.*—Parts* IV *and* V.

Part IV. *The Œstrous Cycle of the Grey Squirrel* (Sciurus carolinensis).

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[Plates 9–14]

Contents.

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I. *Introduction.*

Although it is abundant in many parts of England and of economic importance, singularly little seems to be known about the breeding habits of the grey squirrel (*Sciurus carolinensis*). MIDDLETON (1930) noted that 23 females examined in March and April, 1930, were non-pregnant, although the males were sexually active. Records of litter size quoted by MIDDLETON suggest that four to six is usual. Apparently no true hibernation occurs in the grey squirrel and this species appeared to be very suitable for the extension of œstrous cycle work. Collection of field material was started in October, 1930. In view of the obvious limitations of such material, attempts were immediately made to induce the animals to breed in the laboratory. These were unsuccessful and although some few animals showed signs of approaching œstrus, no pregnancy or ovulation was obtained. Since better quarters are now available, it is hoped that further information may be secured by observation of animals breeding under laboratory conditions. Such information would relate mainly to the time relations of the cycle and to the nature of the changes in the absence of mating.

The work has been extended to the less plentiful native red squirrel, and we expect in time to obtain parallel information for this species. The data up to the present are inadequate to say how far the reproductive processes of the red and the grey are comparable. According to MILLAIS (1905) the red breeds early in the spring and again in July and August.

In the later stages of the work on the grey squirrel we were fortunate in getting into touch with Mr. A. D. MIDDLETON of the Bureau of Animal Population, Oxford, who is engaged in work on the ecology of the species, and who very kindly placed at our disposal certain data with regard to breeding season and fertility. These are incorporated in

Section IV. The present paper is concerned exclusively with the morpho-physiology of the œstrous cycle; no attempt has been made to deal with geographical variations in breeding season and size of litter, or with other problems of an ecological nature.

II. *Material and Methods.*

(*a*) *Collection of Material.*—Much of the material has been obtained from squirrels trapped alive, and killed in the laboratory. Most of such animals were killed as soon as received, to avoid possible changes due to captivity, but a few were kept some weeks before killing. Other animals were shot in the field during organized hunts and dissected and fixed on the spot within 30 minutes; both of these kinds of material are thus good histologically. The remaining animals were fixed whole by game-keepers after removal of the body wall and viscera, though this system naturally gave inferior histological results.

By one means or another animals were obtained from six districts: round London, Oxford, St. Neots (Cambridgeshire), Withyham (Sussex), and Tenterden and Tonbridge (Kent).

(*b*) *Methods.*—Date, body weight, sex, district, and macroscopic condition were recorded as far as possible. Body weight cannot be taken as being very significant in view of the variable condition of the animals and the variation in the gut content, though it distinguishes the small half-grown animals obtained during the summer. Clean body weight would have been a better index, but under field conditions dissection had to be reduced to a minimum. After fixation and transference to 70 per cent. alcohol, the ovaries and uterus were drained and weighed on a torsion balance. Weighing at this stage, though not ideal, makes it possible to include material fixed in the field. The ovaries were decapsulated before weighing, the two uterine cornua being cut off immediately above the cervix and below the Fallopian tube and capsule. Where for any reason the weight of only one ovary could be obtained this was generally doubled for comparative purposes. No attempt was made to weigh the vagina owing to the difficulty of clean removal of the connective tissue. The fœtuses were weighed with and without the membranes, and then measured. Small embryos were weighed intact in the membranes and uterine coat.

The mammary glands from likely squirrels were preserved, and treated by a modification of HAMMOND'S (1925) method. These mammary glands are particularly suitable and good preparations were obtained.

(*c*) *Histological Technique.*—Bouin's picro-formol-acetic, usually the alcoholic variety, was used as a routine fixative. Sections were cut at 10 μ and stained with hæmatoxylin and eosin. Two ovaries with corpora lutea were fixed in Flemming's fluid. As a routine, one ovary only of each pair was sectioned serially. If this initial examination showed an active condition (presence of large follicles, corpora lutea or other features of interest), the remaining ovary was sectioned. This system gave a reserve ovary in the case of important material, and avoided the unnecessary labour of sectioning pairs of similar inactive ovaries. The size of follicles was estimated by taking the mean of two diameters at right angles ; for estimating corpora lutea three diameters were determined and averaged. Except when many dozens of small follicles were present, all normal follicles large enough to have an antrum were measured.

The average size of follicle therefore means the average of all follicles with antra, or, in active ovaries, the average size of the group of developing follicles. Thus the average size of follicle is little below the size of the largest follicle and the latter figure is used in the appendix tables. When both ovaries were sectioned, follicle measurements were made in both if this seemed desirable ; these cases are marked by an asterisk in the tables. Corpora lutea measurements were always determined for both ovaries.

(*d*) *Determination of Stage of Cycle.*—The material described in the present paper includes only two animals kept under observation for an appreciable time before killing. Little or no history is available for the rest, and their seriation is dependent upon examination of the reproductive organs. In some animals the condition is obvious by examination merely of one organ, but in others the genitalia had to be considered as a whole before determination could be made. Several points were obscure till the series was almost complete, but comparison of different specimens and analogy with other species has enabled us to place every specimen. In a few cases this has been more difficult because of the absence of the mammary glands owing to rough dissection by keepers.

The young squirrel is fully grown by the autumn of the year of birth, and one of our early problems was to distinguish first year "immatures" from the older animals during the anœstrus which obviously exists during the last months of the year. It was then observed that the uteri during anœstrus fell into two size groups ; histological examination showed that this was owing to the larger type of uterus possessing masses of fibrosed blood vessels* in the muscular covering. These were obvious indications of a previous pregnancy, and when present were clear proof that the animal was not immature ; conversely, their absence was strong though not conclusive evidence of the animal being first year. All anœstrous animals, therefore, were readily classified as parous or non-parous, though the distinction between the two types becomes much less obvious in the final stages before œstrus. The recognition of the œstrous condition is comparatively easy. Two signs were observed, which by analogy with other species seemed to be indicative of œstrus, (*a*) swelling of the vulva with the production of a patent vaginal orifice, (*b*) distention of the uterus similar to that occurring in the mouse and rat. The latter was found in association with a vaginal plug and with spermatozoa in the uterus, and must therefore be highly characteristic of œstrus. Pregnancy was, of course, readily diagnosed. Two animals with young corpora lutea, but without obvious implantation sites, have been classified as very early pregnancies. We have not secured any obvious case of pseudo-pregnancy (fully developed corpora lutea without

* These structures were present all through the uterus, but were much more abundant in certain places, presumably the old placental sites.

fœtuses), and therefore old corpora lutea have been taken as a sign of the early post-partum phase. These, however, generally dwindle away and disappear soon after parturition, and are not often available. Another sign of the early post-partum phase, the enlarged uterus showing obvious placental sites on macroscopic examination, is definite while it lasts, but, like the presence of corpora lutea, is only transient. The great variability in the subsequent involution of the uterus makes it difficult to seriate accurately later post-partum material. Lactating mammary glands are an obvious criterion of the post-partum phase, but while active they give little evidence of the stage. Our chief difficulty has been in diagnosing (*a*) lactating animals for which mammary glands were not available, and (*b*) one or two later post-partum animals which showed no evidence of lactation. The situation is complicated by the fact that during the involution of the uterus the segments in between the placental sites may show a condition similar to that found during pro-œstrous enlargement. Examination of the uterine glands and reference to the Fallopian tube has been found of assistance in these cases; the Graafian follicles give no clear indication of the post-partum condition. A general classification of this material has been achieved firstly by examination of the mammary glands, and secondly by considering the weight and histological appearance of the uterus; when the glands were not available, classification has occasionally been difficult.

Fig. 1 shows in diagrammatic form the main points involved in arriving at the reproductive state of a given specimen. This diagram covers all observed categories except where the death of a litter results in the association of a regressing mammary gland with an early post-partum uterus.

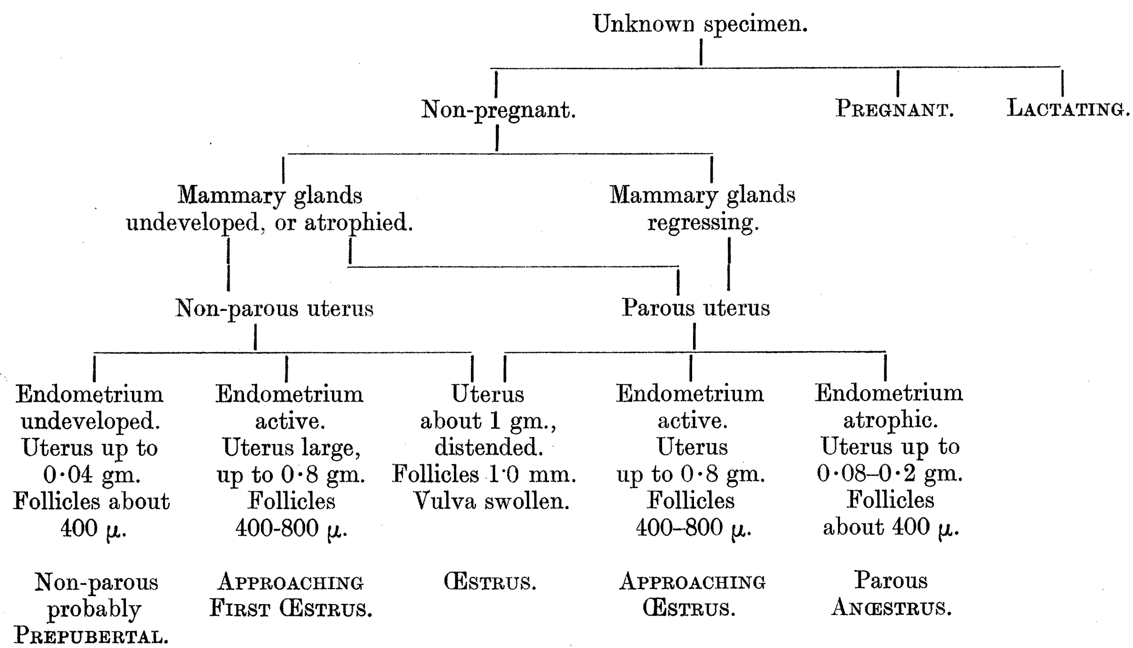

FIG. 1.

III. *General Morphology of the Reproductive Organs.*

The position of the uterus and ovaries in the body cavity varies considerably according to the reproductive state of the animal. During the active phase of the cycle the ovaries lie near the kidneys as is usual in rodents, and the uterine cornua are long and highly vascular. In the prepubertal animal the organs lie in the pelvis, where they are also found in the anœstrous adult. The change of position causes much folding up of the parous uterus, which never returns to the small prepubertal size even during anœstrus. The ovaries are enclosed by a peritoneal capsule, which, however, is not complete as in the rat and mouse. The Fallopian tube is long, up to 50–60 mm., but is much coiled so that the ovary is in close approximation to the tip of the uterine cornua. The cornua, which are flattened during anœstrus, but plump in active phases of the cycle, run down and anastomose their outer coats appreciably above the cervix. They then run side by side, fig. 24, Plate 12, for a short distance (about 0·5 cm. in the œstrous animal), before the circular muscle layers become fused, at which point the external groove between them gradually disappears as the cervix proper is reached. A little behind this the two canals become confluent, and there is a slight swelling externally, which is followed by a marked neck or constriction where the cervix joins the vagina. The two canals show a gradual reduction in size as they run backwards, and in the constricted region, shortly after they have fused, the lumen at its widest is less than 0·5 mm. in an animal approaching œstrus. This narrow lumen persists for a short distance through the greatly thickened musculature before it meets that of the vagina. The cervix does not project into the vagina so that there are no cervical fornices. Both vagina and cervix are very muscular especially at œstrus. The vaginal orifice is not patent during prepuberty and anœstrus.

The urethra runs into the muscular wall of the vagina about half-way between the cervix and the vulva, but it never actually enters the vagina, and retains its own identity to open to the exterior through the clitoris.

Histologically, the ovary shows comparatively little tissue which can reasonably be called interstitial, and is therefore of the type found in the mouse and rat rather than in the rabbit. In certain of the squirrels, probably the older ones, the ovaries contain very large amounts of fibrous tissue.

The mammary glands are similar in distribution and structure to those of the rabbit; there are normally four pairs, two thoracic and two abdominal, though there have been several asymmetrical animals possessing only $3\frac{1}{2}$ pairs.

IV. *General Nature of the Cycle.*

(*a*) *Breeding Season.*—The amount of information to be obtained from animals killed in the field is naturally limited, but nevertheless we have been able to ascertain the chief features of the fertile cycle. In Part V Miss Allanson shows that functional males, with large testes in full spermatogenesis, can be found at all times of year, so that the male grey squirrel cannot be said to have a quiescent period of the type found in certain mammals such as the ferret. The female grey squirrel, on the other hand, definitely has a period of anœstrus during which all the reproductive organs are atrophic. Mr. Middleton has informed us of a pregnant specimen obtained on January 26, 1932, the earliest record of pregnancy he knows, but of the 34 adult animals examined by us from September to January, only three showed signs of recent reproductive activity (Nos. 119, 9, and 122, late lactators taken in September and early in October). From February to August, pregnant and lactating animals were common, the former being most numerous in March and in June and July. Until the end of May, however, there were many squirrels with quite undeveloped uteri (Appendix 2). Histological examination showed that all these were non-parous, and they are apparently the later born young of the previous season not ready to breed until the summer. No well-grown animals of this kind are found in July when pregnancies are common, but 3 to 4 months old young born in the spring of the same year begin to appear. All the evidence points to the conclusion that squirrels do not breed in the year they are born. The reproductive cycle may therefore be summarized as follows.

Young are born in the spring and summer, Table I, and reach adult body weight by the autumn. When rather less than a year old, about February or June, according to their date of birth, they come into œstrus and become pregnant. Anœstrus eventually sets in and continues through the winter. In the spring of the next year these animals again become pregnant. It is not possible to say definitely whether all parous squirrels breed early in the year, possibly some remain quiescent till May or June so that their breeding period overlaps with that of the late first-year animals. No. 280, taken in June, which was pregnant and had recently lactated, provides definite evidence that animals breeding early may also have a second litter in the same year, but it is impossible to say whether this occurs in the majority of squirrels, or whether any breed twice in their first adult year. Those breeding in spring and failing to have a second litter presumably go into an early anœstrus.

Table I.—Grey Squirrel: Duration of Breeding Season.*

Month.	No. of pregnancies.	Month.	No. of pregnancies.
January	1	July	5
February	3	August	—
March	7	September	—
April	2	October	—
May	—	November	—
June	6	December	—
		Total	24

* Including data provided by Mr. Middleton.

These observations make it evident that the squirrel may experience more than one period of œstrus a year, but in the absence of information as to the behaviour of the unmated animal, we are unable to say whether this represents one polyœstrous breeding season or two monœstrous breeding seasons. Since the latter interpretation would involve two unequal periods of anœstrus during the year, the former is more likely.

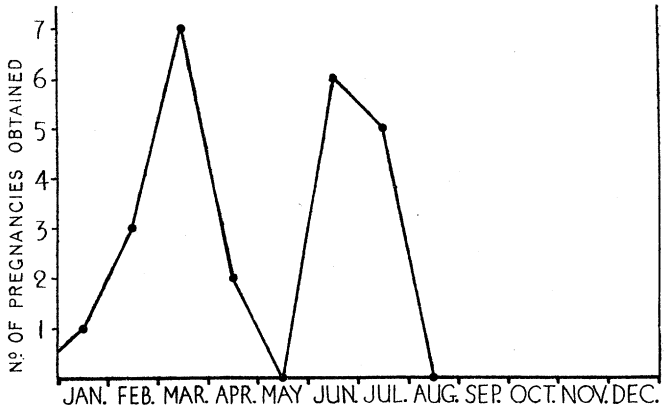

FIG. 2.—Seasonal distribution of pregnancies.

No information is available as to whether ovulation is spontaneous or dependent on copulation, *i.e.*, whether the unmated squirrel would show persistent œstrus or a diœstrous cycle. We are, nevertheless, able to say definitely that there is no post-partum œstrus, and that lactation is characterized by quiescence of the ovaries and uterus. The period of gestation has not been determined.

The young are born hairless and blind ; the weight at birth has not been ascertained, but the largest fœtuses secured averaged 10·3 gm. and the smallest young 23 gm. The duration of lactation is difficult to estimate, especially as the weaning process is usually gradual, but the interval between two litters produced in the same season appears to be about three months.

(*b*) *Fertility and Sex-ratio.*—Fertility in the sense of size of litter can be estimated in our material by five criteria : (*a*) number of ripe follicles at œstrus, (*b*) number of eggs ovulated at one time, as shown by the number of corpora lutea found during or immediately after pregnancy, (*c*) number of fœtuses, (*d*) number of placental sites in the post-partum uterus, and (*e*) number of nest young. The available data on these points is given in Table II. The lower averages for the numbers of post-partum corpora lutea and the numbers of placental sites are probably accounted for by the irregular disappearance of these structures. Judging by the number of fœtuses and the number of nest young the average size of litter in the available material is about 3·6. The close correspondence of this figure with the number of corpora lutea found during pregnancy suggests that in this species there is little loss by non-implantation of ova or fœtal reabsorption. Ineffective copulation appears to be rare (see p. 50).

TABLE II.—Grey Squirrel: Fertility.

Number of ripe follicles, fœtuses, corpora lutea, etc.	Number of animals.					
	Ripe follicles at œstrus, or pro-œstrus.	Corpora lutea (in pregnancy).	Corpora lutea (post-partum).	Fœtuses.*	Placental sites.	Nest young.*
1	—	—	—	—	—	—
2	—	—	4	1	2	1
3	—	4	3	10	2	2
4	2	6	—	7	1	3
5	—	—	—	3	—	1
6	1	—	—	—	—	—
Total animals . . .	3	10	7	21	5	7
Total number of ripe follicles, etc. . .	14	36	17	75	14	25
Average number of ripe follicles, etc. .	4·7	3·6	2·4	3·6	2·8	3·6

* Including data from Mr. MIDDLETON.

The distribution between the two horns of the uterus shows no abnormality. In the eleven pregnancies for which the information is available, 36 fœtuses were evenly divided between the two cornua.

Our data throw little light on the sex-ratio since the number of litters taken from nests is too small to give any information. The catchable population of fully-grown animals seems to be fairly evenly divided between the sexes. In the later stages of the collection, males were not recorded, but up to the time when records were kept of all animals caught, 207 animals were obtained of which 110 were males, a percentage of 53·1.

(*c*) *Nature of the Changes in the Reproductive Organs.*—During anœstrus the vulva is small and the vaginal orifice imperforate. On the approach of œstrus, the surrounding tissue begins to swell, and finally forms a papilla rather over 0·5 cm. wide stretching backwards from the clitoris for about 1 cm.; in the final stage of vulval growth the orifice appears as a slit starting at the clitoral end and rapidly extending backwards. Copulation, as in many other rodents, is followed by the formation of a vaginal plug, which is described by Mr. MIDDLETON as a firm jelly-like mass completely occluding the vaginal lumen. The time occupied by the pro-œstrous swelling of the vulva has not been determined accurately, but judging from specimens kept alive in the laboratory, it is of the order of a fortnight. In our early pregnant animals the vulva has subsided to resting level, but the vaginal lumen remains patent.

The corresponding changes in the vagina consist essentially of hypertrophy and cornification of the epithelium, with subsequent sloughing and regeneration.

The uterus begins its development before any external signs of activity are visible. The initial changes are concerned solely with the development of the endometrium and its glands, followed, in the final condition of œstrus, by distention of the lumen such as occurs in the mouse and rat. To judge from the development occurring before implantation, the pseudo-pregnant condition would be well developed after infertile ovulation. Following post-partum involution, the uterus becomes atrophic during lactation and anœstrus. No unusual feature has been observed in the ovarian cycle, except perhaps the small size of the corpus luteum in relation to that of the ripe follicle.

V. *The Ovarian Cycle.*

(*a*) *Size Changes in the Ovary.*—Apart from the initial growth of the ovary in the young animal, size changes in the ovary of the squirrel are not great. The smallest animals obtained, other than nest young, weighed 250–350 gm., and had minute ovaries of the order of 0·01 gm. The ovaries gradually increase in weight during the next 6–9 months until the first œstrus, when they go up to about 0·04–0·05 gm.; the subsequent appearance of corpora lutea did not cause any regular increase in their weight. The ovaries of the pregnant animal are only about double the weight of the resting ovaries. After parturition the weight of the ovary falls and reaches the lowest anœstrous level in November and December. At this time the ovaries of the parous animal show no significant difference in size from those of the non-parous first-year animal; thus in December, the ovaries of 21 non-parous animals averaged 0·017 gm. and those of 14 parous ones, 0·018 gm.

(*b*) *Initial Growth of the Ovum and Follicle.*—In the grey squirrel, as usual in mammals, very large numbers of small oocytes, about 20 μ in diameter, are present immediately below the germinal epithelium. The subsequent growth of the ovum in relation to its surrounding follicular epithelium is even more rapid than usual (PARKES, 1931). The ovum approaches full size while the follicular epithelium is still only one cell thick. When the ovum reaches full size (about 95 μ in diameter) the whole follicle is only about 150 μ in mean diameter. Soon afterwards the sheath of the follicle differentiates into theca externa and theca interna, the latter rapidly becoming 3–4 cells thick. At 250–300 μ in diameter the follicle develops an antrum.

The growth relations of the ovum and follicle are thus similar to those shown by BRAMBELL (1928) for the mouse and by PARKES (1931) for other species.

(*c*) *The Prepubertal Ovary.*—Female squirrels born between January and July remain in the prepubertal state until the following spring or summer, according to whether the first œstrus occurs early or late in the breeding season (see p. 53). In both types the history of the prepubertal ovary appears to be similar. Owing to its far greater range, the weight of the uterus is a better index of approach to puberty than that of the ovary; in our experience non-parous uteri with a weight of more than

0·05 gm. are showing signs of development. Considering the 43 non-parous animals with uterus weights below this figure, it will be seen that the diameter of the largest follicle (Appendices 1 and 2) is about 600 μ, half that of the mature follicle, and it may therefore be taken as about the maximum size reached by the follicle before the onset of the initial changes preceding the breeding season. Nineteen of the non-parous animals possessed no follicles with antra, fig. 5, Plate 9. This condition shows a slight connection with the body weight of the animal and is probably more common in the younger animals (but see p. 56). It is difficult to say from the existing material whether steady or intermittent growth brings about the enlargement of the originally small solid follicle till it has the mean diameter of 600 μ. The presence of crops of atretic follicles of about 400–600 μ in many of the ovaries makes it possible that successive batches of follicles grow and become atretic until the onset of the first pro-œstrus, when growth is continued.

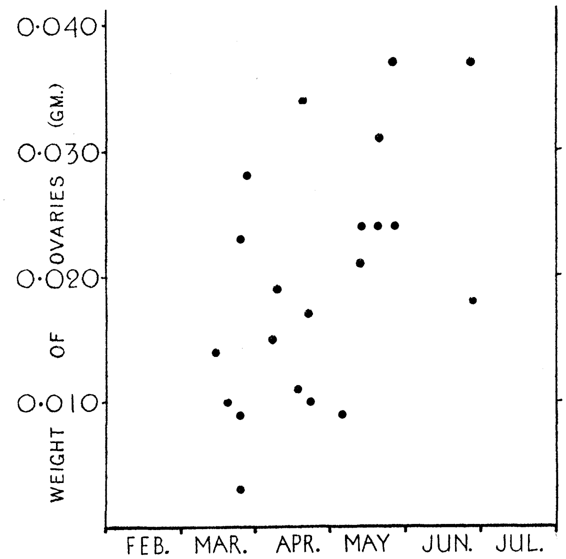

Fig. 3.—Weight of ovaries of prepubertal squirrels coming up for summer breeding.

Owing to the fact that the prepubertal animals are of two types according to when they are destined to have their first œstrus, they do not make a very uniform group considered together. There is, however, one set of animals forming a really homogeneous group, *i.e.*, the prepubertal ones which fail to come into œstrus in the spring and reach puberty in May or June. These animals are easily recognizable after February. Table III shows the monthly averages for the weights of ovaries and uteri in this group. The individual ovary weights are shown in Appendix 2 and fig. 3.

The ovarian development during this time is very largely follicular. Of the six March animals only two had follicles with antra, while only one animal in each of the other two months had no follicles of this type.

(*d*) *The Anœstrous Ovary.*—In view of the complete cessation of reproductive activity in the female squirrel during the last months of the year, one would expect to find an inactive ovary at these times. It is, however, becoming increasingly evident that when

TABLE III.—Weight of Ovaries and Uterus in Prepubertal Squirrels coming up for Summer Breeding.

Month.	Number of animals.	Average weight of ovaries (gm.)	Average weight of uteri (gm.)
March	6	0·015	0·027
April	6	0·018	0·034
May	7	0·026	0·306

it occurs, the anœstrous period is made up of two phases, (*a*) regression after the last breeding season, and (*b*) initial preparations for the next; thus the actual period of quiescence is short. This is certainly true of the male ferret (ALLANSON, 1932) and, judging by HAMMOND and MARSHALL'S (1930) table of follicle size, also of the female ferret. In the grey squirrel the same sequence is found. From August to January inclusive, only three squirrels were obtained with corpora lutea (see Appendix 7), and these were post-partum animals with the remains of the old corpora lutea of pregnancy, but nevertheless, there is considerable variation in the state of the ovary during anœstrus. If the percentage of animals possessing follicles with antra is taken as the index of follicular activity in a given month, October and November would appear to be the time of minimal development during anœstrus. There were follicles with antra in all the five parous animals obtained in August and September, in only six of the 16 in October and November, but in 13 of the 16 in December and January. Since the follicle is not such a dominating feature of the parous ovary as of the prepubertal, the ovarian weights do not show any significant variation during anœstrus. The size of the largest follicle is also irregular.

If the ovary of the parous anœstrous animal is compared with that of the fully-grown but prepubertal animal obtained at the same time, a slightly greater development is found in the former. During December, a large number of animals were obtained at the same time from the same source. It was found that only 11 of the 21 prepubertal animals possessed follicles with antra, as compared with 11 out of the 14 parous ones. The size of largest follicle also tends to be greater in the latter. In few cases, however, is the diameter of largest follicle in excess of the 600 μ (Appendices 1 and 8).

(*e*) *Follicular Maturation.*—We may assume, therefore, that the maximum diameter attained by the follicle in the prepubertal or anœstrous squirrel is little more than 600 μ. This, though not necessarily indicating a well defined quiescent condition, may conveniently be called the resting size. In contrast, the four large follicles in our œstrous specimen (No. 283) averaged 1010 μ, the largest being 1100 μ (Table IV), and since the specimen was obtained after copulation, this size may reasonably be taken as closely approaching that of the follicle at ovulation. Comparatively few intermediate stages between the resting and mature follicles were obtained, and it is evident that in comparison with the growth of the uterus, the maturation of the follicle is abrupt. Thus in No. 275 the uterus was 0·726 gm., about 15 times the resting prepubertal weight, whereas the diameter of the largest follicle had merely increased to 750 μ.

The maturation of the follicle appears to follow quite the usual course. In the 500–600 μ diameter resting follicle the granulosa is about 70–80 μ thick, leaving the antrum a diameter of about 400 μ, the ovum tends to be situated centrally and the surrounding discus is joined to several points of the periphery by strands of granulosa in addition to the main point of attachment. The theca interna is 3–4 cells thick and well developed. In the maturing follicle the granulosa remains about the same thickness as in the resting state, and thus constitutes a smaller proportion of the follicle, though it is increasing considerably in volume. The secondary attachments of the discus disappear and the ovum loses its central position. The innermost cells of the discus crowd together to form a dense even layer surrounding the ovum, and subsequent retraction of this layer forms the corona radiata of the ripe follicle. There is no increase in granulosa cell size during maturation, the increased volume of the tissue being brought about as usual by cell division, fig. 6, Plate 9.

The development of the group of follicles due to ovulate appears to be associated with the degeneration of remaining resting follicles. In No. 275 (largest follicle 780 μ) and No. 271 (largest follicle 740 μ) many of the smaller follicles were becoming atretic, but in No. 87 (largest follicle 860 μ) all follicles with antra except those maturing were in atresia. No. 283 had no follicles with antra whatever except the four mature ones. Table IV shows the size of the normal follicles in squirrels coming up to œstrus as compared with those of a typical parous anœstrous animal (No. 146), and indicates well the disappearance of the smaller follicles towards œstrus.

TABLE IV.—Diameter in μ of Follicles in Squirrels Developing to Œstrus.

No. 146.	No. 209.	No. 213.	No. 196.	No. 287.	No. 271.	No. 275.*	No. 87.*	No. 283.*
590	640	660	690	730	740	780	860	1100
570	630	640	630	730	670	770	820	1050
570	610	620	620	690	610	770	810	950
560	610	610	610	680	600	710	780	940
530	600	590	590	680	570	700		
510	580	540	580	670	550	690		
510	570	530	550	630	530	440		
500	540	520		620	510	410		
490	530	510		610				
490	530	480		600				
480	500	450						
470	500							
440	490							
	460							

* Follicles measured in both ovaries.

If 1000–1100 μ is taken as the diameter of the follicle at ovulation, it would seem that in the grey squirrel the mature follicle has about twice the diameter (or about eight times the volume) of the resting follicle. In the mouse the corresponding figure is about 1·5 (BRAMBELL and PARKES, 1927) and in the baboon (ZUCKERMAN and PARKES, 1932) about 6·0. Thus the ratio between the sizes of the mature and the resting follicle appears to increase with the body weight of the animal, *i.e.*, with increasing body weight of the species, the size of the resting follicle does not keep pace with the greatly increased size of the mature follicle.

(*f*) *Growth and Regression of the Corpus Luteum.*—Corpora lutea were found in the ovaries of only 18 squirrels; all these were killed during pregnancy, or after parturition, in the months of March, June, July, August and September. No post-partum ovulation had occurred in any of the 22 lactating squirrels examined, and in only 7 of these were the remains of the corpora lutea of pregnancy still to be seen. Almost all the corpora lutea were measured in section (Appendix 6), but owing to the fact that many were irregular in shape the mean diameters obtained by the method described can only give approximate indications of the size of the body. Taken in conjunction with direct comparison of lutein cell size, however, these measurements showed that the corpus luteum reaches its maximum size rapidly, and then has a mean diameter 1·0–1·3 mm. The corpus luteum begins to regress about half-way through pregnancy; in No. 93, where an embryo weighed 1·07 gm., signs of shrinkage and degeneration were already apparent and in No. 107 having embryos weighing 1·87 gm. the mean diameter of the corpus luteum was only 0·74 mm., obvious shrinkage having taken place.

The most recently formed corpora lutea in the present series of ovaries are those of No. 73, fig. 7, Plate 9; they are solid but project irregularly from the surface of the ovary and portions of the theca interna can still be distinguished. The spindle-shaped fibroblastic cells forming the framework of the vascular tissue resemble those of the rabbit and other rodents. The nuclei of the lutein cells are only slightly larger than before rupture of the follicle, but they have attained their full size, with a diameter of 6 μ. The lutein cells are not quite fully developed. In one ovary of No. 73, a follicle which has failed to ovulate shows luteinization of the granulosa similar to that in the developing corpora lutea. In No. 66 no definite nodules have formed in the uterus but progestational development of the endometrium is well advanced, fig. 17, Plate 11. In this animal the corpora lutea have already reached their full size, the lutein cells, which are irregular and not well defined, having a diameter of about 10–12 μ. Nos. 80, 129, 284 and 92 have similar corpora lutea, fig. 8, Plate 9. One ovary of No. 284 was fixed in Flemming's fluid, and the corpus luteum contained little osmicated fat. In the latest pregnancy of this group the embryos had an average weight of 0·3 gm. In No. 93 leucocytes had tended to accumulate among lutein cells which had lost their regular outline, but not all the corpora lutea were equally affected. In Nos. 107 and 102, the two latest pregnancies of the series (Appendix 6), containing embryos averaging 1·87 gm. and 10·34 gm. respectively, the diameter of the corpora lutea in section was reduced by more than a third and the lutein cells were shrunken and irregular.

In the earliest post-partum squirrel obtained, No. 219, the corpora lutea were similar to those of No. 102 and probably only a few days older. In the remaining lactating squirrels, where the corpora lutea of pregnancy could still be distinguished, further shrinkage had taken place, the mean diameter falling from 0·5 mm. to 0·27 mm., so far as the irregularity of the remains permitted measurement.

(*g*) *The Follicle during Pregnancy and Lactation.*—The earliest post-ovulation animal had only very small follicles, as might be expected from the disappearance of resting follicles during maturation, while the remaining pregnant squirrels had follicles up to the largest resting size or above; it is evident, therefore, that follicular growth must occur at least during early pregnancy. Three of the animals (Nos. 66, 284, 102) had follicles very definitely above resting size, the ovaries of No. 66 in particular were remarkable; in addition to the four corpora lutea, they contained 20 follicles between 800 μ and 570 μ. In No. 284, the one ovary measured contained eight follicles between 900 μ and 540 μ, while one ovary of No. 102 contained eight between 830 μ and 370 μ; no other ovaries in the whole series of squirrels examined showed such large follicles in conjunction with an abundance of normal smaller ones. These three animals represent very different stages of pregnancy, and unless they are isolated curiosities, it is necessary to suppose that bursts of follicular growth, dissimilar to œstrous maturation in being unaccompanied by the degeneration of smaller follicles, may occur during pregnancy. In the absence of a post-partum ovulation, the ultimate fate of these large follicles is presumably atresia, of which a good deal is found in some of the ovaries of pregnancy.

The follicles of the lactating animals are usually of the typical resting size, and so far as we have been able to seriate the post-partum material, there is no significant variation in the size of the follicle at different stages of lactation or even as lactation passes into anœstrus.

VI. *The Cycle in the Fallopian Tube.*

The Fallopian tube, examined in 23 squirrels at characteristic stages of the cycle, showed a marked difference in diameter and appearance between its œstrous and anœstrous condition, Plate 13. No attempt will be made to give a systematic description of the differences between different parts of the tube; as far as possible the regions described are comparable ones from the mid part of the tube.

In well-grown prepubertal squirrels showing no approach to œstrus, the Fallopian tube is lined by a single fairly regular layer of epithelium, 7–10 μ in height. The cells are almost entirely filled by the oval nuclei, and no cilia can be distinguished; traces of secretion sometimes occur in the lumen. In parous anœstrous squirrels the epithelium is very similar but tends to be more irregular, fig. 25, Plate 13. A certain number of ciliated cells persist. At the end of the breeding season, cell debris may be found in the lumen of the Fallopian tube, including agglomerations of giant nuclei produced by the breakdown of the epithelium.

No. 196 (non-parous), taken in January, shows the earliest definite stage in the transition to the active Fallopian tube. In this specimen the height of the epithelial cells is about 16 μ and they show a clear cytoplasmic border next the lumen.

Nos. 209, 213 and No. 271 (parous), fig. 26, Plate 13, taken in February and May with enlarged uteri, show further growth stages, the Fallopian tube of No. 213 being the most advanced. The cells vary in height from 24–30 μ, many are ciliated, and both oval and rod-shaped nuclei occur. The diameter of the tube has enlarged, the villi are branched in some regions; degenerating cells are being sloughed into the lumen, and there appears to be fairly active secretion.

The maximum growth in the Fallopian tube is shown by Nos. 87 and 275, both of which have large pro-œstrous uteri; the epithelial cells have reached a height of 30–40 μ. The nuclei vary in size but usually occupy only the quarter of the cell next the basement membrane. The cell cytoplasm is frayed out at the edge, and there is secretion in the lumen; mitoses can be found, fig. 27, Plate 13.

The fixation of No. 283, killed on œstrus, was unfortunately poor, but the epithelium of the Fallopian tube appears to be about the same height as in No. 87, though there is more secretion in the lumen.

The post-ovulation condition of the tube is shown by No. 80, fig. 28, Plate 13; in sections it contrasts markedly with the œstrous example. The epithelial cells have lost their cytoplasmic border and seem disarranged, and secretion has vanished from the lumen, while the reduced appearance of the villi suggests that a considerable amount of sloughing has taken place. The average height of the epithelium is 10–13 μ, some of the cells being ciliated; degeneration is not as marked in the region of the fimbriæ.

In No. 102 at the end of pregnancy the epithelial cells are still low, from 7–13 μ, but they show a narrow cytoplasmic border.

The Fallopian tubes of four squirrels were examined during lactation and in all these the epithelium resembled that of No. 102. The diameter of the tube appears to undergo reduction. Other squirrels at the end of lactation, taken in October and November, also showed regression of the Fallopian tube and a return to the anœstrous appearance, the cells and nuclei undergoing further shrinkage at the end of the breeding season. In No. 26 (November) the epithelium in many parts of the tube is no longer columnar and may be as low as 6 μ.

VII. *The Uterine Cycle.*

(*a*) *The Immature Uterus.*—In most squirrels the uterus remains very small until it undergoes enlargement before puberty, probably when the animal is nearly a year old. Two cases, however, suggest that there may sometimes be a slight development in the 6–8 months old animal (Nos. 112 and 8, August and October), but the evidence indicates conclusively that any growth at this time is abortive, and does not lead to puberty. In prepubertal animals destined to breed in spring, uterine activity is initiated in

December, 6–8 weeks before œstrus, while, in those breeding for the first time in the summer, signs of activity are not common until the end of May.

In 37 non-parous squirrels taken at all times of the year and showing no indications of activity, the uteri had an average weight of 0·028 gm. (Appendices 1, 2). Fig. 9, Plate 10, shows a section through a uterus of this type; the stroma is dense and poorly vascularized, the glands are few and small and the epithelium round the slit-like lumen has a height of 7–12 μ. The glands vary slightly in development with the size of the uterus but hardly any show signs of secretory activity. They are simple tubular outgrowths of the epithelium with a diameter in cross-section of 30–40 μ; the cells measure 7–12 μ and have hardly any cytoplasm round their nuclei.

(*b*) *The Anœstrous Uterus.*—The uteri of parous squirrels in anœstrus are essentially similar to those just described except for the presence in the serosa of hyalinized blood vessels, the persistence of which makes the uteri of parous animals in anœstrus always heavier than those of prepubertal animals. The average weight of 36 such uteri (excluding ones which had not fully involuted after parturition) was 0·122 gm. (Appendices 4 and 8).

Fig. 10, Plate 10, shows a typical section through a parous uterus in winter. The stroma is very dense, the glands and gland cells small and apparently inactive, though traces of secretion sometimes persist in the lumen of the glands. Hyalinized blood vessels can be seen in the serosa.

(*c*) *Development to Œstrus.*—Appendices 2 and 3 (especially the February and May squirrels), show the enormous increase in the weight of the uterus associated with the œstrous changes. Growth of all the uterine tissues takes place and striking changes occur in the stroma; its nuclei enlarge and the whole tissue becomes œdematous. The final increase in weight of the uterus is due, as in other rodents, to the accumulation of fluid in sufficient quantity to distend the walls of the organ.

The first clear signs of the approach of puberty are found in non-parous squirrel uteri weighing 0·050–0·090 gm., although histological changes can sometimes be detected below this size. Fig. 11, Plate 10, shows the uterus of No. 196 taken in January; it weighed 0·089 gm. The uterine stroma is distended and very vascular; the gland tubules have increased in number and size, and the height of the epithelium is now 15–20 μ. The lumen is larger and less slit-like, and the gland cells show a border of cytoplasm next the lumen of the tubules.

In parous anœstrous squirrels, owing to the complication of previous pregnancy, the earliest signs of activity are less easy to distinguish than in prepubertal animals, though they appear to be essentially similar. Nos. 38 and 43 have uteri in which the lumen is distended and the stroma somewhat œdematous; both are well vascularized. The uterine epithelium has a height of about 10 μ; the glands are not yet very active. No. 287 taken in May is of a rather similar type, but No. 65 taken in June shows greater œdema of the stroma and glandular development; the epithelium varies from 15–20 μ in height. No. 271 with a uterus weight of 0·294 gm. shows a greater activity; the epithelium is still 15–20 μ in height, but the gland cells have enlarged and have a definite border of cytoplasm next the lumen ; they are 12–15 μ in height. The stroma is œdematous.

No. 209, a parous squirrel taken in February, has a uterus weighing 0·302 gm. Its stroma cells show greater enlargement than those of No. 271 and the height of the uterine epithelium is 20–25 μ. Fresh gland tubules are growing back into the stroma.

The uterus of No. 213, February (0·442 gm. parous), shows a continuation of the enlargement but is not essentially different ; the epithelial cells have increased in height to 30–40 μ and have a cytoplasmic border next the lumen. The stroma is again less dense. Leucocytes can be seen migrating through the epithelium and in the wide lumen. The gland cells appear to be actively secreting.

The latest stages available of enlargement of the uterus, before its final distention with fluid at œstrus, are provided by two similar non-parous squirrels, No. 275 taken in May and No. 87 taken in June, figs. 12 and 15, Plates 10 and 11. No. 275 had a uterus weight of 0·73 gm. ; its largest follicle had a diameter of 0·78 mm. while that of No. 87 was 0·86 mm. Comparison with No. 213 shows a further increase in the height and volume of the epithelium ; folds of it now project into the lumen and the cells reach a height of 50 μ ; their oval nuclei still lie near the base, leaving in section a deep border of light cytoplasm next the lumen. The gland cells are similar and have a height of 15 μ. The coiled, obliquely running gland tubules reach far into the stroma ; secretion can be seen in the uterine lumen but it is not very abundant.

The œstrous uterus obtained from No. 283, shot just after mating, weighed 1·1 gm. ; it contained spermatozoa and was distended with fluid so that the wall was far thinner than in the preceding specimens. (Fig. 13, Plate 10.) Owing to the fact that it was not fixed till 15 hours after death a detailed histological description is not possible. The distended epithelium is not quite so high as in No. 87, but it averages about 40 μ (assuming it to be unaffected by post-mortem changes). The epithelial cells seem to be actively secreting.

(*d*) *Changes before Implantation.*—Fig. 16, Plate 11, shows the appearance of the uterine mucosa associated with the youngest corpus luteum of the series, that of No. 73 illustrated in fig. 7, Plate 9. This was probably 1–2 days after ovulation. The uterus of No. 73 weighed 0·78 gm. ; there were no external signs of implantation. Fig. 16, Plate 11, shows how the uterine endometrium has proliferated and grown into a series of folds deeper and more regular than those found just before œstrus. The actual epithelial cells show a decrease in height to 25–30 μ ; the glands have also proliferated and appear more coiled but are not actively secreting. They open into depressions between the epithelial folds.

Fig. 14, Plate 10, and fig. 17, Plate 11, show a slightly later condition of uterine proliferation, when the corpus luteum has reached its full size, although implantation has not yet taken place. This uterus (No. 66) was the largest of the series, weighing 2·55 gm. The endometrium has grown out into numerous tapering projections which interlace with each other and form a fringe round the lumen. The actual epithelial cells are further decreased in size and now have a height of 10–12 μ. Leucocytes are fairly abundant in the stroma.

No. 80 shows the earliest implantation sites; in sections through the uterus between these regions there is a further complication and interlacing of the endometrium; all but the most deeply lying glands appear to be involved in the process. Later in pregnancy, sections between the embryos reveal active secretion of the endometrium.

(*e*) *The Uterus after Parturition and during Lactation.*—No. 219 indicates the condition of the uterus very shortly after parturition; one of the young of this squirrel weighed only 23 gm. The corpora lutea resemble those of No. 102 killed in late pregnancy. Each uterine horn has one placental site; sections through the latter show post-partum disorganization, absence of glands in the stroma and sloughing of the inner cell layers including groups of decidual cells. There is pigment in the degenerating blood vessels of the stroma. Away from the placental sites the uterine endometrium has returned to the active appearance characteristic of the enlarging uterus having œdematous stroma and large well-formed glands, with gland cells 12–15 μ in height. There is no trace of the progestational proliferation which still persisted in the later stages of pregnancy. The epithelial cells have a height of 25–32 μ, and their nuclei instead of lying, as before, at the base of the cells are situated nearer the middle, while the edge of the cytoplasm appears to be fraying into the lumen. Leucocytes are migrating through the epithelium from the stroma.

The next earliest post-partum squirrels in the series are No. 230 and No. 226 in which the uteri weighed 1 gm. and 0·92 gm. respectively, and the mammary glands were not fully developed. As in No. 219, a distinction must be made between the condition of the placental sites and that of the other parts of the uterus. In the latter the stroma is much less œdematous than it was in No. 230, the glands, too, are diminished in volume. There is a decrease in the epithelial cytoplasm; the cells now have a height of only 20 μ and their nuclei lie nearer to the lumen than in the previous stage. Leucocytes and cell debris can be seen in the stroma and in the lumen.

Later post-partum squirrels can be arranged only in approximate order of time after parturition since the number of young born and suckling will affect the size of the uterus, and the rate at which it changes in structure. In general the uterine changes during lactation consist of progressive shrinkage and loss of œdema; at the placental sites resorption of cell debris and growth of fresh epithelium take place, followed by the redevelopment of endometrial glands. The placental sites can be distinguished histologically for a considerable time owing to the difference in the stroma and glands and the greater number of hyalinized blood vessels in the serosa and circular muscle. Pigment often persists in these regions.

In No. 228 the uterus, weighing 0·236 gm., is typical of early lactation, although the mammary gland was not fully developed. One of the young weighed 47 gm. Considerable contraction of the uterine tissues has taken place and the stroma nuclei are closely aggregated and smaller than in earlier post-partum stages. The gland tubules show a decrease in size but still contain secretion in the lumen. The epithelium varies in height from 12–20 μ, and its nuclei lie almost at the free surface of the cells so that there is a clear cytoplasmic zone next the stroma; this contrasts with the condition in the developing uterus.

In No. 266, which had a very well developed mammary gland, the uterus weighed 0·194 gm., though placental sites are still distinguishable in section. The stroma is very dense and the glands are no larger than in anœstrus, but they still contain secretion; the cells have lost their cytoplasmic border.

Animals killed later in lactation, and when the mammary gland was atrophying, show a decrease in the size of the uterus. In section the endometrium appears very dense;

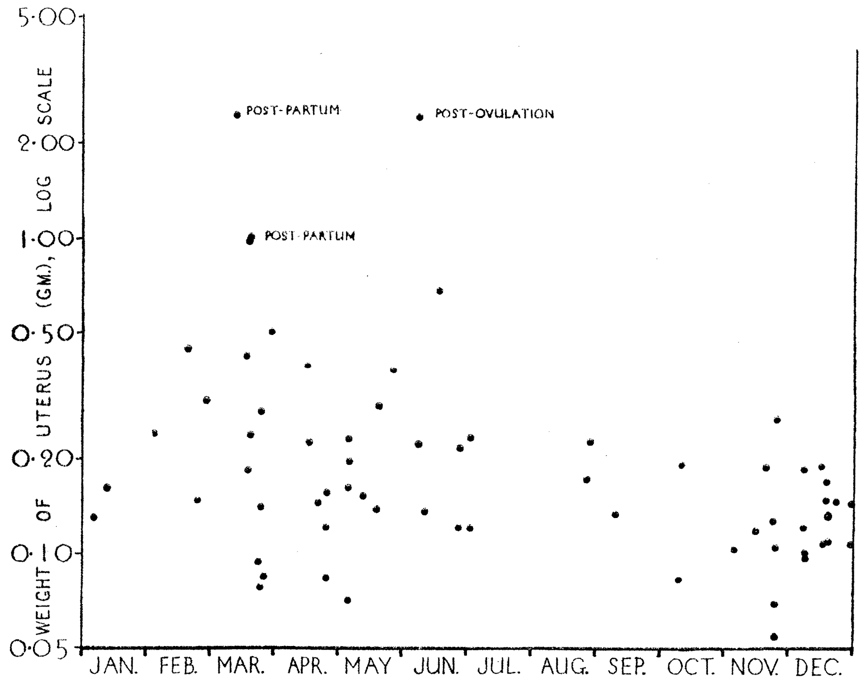

FIG. 4.—Uterus weight of parous squirrels.

hyalinized blood vessels stand out sharply in the serosa. Once they have regressed in size the uteri at the end of lactation resemble the anœstrous uteri of other parous animals, except where traces of repair can be distinguished at the placental sites.

(*f*) *Changes in the Cervix.*—Histological examination of the cervix in anœstrus shows that the transition from the uterine to the cervical epithelium occurs rather abruptly just behind the external junction of the two cornua, before the circular muscle layers have fused. The uterine glands disappear and the columnar uterine epithelium is replaced by a layer of columnar, cuboid or flattened cells resting on a basement layer. Serial sections cut horizontally through an anœstrous cervix show local variations in the height of the epithelium and a tendency for the cells to become more flattened towards the vagina. In No. 168 the cervical epithelium, before the fusion of the two canals, is 10–15 μ thick and columnar, but in No. 155 the epithelium is cuboid and only 7–10 μ in about the same region. In No. 164 the epithelium in the constricted part of the cervix has a height of only 10 μ ; the lumen is very small.

A study of the cervix during reproductive activity shows that in the upper region the changes closely parallel those found in the uterus. As œstrus approaches the epithelium increases in height and proliferates ; in No. 213, fig. 24, Plate 12, the cervical epithelium both before and immediately behind the point of fusion of the cervical canals is composed of narrow columnar cells, 16–20 μ high, which form irregular papillæ projecting into the lumen. In most regions they do not yet appear to be secreting. Further back in the same cervix where the lumen is very small the epithelium is variable, containing both columnar and flattened cells ranging in height from 6–12 μ.

The cervix of the œstrous squirrel, No. 283, cut sagittally, appears to show the papillæ of columnar epithelium in process of desquamation. A similar section through the cervix of the pregnant No. 284 shows a glandular proliferation resembling that of the uterus in the anterior region and low cubical epithelium (10–13 μ) further back. In this part there is a leucocytic infiltration probably associated with the cell desquamation which apparently occurs at œstrus.

During lactation the cervical epithelium reverts to its anœstrous appearance.

VIII. *The Vaginal Cycle.*

(*a*) *The Resting and Active Vagina.*—The vagina of the squirrel enlarges coincidently with the development of the uterus, the greater part of the enlargement appearing to be caused by œdema of the sub-epithelial stroma. Although the vaginal epithelium is not completely uniform from the cervix to the vulva (variations being more marked during periods of reproductive activity), no attempt has been made to describe these minor regional differences. Normally sections were made through two or three different parts of each vagina, although in the pro-œstrous condition and sometimes during pregnancy the organ was difficult to cut.

In anœstrus the vaginal lumen, where present, is slit-like and surrounded by an epithelium 7–10 μ thick, consisting of 2–3 layers of flattened or cuboid cells, fig. 18, Plate 11. In No. 196, showing early signs of activity, the vaginal epithelium consists of a single layer of well-defined cuboid cells 10–15 μ in height resting on a flattened basement layer. In No. 276, which is more advanced, the vaginal epithelium is 20–30 μ thick, four layered, and already stratified ; its surface cells are flattened and have thin rod-like nuclei lying parallel to the lumen, fig. 19, Plate 11. In this specimen the epithelium has increased greatly in extent and is thrown into folds. No. 271 has a vaginal epithelium of the same thickness as No. 276 but its surface cell layers are vacuolated, flattened and degenerating. The lumen did not extend through the whole of this vagina although pro-œstrous changes had begun. In No. 209, which had a uterus similar in weight to No. 271, the vaginal epithelium in the section examined was still low, 10–20 μ, although it had extended considerably. No. 213 shows cornified vaginal epithelium about 100 μ in height; sloughing of the outer cell layers is in progress.

In the absence of the vagina of No. 283 the nearest approach to œstrus is shown by that of No. 275. The stratified epithelium shows enormous growth to a thickness of about 150 μ; the surface layers are cornified and sloughing into the lumen, fig. 20, Plate 12. The epithelium is much folded and the stroma very œdematous.

(*b*) *The Vagina during Pregnancy.*—In the present series the earliest stage after ovulation is shown by No. 73; almost all the vaginal epithelium except the basement layer has been sloughed off since œstrus; the lumen contains only small quantities of cornified debris. The remaining epithelium varies in thickness from 20–30 μ and the lining cells do not form a regular layer. Numerous polymorphs can be seen under the epithelium and also migrating through it into the lumen. In No. 66, which had a slightly older corpus luteum, the vagina is very similar. The stroma seems less œdematous than at œstrus, but there is a cornified epithelial layer. In Nos. 80 and 129 the epithelium has a height of only 7–15 μ; the nuclei next the lumen are degenerating, fig. 21, Plate 12.

Later in pregnancy (Nos. 92, 93) a regular two-layered epithelium, 10–15 μ thick, lines the vagina of which the upper cell layer becomes cuboid, fig. 22, Plate 12. These cells apparently continue to enlarge so that in more advanced pregnancies (No. 102) the vagina is lined by a single irregular columnar cell layer, 10–16 μ thick, resting on the flattened basement cells. In some parts of the vagina these cells form epithelial papillæ projecting into the lumen, fig. 23, Plate 12; there are some indications of secretion and it is possible that this type of epithelium corresponds to the mucin cells found in certain other mammals at the end of pregnancy. In some sections numerous leucocytes can be seen in the epithelium and lumen.

(*c*) *The Vagina during Lactation.*—The vagina regresses during lactation and there is no post-partum activity. The columnar cells found in late pregnancy revert first to a cuboid and subsequently to a flattened condition, though retaining for some time their round vesicular nuclei. In No. 230 the vaginal epithelium is 20–30 μ thick, but in Nos. 226 and 266 only 10–20 μ. There are a number of migrating leucocytes. In No. 110 the epithelium is similar but only about 10 μ thick, while in No. 113 the epithelium is approaching the flattened type, and in No. 9, a late lactating animal, the epithelium is only 7–10 μ thick, as in anœstrus, and the nuclei of the surface layer are flattened.

IX. *The Mammary Gland.*

The earliest stage of mammary gland development we have considered is that from No. 275, approaching œstrus. At this time the glands are barely detectable. The earliest gland obtained during pregnancy (from No. 284) shows considerable advance on this condition; development has definitely begun, the largest mammary area measuring about $1 \cdot 3 \times 1 \cdot 7$ cm., fig. 29, Plate 14. The next stage available, not complicated by a previous pregnancy, is from No. 102, fig. 30, Plate 14, and from the fœtus weight (10·3 gm.) this must represent a late stage of pregnancy. The glands are still rather undeveloped, consisting of a fine network measuring only 2·3 × 3·2 cm. across the largest nipple area. The upper thoracic pair are no bigger than at the previous stage, further, the individual areas are not confluent as is usual at the end of pregnancy. The best lactating gland obtained shows great activity; the areas have grown together to form solid strips of secreting tissue about 0·3 cm. thick, fig. 31, Plate 14. Every stage of activity and atrophy between this and the parous anœstrous condition has been found; the typical atrophied gland (No. 10) consists of a number of fairly long ducts radiating up to 2 cm. round the nipple, but quite devoid of alveoli.

The most interesting gland obtained was from No. 280, secured on June 3. This was an early pregnancy, but rather later than No. 284. The glands are thin, but large, measuring about 4·5 × 3·5 cm., the middle ones being almost rectangular. The edges, though discrete, are in close contact. Comparison, fig. 32, Plate 14, with the glands from Nos. 284, 102 and 10 shows that pregnancy alone would not produce this type of gland, nor would an atrophic gland from the previous year have persisted in this condition; it is, therefore, necessary to suppose that No. 280 had recently lactated before the existing pregnancy. This constitutes our only conclusive proof that squirrels which breed in the spring may have a second litter in the summer.

Our very best thanks are due to Mr. A. D. MIDDLETON of the Bureau of Animal Population, Oxford, who has placed records at our disposal, provided us with material, and assisted with the collection. Dr. S. ZUCKERMAN has also very kindly obtained a number of animals, and we have to thank Professor F. W. ROGERS BRAMBELL for measuring the fœtuses.

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X. *Summary.*

(1) One hundred and forty-one female grey squirrels (*Sciurus carolinensis*) have been collected over a period of two years to determine the limits of the breeding season and the salient features of the changes in the reproductive organs.

(2) Twenty-four pregnant animals were obtained or recorded; 13 between January and April, and 11 in June and July. The average number of fœtuses was 3·6, agreeing well with records of nest young.

(3) From August to the middle of January all animals obtained were prepubertal or anœstrous, except five still lactating in autumn. Parous squirrels commonly breed in spring and may do so again in summer; of the first year ones, some breed in spring, others not until the summer. There is no œstrus immediately after parturition or during lactation.

(4) During prepuberty and anœstrus the largest follicles found are a little over 600 μ. Comparatively late in œstrous development the follicle grows rapidly and reaches a diameter of about 1·0 mm. The corpus luteum soon attains full size, about 1·2 mm. in diameter, and is considerably shrunken before the end of pregnancy. The old corpora lutea disappear rapidly after parturition.

(5) The uterus of the prepubertal animal is very small, the average weight being 0·028 gm. before the onset of the changes which ultimately lead to the œstrous condition, when the weight is about 1 gm. This increase is caused by growth and glandular development, and in later stages, by distention of the lumen with fluid such as occurs in the rat and mouse. The immediate post-ovulation changes in the uterus consist of further development of the endometrial glands; in the absence of pseudo-pregnant material it has not been possible to study these changes without the complication of embryos. Following parturition, the uterus undergoes involution and becomes quiescent.

(6) The vagina during prepuberty and anœstrus is lined by a thin layer of flattened epithelium. As œstrus approaches the vagina enlarges and the epithelium thickens and becomes cornified; the vulva swells and becomes patent. In late pregnancy the vaginal epithelium becomes columnar; during lactation it returns to the resting condition.

(7) Cyclic changes also take place in the Fallopian tube, cervix and mammary glands.

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DESCRIPTION OF PLATES.

Plate 9.

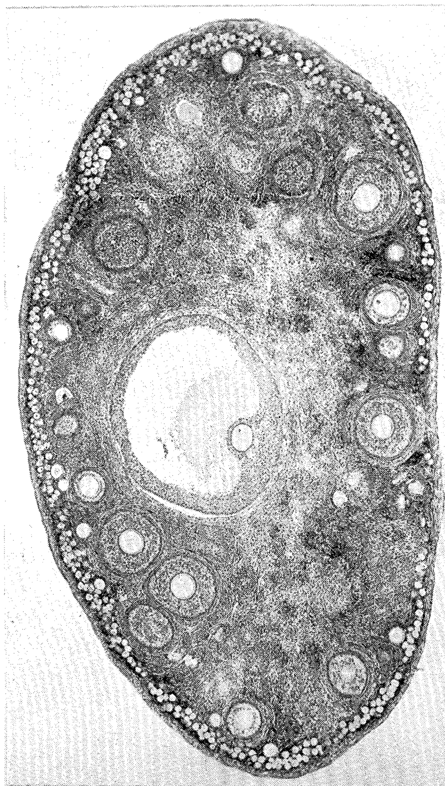
Ovary.

Fig. 5.—No. 158. Anœstrous ovary, December, showing absence of large follicles, and of organized interstitial tissue. × 34.

Fig. 6.—No. 87. Pro-œstrous ovary, June, showing large follicle. × 34.

Fig. 7.—No. 73. Developing corpus luteum, showing theca interna. × 68.

Fig. 8.—No. 80. Corpora lutea during early pregnancy. × 68.



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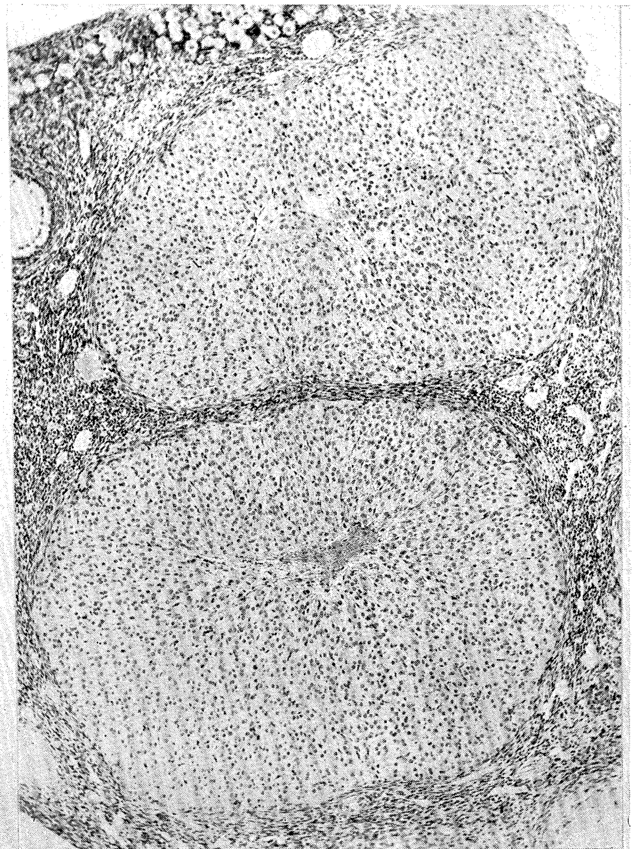

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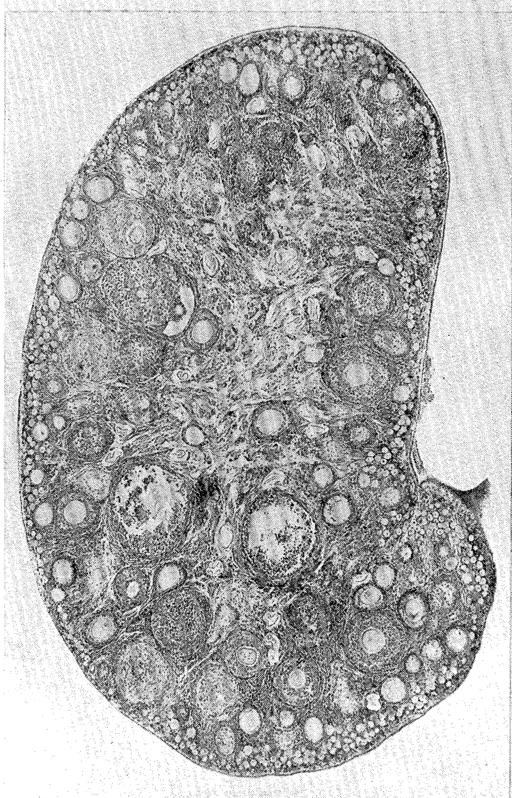

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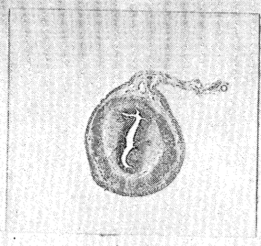

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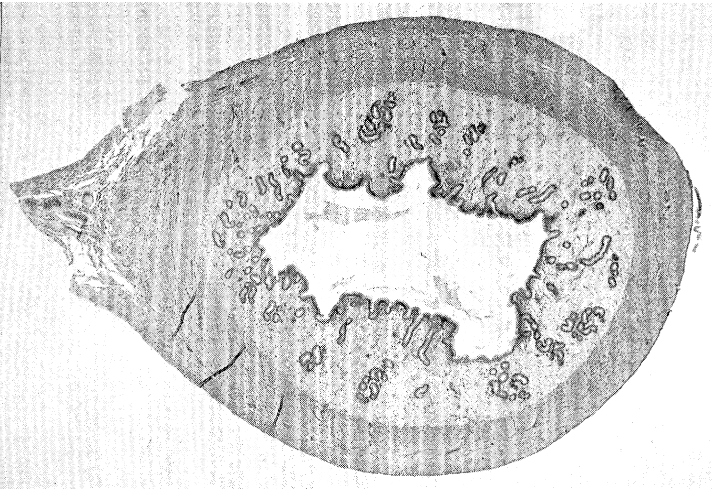

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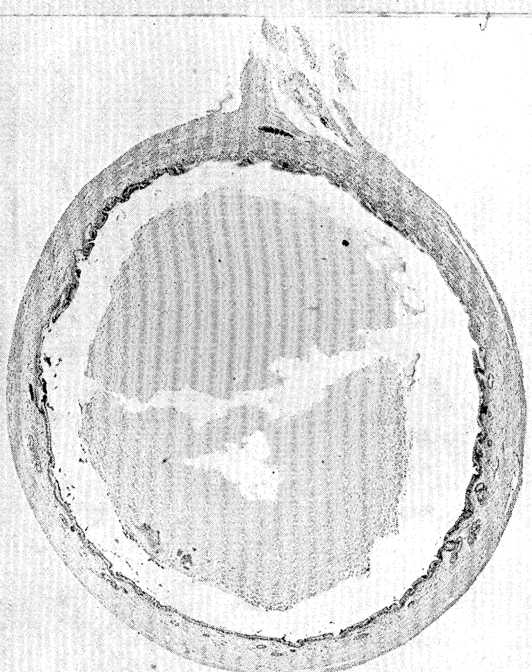

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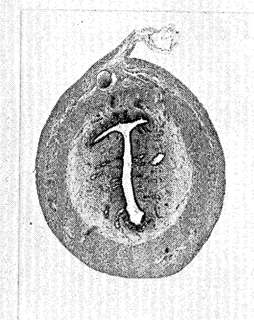

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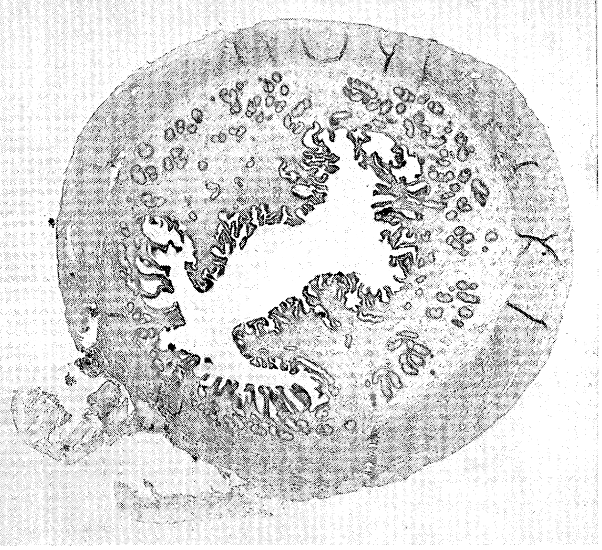

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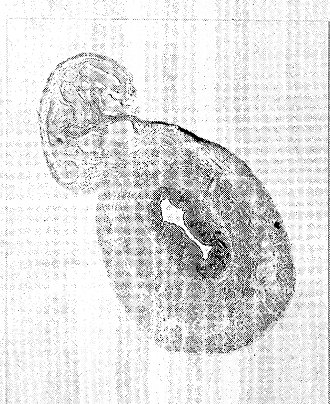

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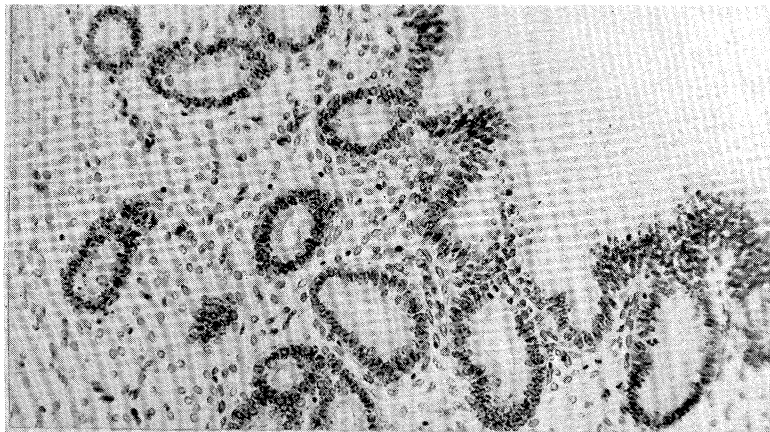

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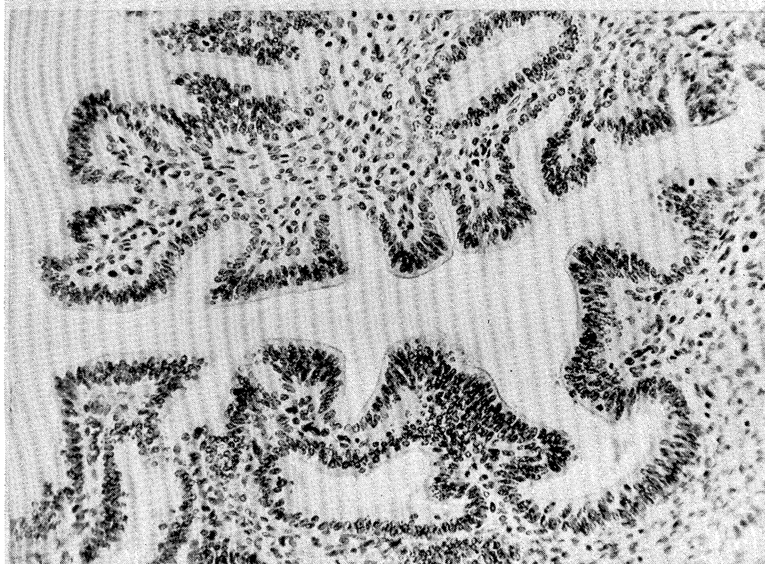

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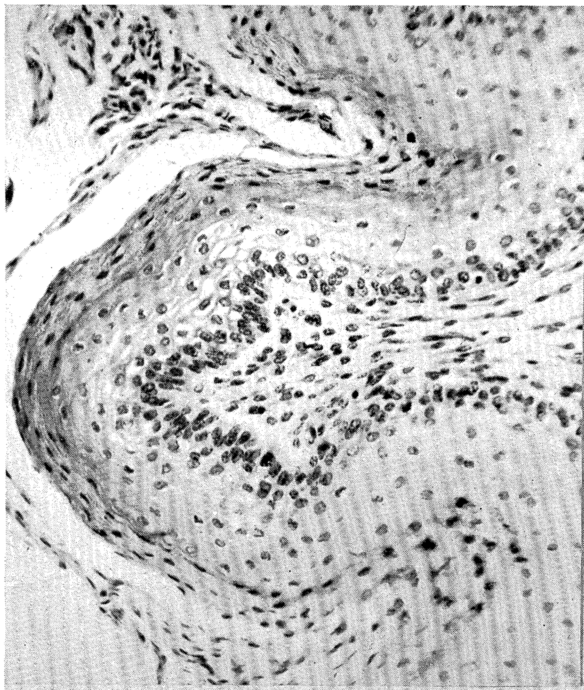

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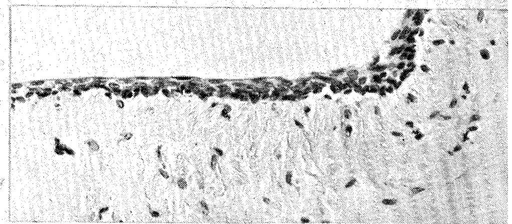

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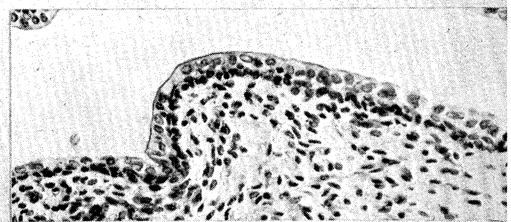

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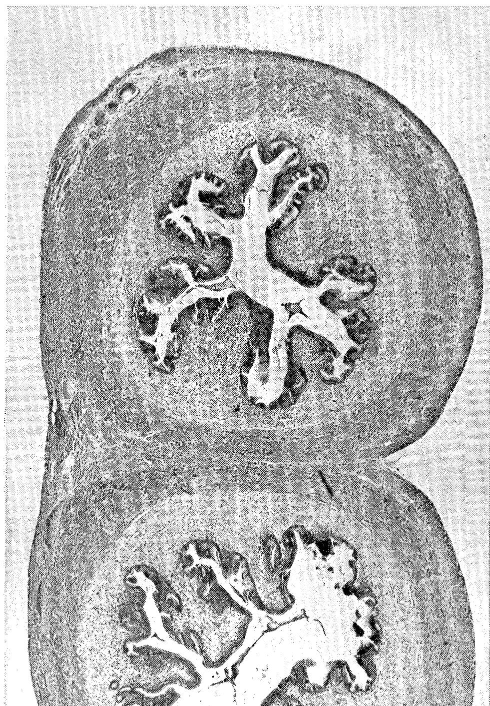

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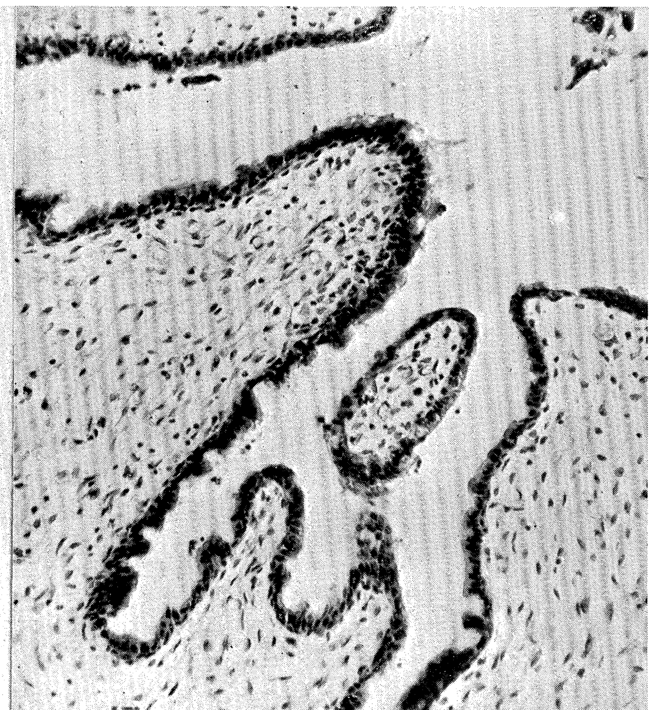

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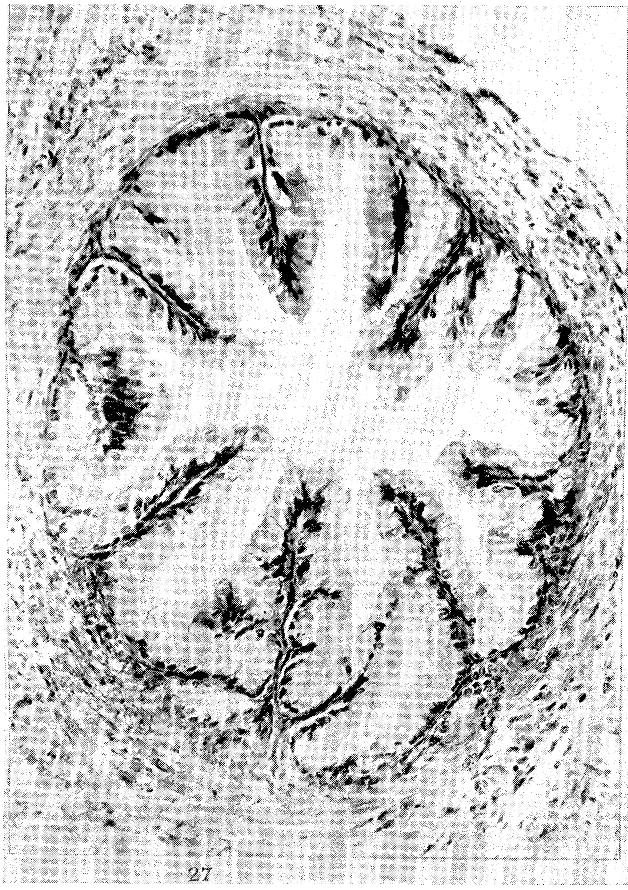

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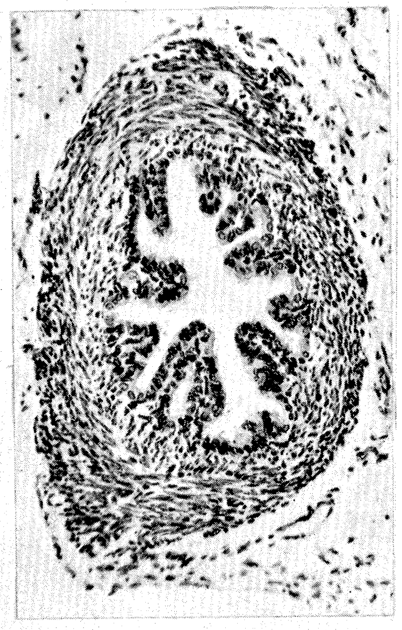

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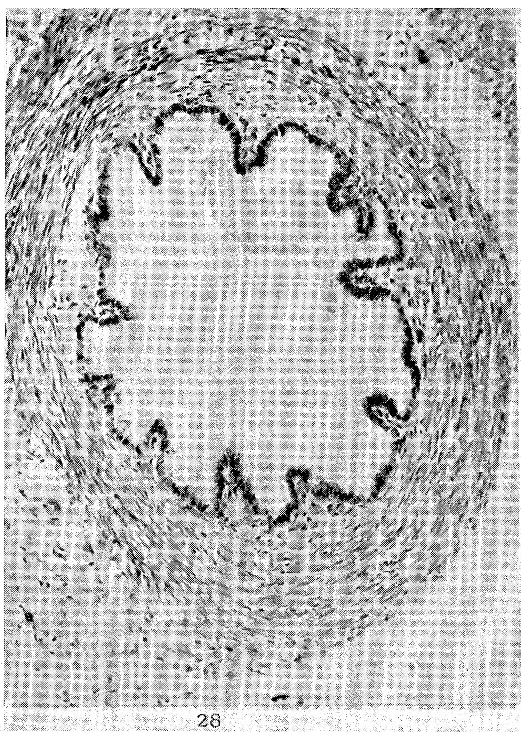

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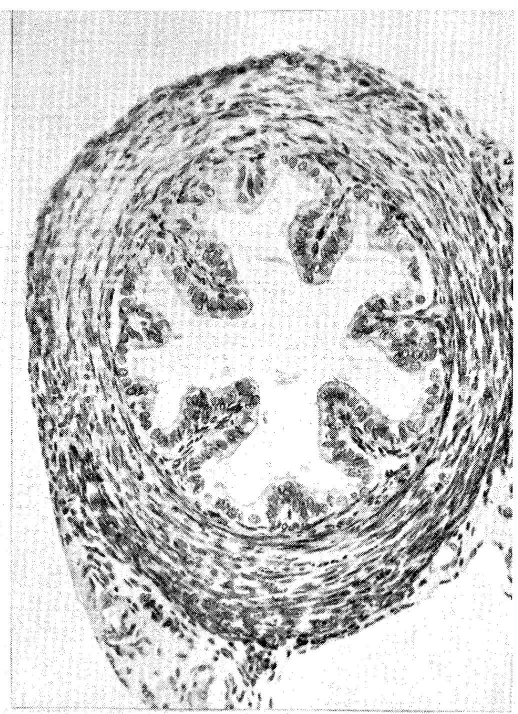

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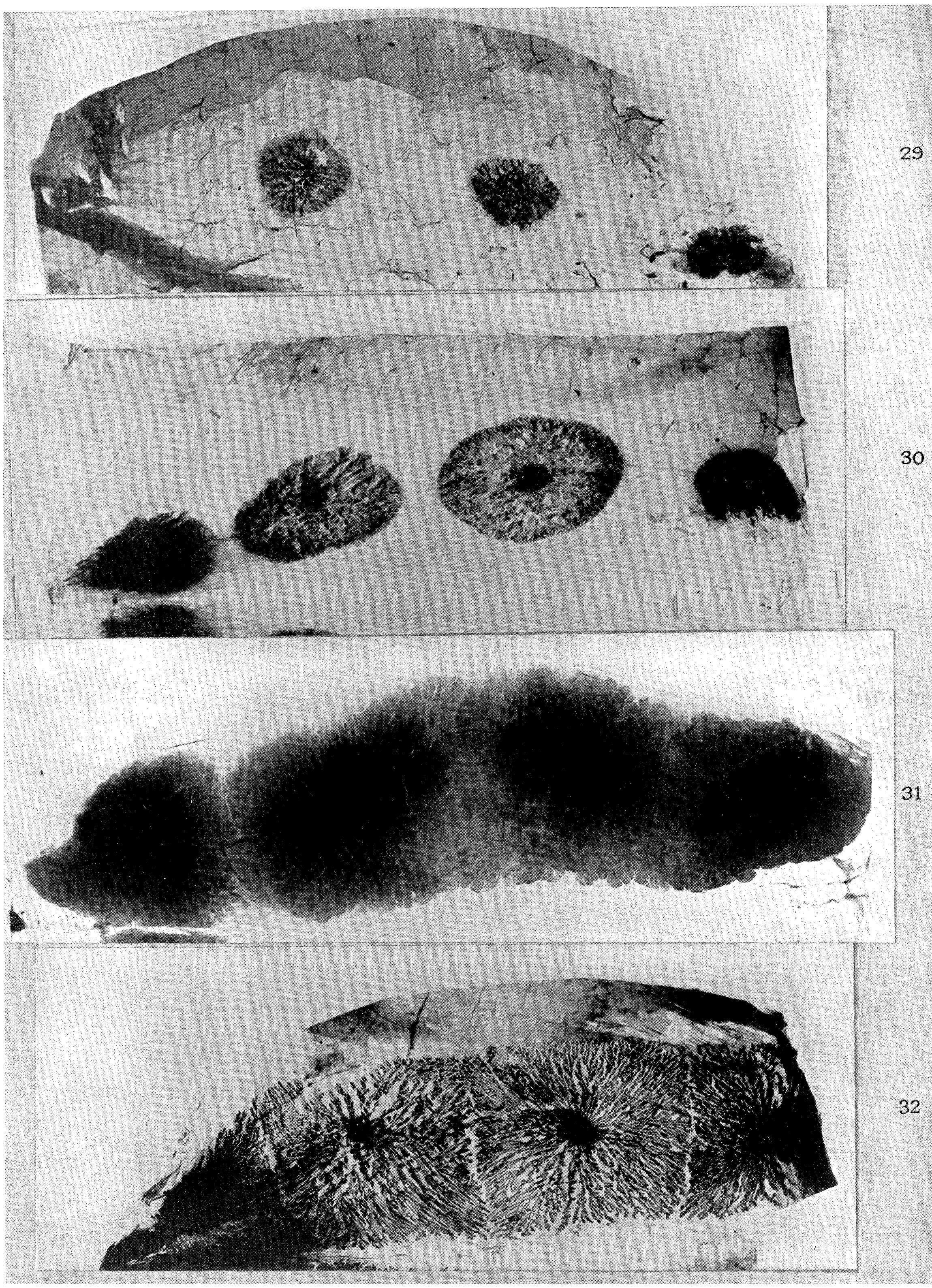

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PLATE 10.

Uterus. × 16.

FIG. 9.—No. 159. December, non-parous.

FIG. 10.—No. 143. December, parous, anœstrous, showing dense stroma and thick wall.

FIG. 11.—No. 196. January, non-parous, showing enlargement of uterus, growth of the glands and œdema of the stroma.

FIG. 12.—No. 87. June, pro-œstrous uterus, showing great increase in size, growth of the epithelium and glands, and œdema of the stroma.

FIG. 13.—No. 283. May, œstrous uterus, showing distention of the lumen and presence of spermatozoa.

FIG. 14.—No. 66. June, post-ovulation uterus, showing progestational proliferation of the endometrium.

PLATE 11.

Uterus and Vagina. × 165.

FIG. 15.—No. 87. June, part of section shown in fig. 12, Plate 10, showing height of epithelium and gland cells and œdema of the stroma.

FIG. 16.—No. 73. June, progestational proliferation of the uterine endometrium corresponding to the developing corpus luteum shown in fig. 7, Plate 9.

FIG. 17.—No. 66. June, part of fig. 14, Plate 10.

FIG. 18.—No. 177. December, anœstrous vagina, showing the low epithelium.

FIG. 19.—No. 276. May, vagina, beginning of œstrous activity, epithelium thickened and beginning to be stratified.

PLATE 12.

Vagina. × 165. *Cervix.* × 17.

FIG. 20.—No. 275. May, pro-œstrous vagina, cornified cells sloughing into lumen.

FIG. 21.—No. 129. June, very early pregnancy, vaginal epithelium low.

FIG. 22.—No. 93. July, pregnancy, vaginal epithelium, showing development of layer of cuboid cells.

FIG. 23.—No. 102. July, late pregnancy, showing vaginal epithelium, irregular columnar cells.

FIG. 24.—No. 213. March, cervix showing early pro-œstrous growth of epithelium.

PLATE 13.

Fallopian Tube. × 154.

FIG. 25.—No. 148. December, anœstrous condition.

FIG. 26.—No. 271. May, early stage of œstrous growth of the epithelium. Tube increased in diameter.

FIG. 27.—No. 87. June, condition of tube just before œstrus. Further increase in size of tube ; epithelium high, with cilia.

FIG. 28.—No. 80. June, early pregnancy. Epithelium shrunk and disorganized, and traces of secretion in the lumen.

PLATE 14.

Mammary Gland.

FIG. 29.—No. 284. June, early pregnancy.

FIG. 30.—No. 102. July, late pregnancy.

FIG. 31.—No. 9. October, lactating.

FIG. 32.—No. 280. June, early pregnancy superimposed on recent lactation.

APPENDICES.

Tables showing the date obtained, body weight, ovary weight, diameter of largest follicle, and uterus weight of all squirrels referred to in the text.

N.A. = no follicles with antrum ; N.Y. = nest young ; O.C.L. = old corpus luteum ; P.S. = placental site.

An * against the "diameter of largest follicle" indicates that follicles were measured in both ovaries.

Appendix 1.

All prepubertal animals (3–11 months old) which may reach puberty in time for spring breeding. Arranged according to month and uterus weight.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).	Condition.
			Weight (gm.).	Diameter of largest follicle (μ).		
281	June 4, 1932	—	0·012	N.A.	0·018	Undeveloped.
112	August 28, 1931	—	0·015	N.A.	0·049	,,
1	October 11, 1930	200	—	N.A.*	—	,,
2	October 17, 1930	250	—	N.A.*	—	,,
7	October 8, 1930	370	—	N.A.*	—	,,
8	October 8, 1930	400	—	N.A.	—	Signs of activity.
124	October 26, 1931	300	—	—	0·033	Undeveloped.
133	November 26, 1931	320	0·021	380	0·017	,,
16	November 12, 1930	450	0·016	N.A.	0·031	,,
68	November —, 1931	400	—	N.A.	—	,,
25	November 24, 1930	500	0·008	N.A.	0·037	,,
14	November 3, 1930	—	—	520	—	,,
176	December 18, 1931	380	0·009	N.A.	0·018	,,
159	December 12, 1931	310	0·009	N.A.	0·019	,,
175	December 18, 1931	450	0·010	N.A.	0·019	,,
179	December 18, 1931	470	0·012	N.A.	0·019	,,
200	December 29, 1931	—	0·013	N.A.	0·019	,,
158	December 12, 1931	340	0·019	430	0·019	,,
174	December 17, 1931	470	0·010	N.A.	0·023	,,
172	December 17, 1931	560	0·015	550	0·023	,,
153	December 7, 1931	—	0·018	N.A.	0·023	,,
155	December 7, 1931	—	0·013	N.A.	0·024	,,
169	December 17, 1931	460	0·011	N.A.	0·024	,,
165	December 15, 1931	440	0·013	450	0·024	,,
197	December 29, 1931	—	0·012	N.A.	0·026	,,
167	December 15, 1931	400	0·025	540	0·027	,,
160	December 12, 1931	360	0·020	N.A.	0·031	,,
184	December 16, 1931	—	0·010	580	0·036	,,
168	December 17, 1931	540	0·024	570	0·038	,,
152	December 11, 1931	500	0·034	500	0·046	Signs of activity.
146	December 11, 1931	530	0·030	590	0·047	Undeveloped.
193	December 29, 1931	440	0·029	560	0·054	Signs of activity.
144	December 7, 1931	430	0·018	620	0·067	,,
195	January 2, 1932	480	0·029	520	0·024	,,
196	January 2, 1932	500	0·026	690	0·089	Active.

Appendix 2.

Prepubertal animals (8–15 months old) which failed to reach puberty in the spring and are coming up for summer breeding. Arranged according to month and uterus weight.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).	Condition.
			Weight (gm.).	Diameter of largest follicle (μ).		
246	March 24, 1932 . . .	—	0·006	N.A.	0·008	Undeveloped.
242	March 24, 1932 . . .	—	0·009	N.A.	0·016	,,
227	March 19, 1932 . . .	480	0·010	N.A.	0·020	,,
255	March 26, 1932 . . .	—	0·028	590	0·023	,,
221	March 14, 1932 . . .	410	0·014	N.A.	0·028	,,
245	March 24, 1932 . . .	—	0·023	580	0·066	Signs of activity.
253	April 7, 1932	—	0·015	N.A.	0·024	Undeveloped.
249	April 17, 1932 . . .	390	0·011	520	0·025	,,
257	April 21, 1932 . . .	610	0·017	600	0·033	,,
251	April 9, 1932	—	0·019	590	0·038	,,
286	April 19, 1932 . . .	—	0·034	600	0·039	Signs of activity.
285	April 22, 1932 . . .	—	0·010	520	0·044	Undeveloped.
264	May 5, 1932	400	0·009	N.A.	0·021	,,
269	May 12, 1932 . . .	480	0·021	600	0·036	,,
282	May 26, 1932 . . .	—	0·024	620	0·056	Signs of activity.
268	May 12, 1932 . . .	580	0·024	620	0·064	,,
276	May 19, 1932 . . .	490	0·031	600	0·071	Active.
275	May 19, 1932 . . .	600	0·024	780*	0·726	Pro-œstrous.
283	May 25, 1932 . . .	—	0·050	1·10	1·170	Œstrous.
85	June 27, 1931 . . .	—	0·018	450*	0·034	Undeveloped.
87	June 26, 1931 . . .	520	0·037	860*	—	Pro-œstrous (uterus similar to No. 275).

Appendix 3.

Parous squirrels coming up for spring breeding.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).	Condition.
			Weight (gm.).	Diameter of largest follicle (μ).		
38	February 25, 1931 .	—	0·025	620	0·147	Beginning to be active.
43	March 18, 1931 . . .	700	0·040	600	0·182	,, ,,
209	February 29, 1932 .	540	0·032	640	0·302	Half-developed.
213	February 20, 1932 .	590	—	660	0·442	,,

APPENDIX 4.

Parous animals from February to July, which are lactating or quiescent, presumably post-lactation. Arranged in descending order of uterus weight to show involution. Some of the quiescent animals may have gone into early anœstrus.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).	Condition.	Notes.
			Weight (gm.).	Diameter of largest follicle (μ).			
219	March 12, 1932	—	0·032	630	2·55	Lactating	3 P.S. 2 O.C.L. 1 N.Y. of 23 gm.
230	March 19, 1932	580	0·029	550	1·00	,,	2 O.C.L.
226	March 19, 1932	520	0·023	560	0·92	,,	2 P.S. 2 O.C.L.
252	March 29, 1932	—	0·027	650	0·497	,,	No O.C.L.
238	March 17, 1932	630	0·027	590	0·418	,,	4 P.S.
47	April 16, 1932	440	0·024	450	0·387	Early post-partum	Uterus involuting.
63	May 26, 1932	400	0·015	N.A.	0·377	,,	Uterus involuting. Mammary gland not in use.
248	March 24, 1932	—	0·027	590	0·285	Lactating	—
44	February 4, 1931	500	0·029	560	0·238	Post-partum	Uterus involuting.
228	March 19, 1932	530	0·018	570	0·236	Lactating	3 P.S. 3 N.Y. of Av. 47 gm. No O.C.L.
95	July 2, 1931	—	0·022	N.A.	0·231	Quiescent	—
244	March 24, 1932	—	0·023	530	0·230	Early post-partum	2 O.C.L.
48	May 5, 1932	500	0·026	500	0·230	Post-partum	Uterus involuting.
250	April 17, 1932	480	0·016	N.A.	0·223	Lactating	—
84	June 27, 1931	—	0·023	490	0·215	,,	Uterus involuting.
266	May 5, 1932	640	0·054	660	0·194	,,	3 P.S.
265	May 5, 1932	660	0·031	620	0·161	Quiescent	—
263	April 25, 1932	420	0·018	430	0·156	Lactating	—
256	April 21, 1932	560	0·019	670	0·145	Quiescent	Mammary gland atrophic.
82	June 27, 1931	—	0·022	N.A.	0·141	,,	Post lactation.
243	March 24, 1932	—	0·020	560	0·140	,,	—
94	July 2, 1931	—	0·021	510	0·120	,,	—
62	May 19, 1931	—	0·022	560	0·139	,,	Mammary gland atrophic.
72	June 17, 1931	—	0·022	570	0·135	,,	—
262	April 26, 1932	450	0·018	550	0·121	,,	Mammary gland atrophic.
233	March 23, 1932	480	0·018	570	0·094	Post-partum	Uterus involuting.
254	March 26, 1932	—	0·024	570	0·084	Quiescent	Doubtful if recent litter.
261	April 25, 1932	460	0·028	520	0·083	,,	—
247	March 24, 1932	—	0·024	520	0·078	,,	—
57	May 5, 1931	400	0·019	570	0·071	,,	—
53	April 24, 1931	—	—	450	—	,,	—

Notes on Appendix 4.—The term "lactating" is reserved for squirrels definitely known to be suckling; "post-partum" for those showing post-partum involution of the uterus, etc., where the mammary glands were not available; "quiescent" for those having no signs of reproductive activity but in some cases known to be post-lactation by the mammary glands.

APPENDIX 5.

Parous animals coming up for summer breeding. These have probably had a litter in the spring. All three are early stages of development from lactation quiescence.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).	Condition.
			Weight (gm.).	Diameter of largest follicle (μ).		
287	May 12, 1932 . . .	—	0·038	730	0·151	Signs of activity.
65	June 8, 1931 . . .	400	0·028	570	0·222	Active.
271	May 19, 1932 . . .	570	0·025	740	0·294	,,

Appendix 6.

Pregnant animals.

No. of animal.	Date.	Body weight (gm.).	Ovaries.				Fœtuses.						Notes.
			Weight (gm.).	Diameter of largest follicle (μ).	No. of corpora lutea.	Average diameter mm. of corpora lutea.	No.	Right.	Left.	Average length (mm.).	Average weight of nodule (gm.).	Average weight of fœtuses (gm.).	
73	June 17, 1931	—	0·035	420	2 + 1	1·13	—	—	—	—	—	—	—
66	June 8, 1931	—	0·035	800	3 + 1	1·01	—	—	—	—	—	—	—
80	June 22, 1931	400	0·029	670	2 + 1	0·99	3	2	1	—	0·179	—	—
284	June 24, 1932	580	0·038	970	1 + 3	1·29	4	1	3	—	0·198	—	—
129	June 20, 1931	—	0·028	640	1 (1 ovary)	1·18	1 (1 horn)	—	—	—	0·305	—	—
280	June 4, 1932	—	0·044	650	2 + 1	1·09	3	2	1	—	1·08	—	—
92	July 2, 1931	—	0·027	600	1 + 3	1·00	4	1	3	13·5	2·45	0·294	—
93	July 2, 1931	—	0·036	600	2 + 1	1·07	3	2	1	17·5	—	0·87	—
103	July 24, 1931	470	0·028	610	1 + 3	0·88	2	1	1	—	—	—	No fœtuses. Probably re-absorbing.
107	July 29, 1931	—	0·039	640	2 + 2	0·74	3	2	1	25·6	5·42	1·87	
102	July 17, 1931	—	0·030	830	1 + 3	0·74	4	1	3	40·0	—	10·34	

APPENDIX 7.

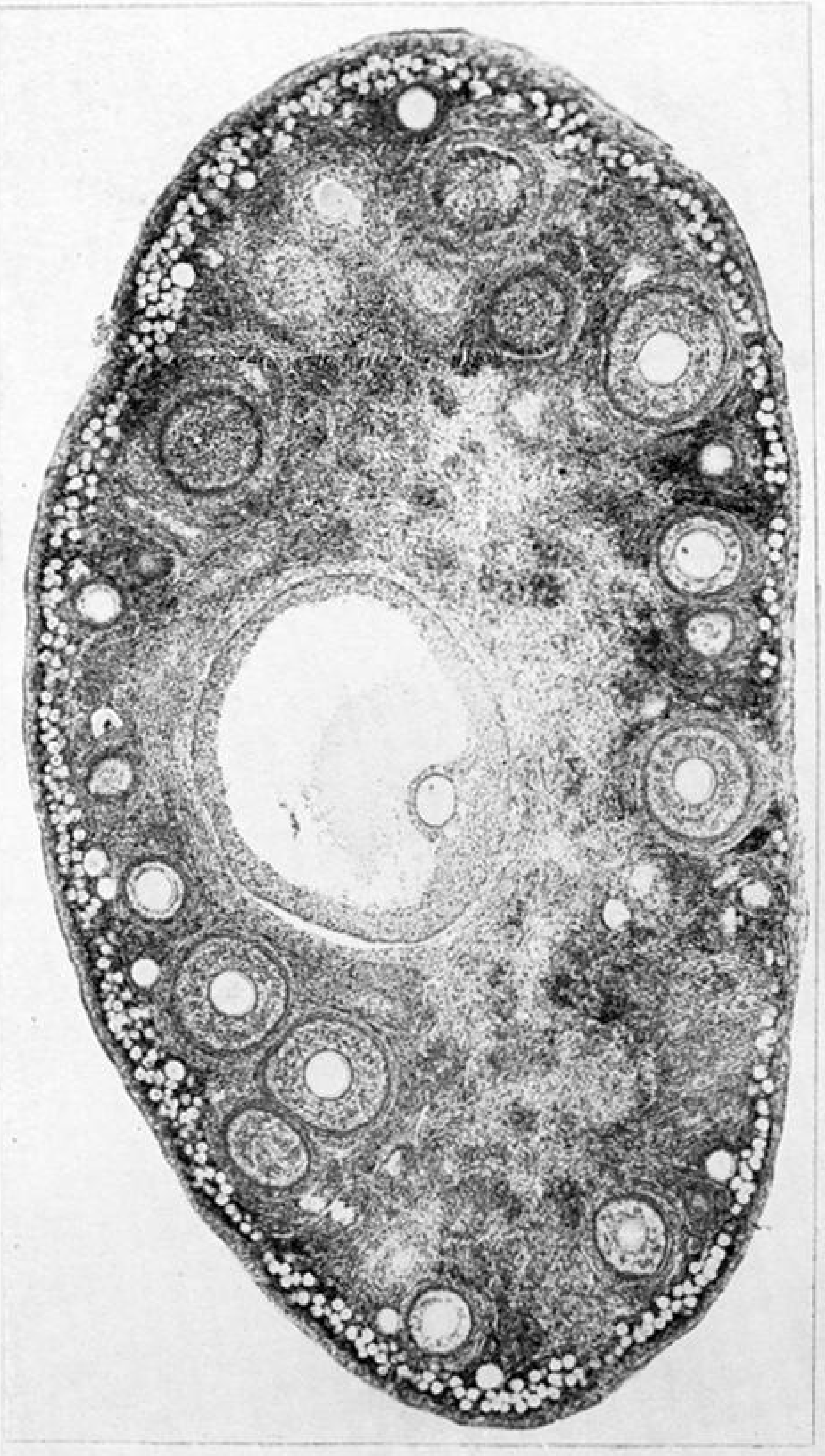
Parous animals lactating (or early post-partum) after summer breeding. Arranged in descending order of uterus weights.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).	Notes.
			Weight (gm.).	Diameter of largest follicle (μ).		
113	August 28, 1931 . .	—	0·026	630	0·223	Post-partum. 3 O.C.L.
122	October 10, 1931 . .	—	0·021	600	0·191	Lactating.
110	August 26, 1931 . .	400	0·020	310	0·171	Post-partum. 3 O.C.L.
119	September 10, 1931	—	0·025	610	0·161	Post-partum. 3 O.C.L.
9	October 8, 1930 . .	610	—	N.A.	—	Lactating.

Appendix 8.

Anœstrous parous animals from September to January showing inactivity of the reproductive tract. The list includes all animals obtained during these months except one in September and two in October, which were lactating or post-partum. It is possible that a few late lactators are included in this list among those for which mammary glands are not available.

No. of animal.	Date.	Body weight (gm.).	Ovaries.		Uterus weight (gm.).
			Weight (gm.).	Diameter of largest follicle (μ).	
117	September 10, 1931 . .	—	0·022	680	0·132
118	September 10, 1931 . .	—	0·023	560	—
121	October 10, 1931	—	0·019	590	0·083
3	October 7, 1930	450	—	N.A.*	—
10	October 10, 1930	710	—	N.A.*	—
11	October 10, 1930	530	—	N.A.	—
12	October 10, 1930	570	—	N.A.	—
26	November 24, 1930 . . .	—	0·010	N.A.	0·263
19	November 17, 1930 . . .	—	0·018	N.A.	0·185
131	November 23, 1931 . . .	470	0·019	590	0·126
69	November —, 1930 . . .	450	0·019	530	0·118
24	November 24, 1930 . . .	700	0·016	490	0·103
4	November 5, 1930 . . .	550	0·026	680	0·103
23	November 24, 1930 . . .	700	0·013	N.A.	0·069
27	November 24, 1930 . . .	600	0·007	N.A.	0·054
148	December 11, 1931 . . .	560	0·009	N.A.	0·097
29	December 11, 1930 . . .	500	0·013	N.A.	0·100
198	December 29, 1931 . . .	—	0·025	590	0·106
185	December 16, 1931 . . .	—	0·013	690	0·108
178	December 18, 1931 . . .	490	0·013	530	0·109
151	December 11, 1931 . . .	540	0·022	570	—
154	December 7, 1931 . . .	—	0·019	600	0·120
177	December 18, 1931 . . .	640	0·019	N.A.	0·130
199	December 29, 1931 . . .	—	0·020	610	0·142
180	December 22, 1931 . . .	450	0·024	590	0·145
173	December 17, 1931 . . .	580	0·022	640	0·147
171	December 17, 1931 . . .	640	0·014	680	0·169
143	December 7, 1931 . . .	450	0·012	N.A.	0·184
164	December 15, 1931 . . .	460	0·022	620	0·188
31	January 6, 1930	500	0·024	570	0·103
32	January 12, 1930 . . .	—	0·022	550	0·161



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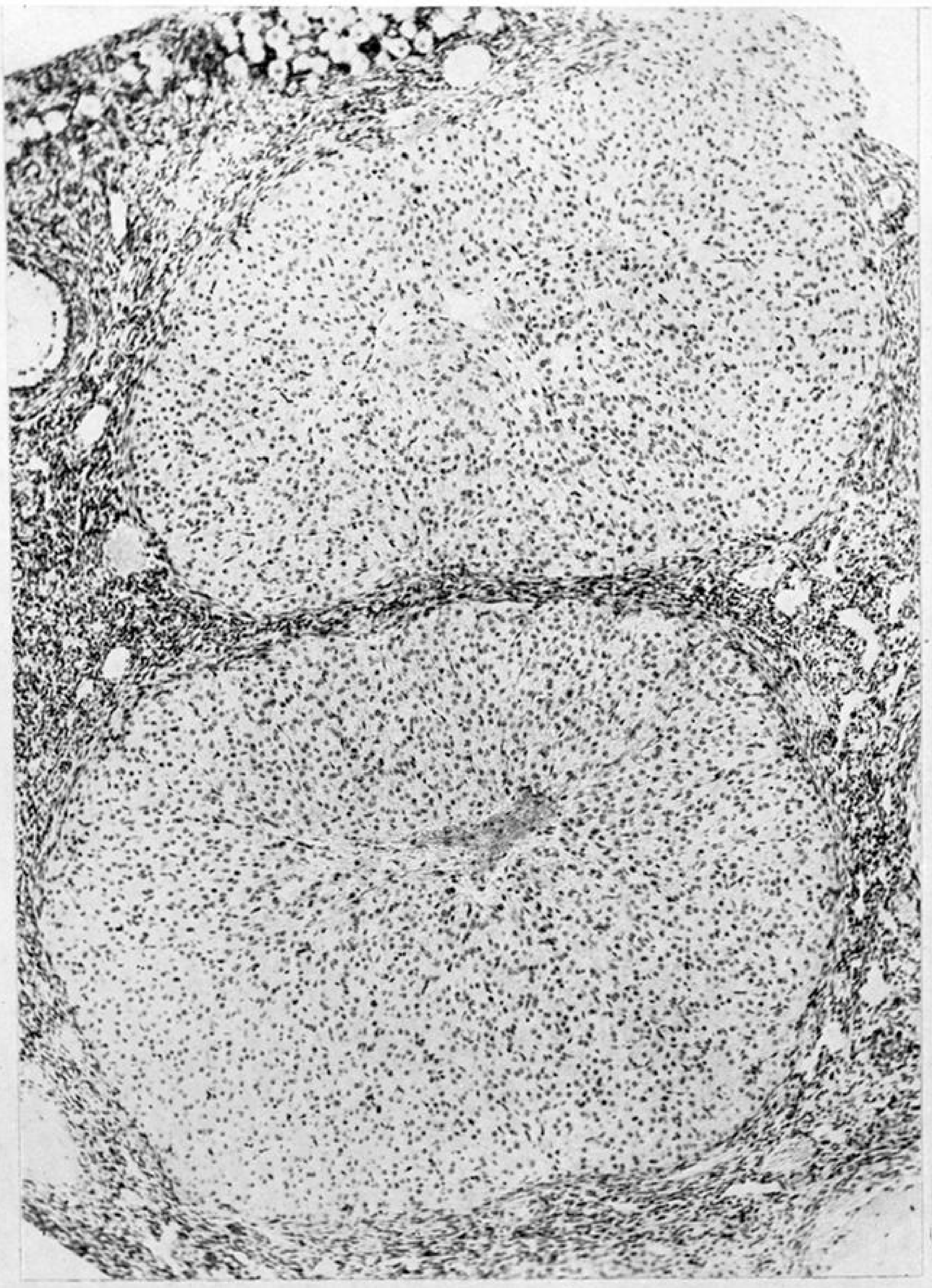

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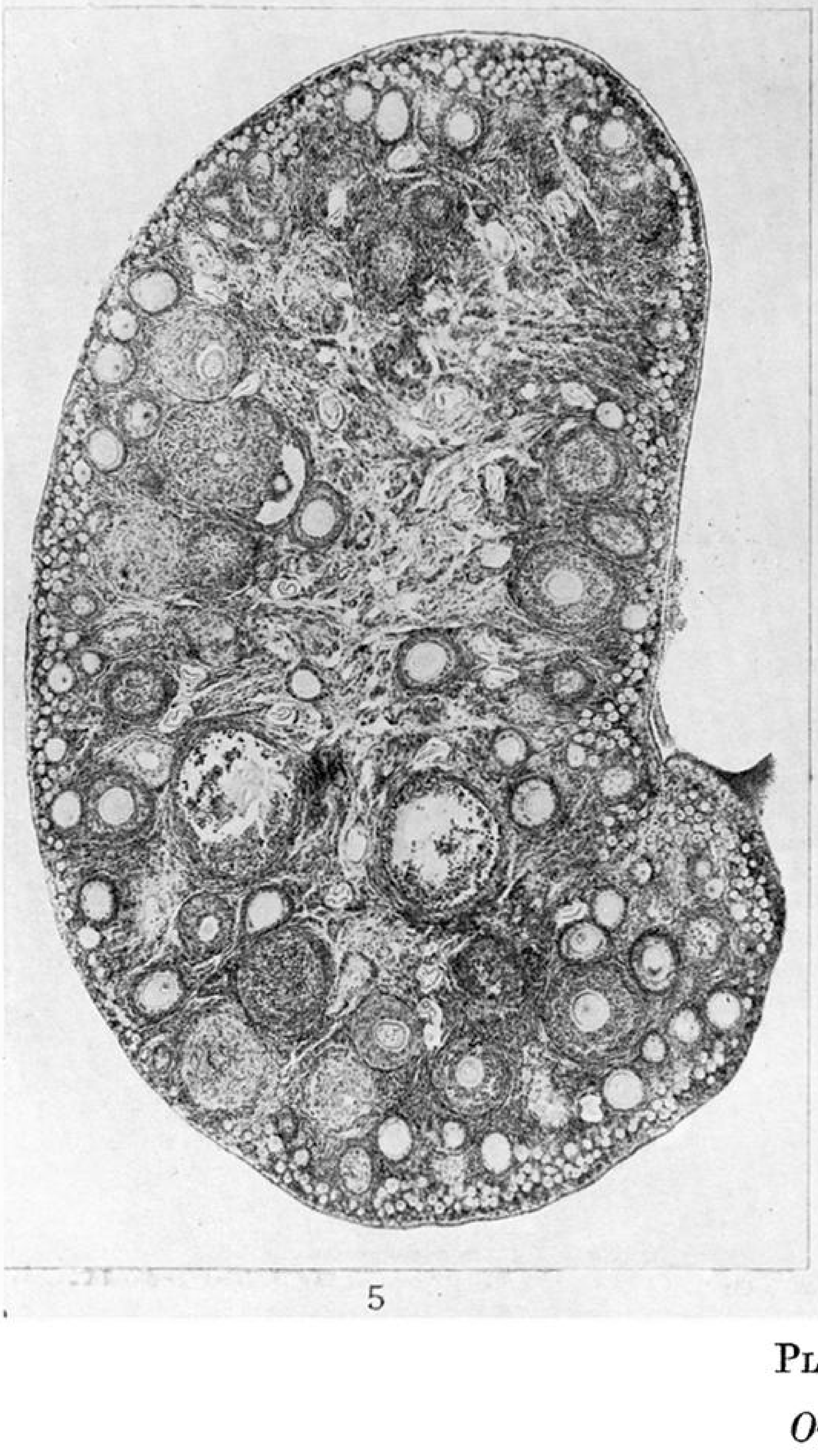

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PLATE 9.

Ovary.

FIG. 5.—No. 158. Anœstrous ovary, December, showing absence of large follicles, and of organized interstitial tissue. × 34.

FIG. 6.—No. 87. Pro-œstrous ovary, June, showing large follicle. × 34.

FIG. 7.—No. 73. Developing corpus luteum, showing theca interna. × 68.

FIG. 8.—No. 80. Corpora lutea during early pregnancy. × 68.

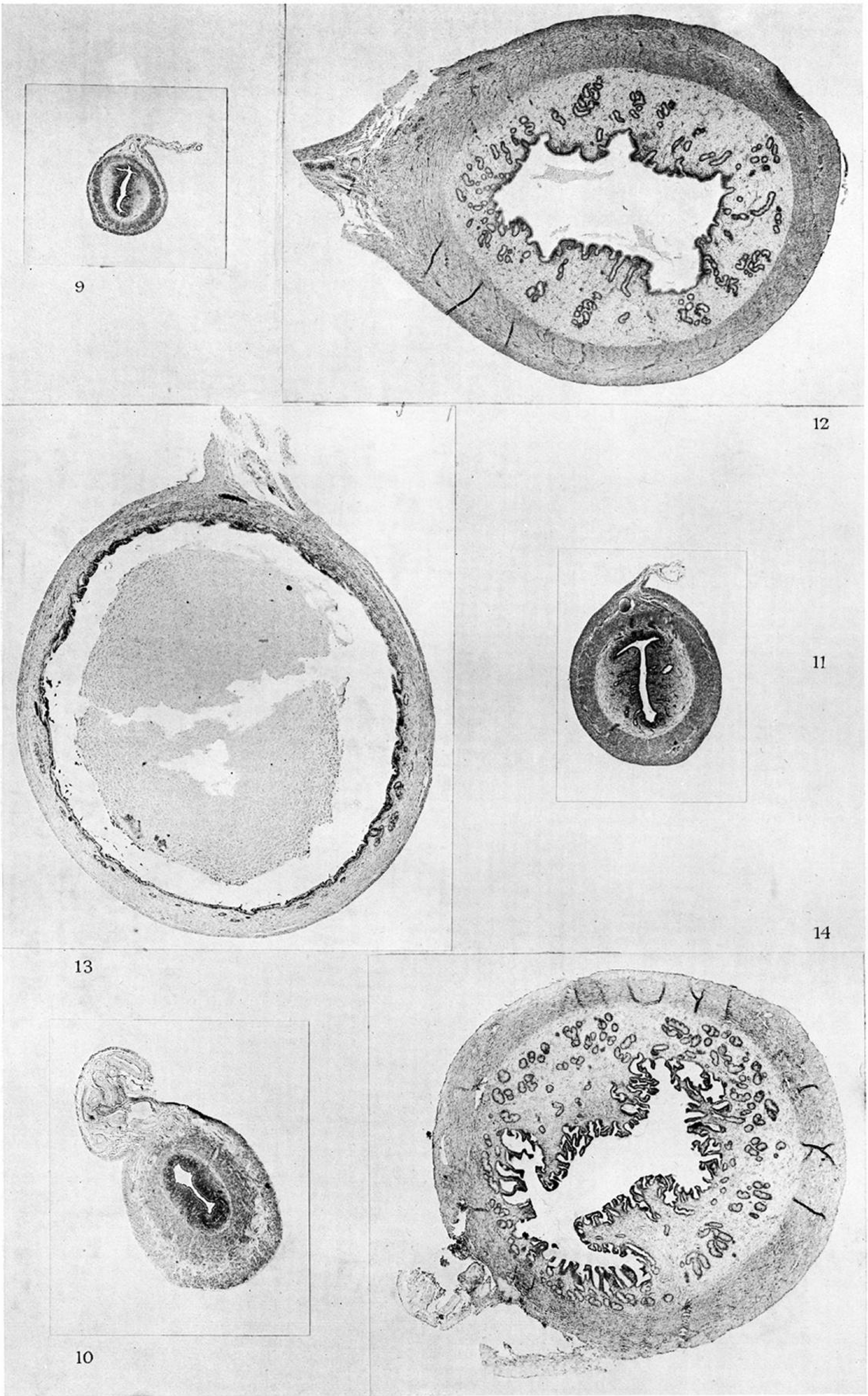


Plate 10.

Uterus. × 16.

Fig. 9.—No. 159. December, non-parous.

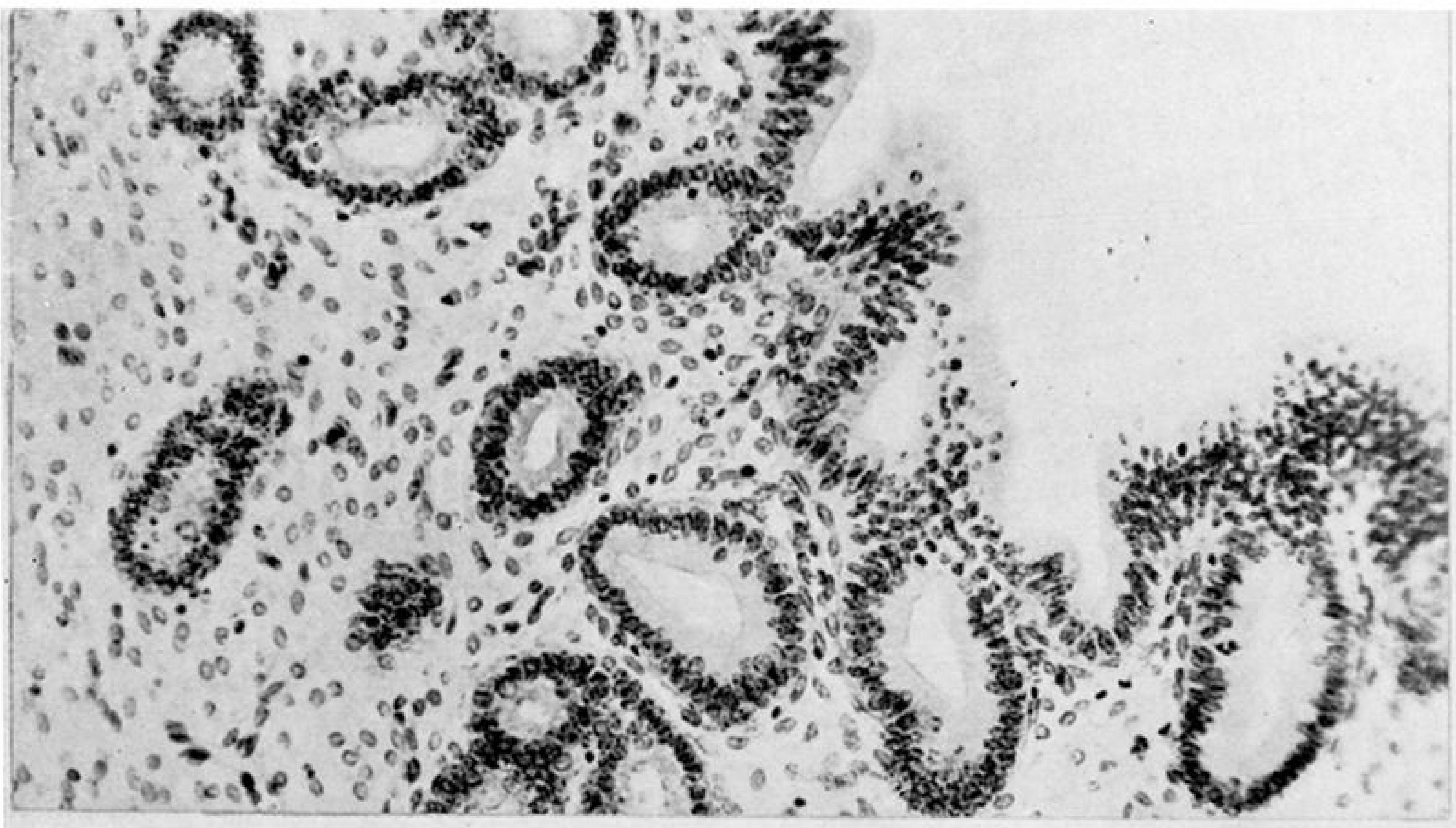
Fig. 10.—No. 143. December, parous, anœstrous, showing dense stroma and thick wall.

Fig. 11.—No. 196. January, non-parous, showing enlargement of uterus, growth of the glands and œdema of the stroma.

Fig. 12.—No. 87. June, pro-œstrous uterus, showing great increase in size, growth of the epithelium and glands, and œdema of the stroma.

Fig. 13.—No. 283. May, œstrous uterus, showing distention of the lumen and presence of spermatozoa.

Fig. 14.—No. 66. June, post-ovulation uterus, showing progestational proliferation of the endometrium.



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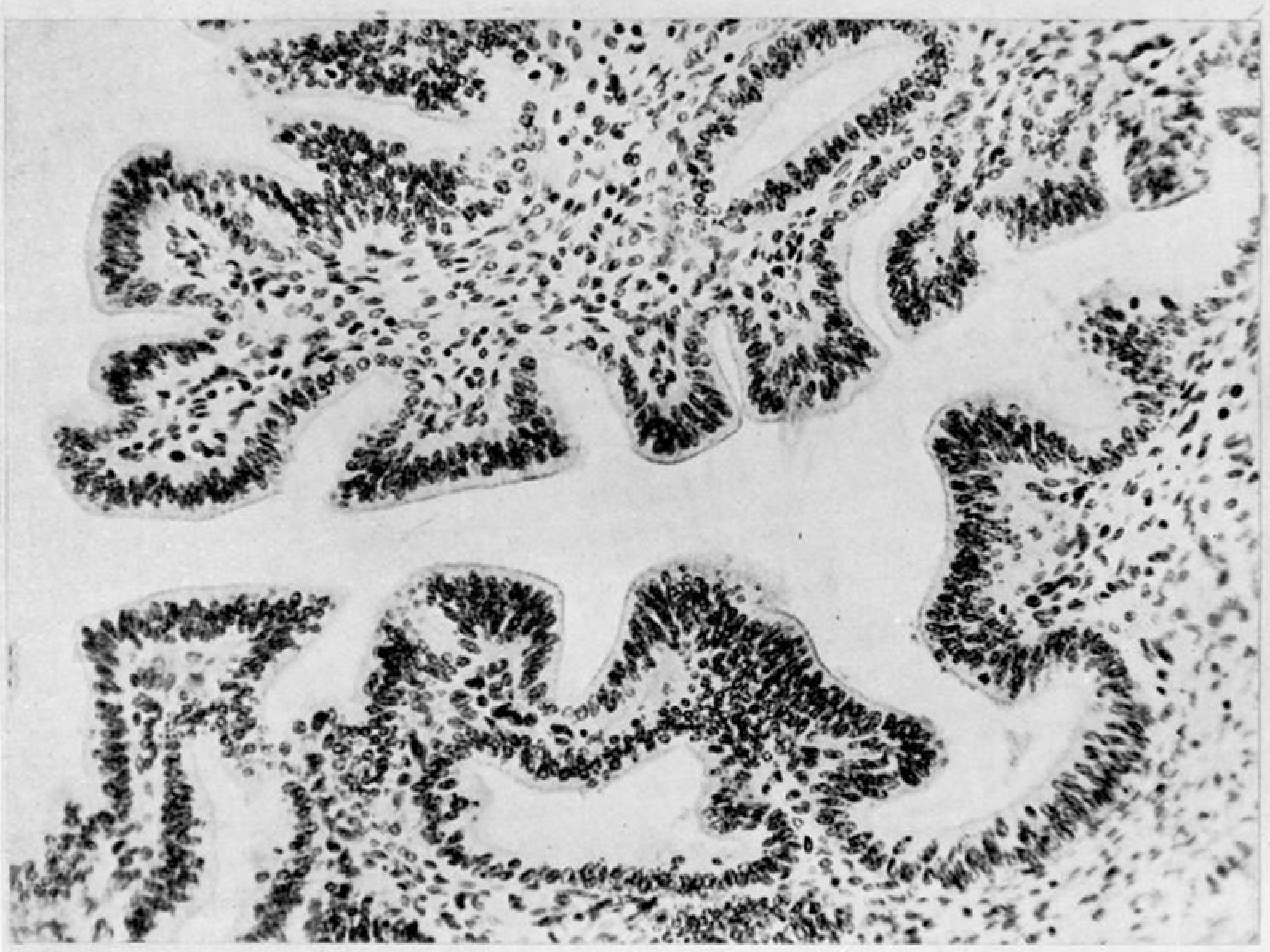

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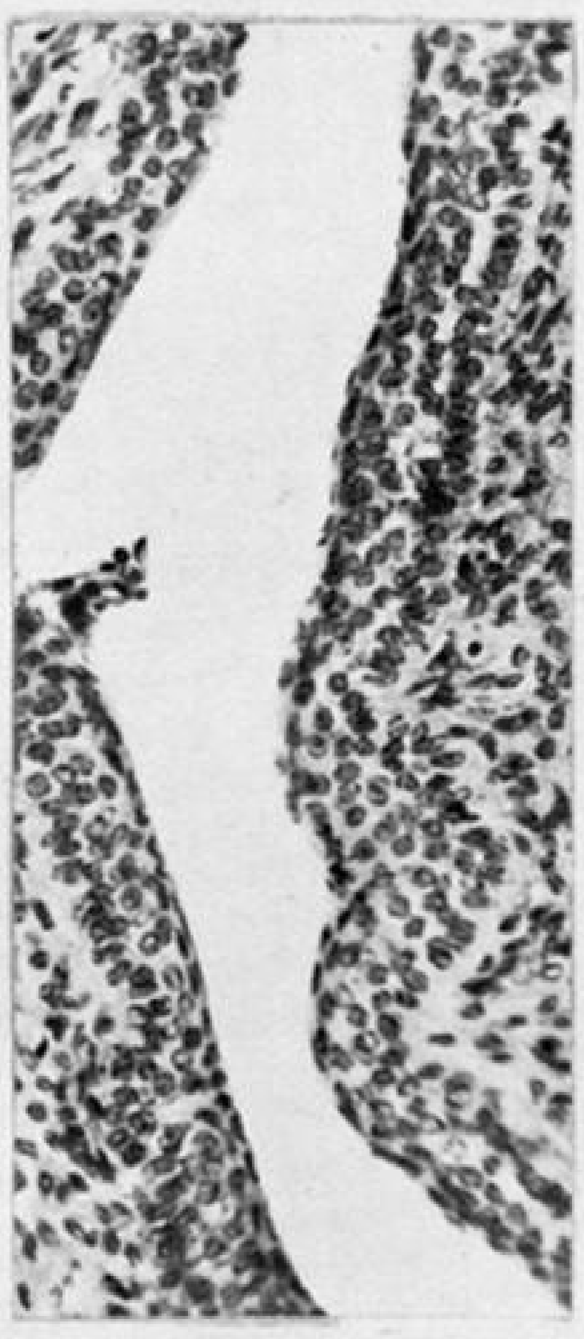

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Plate 11.

Uterus and Vagina. × 165.

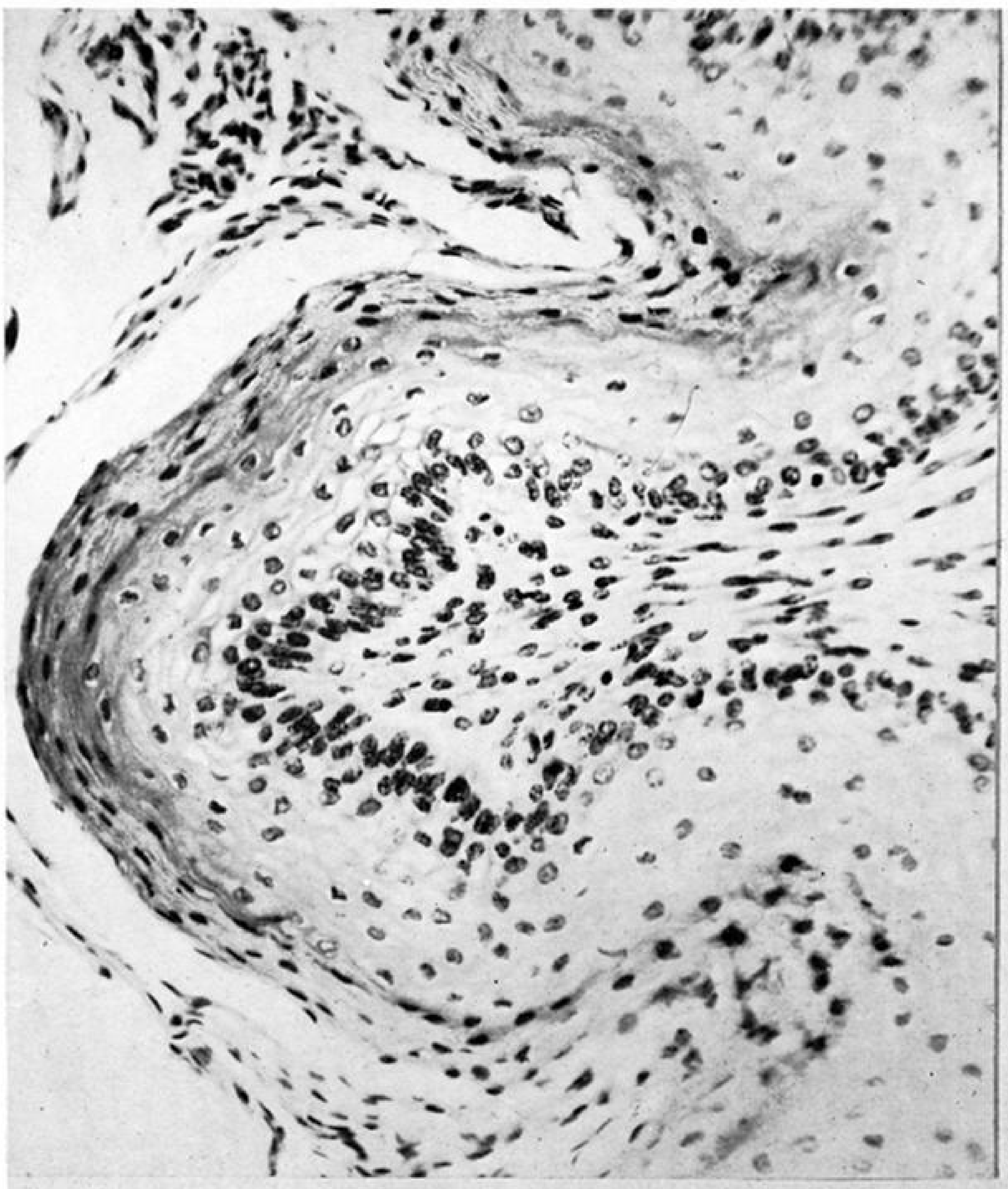
Fig. 15.—No. 87. June, part of section shown in fig. 12, Plate 10, showing height of epithelium and gland cells and œdema of the stroma.

Fig. 16.—No. 73. June, progestational proliferation of the uterine endometrium corresponding to the developing corpus luteum shown in fig. 7, Plate 9.

Fig. 17.—No. 66. June, part of fig. 14, Plate 10.

Fig. 18.—No. 177. December, anœstrous vagina, showing the low epithelium.

Fig. 19.—No. 276. May, vagina, beginning of œstrous activity, epithelium thickened and beginning to be stratified.



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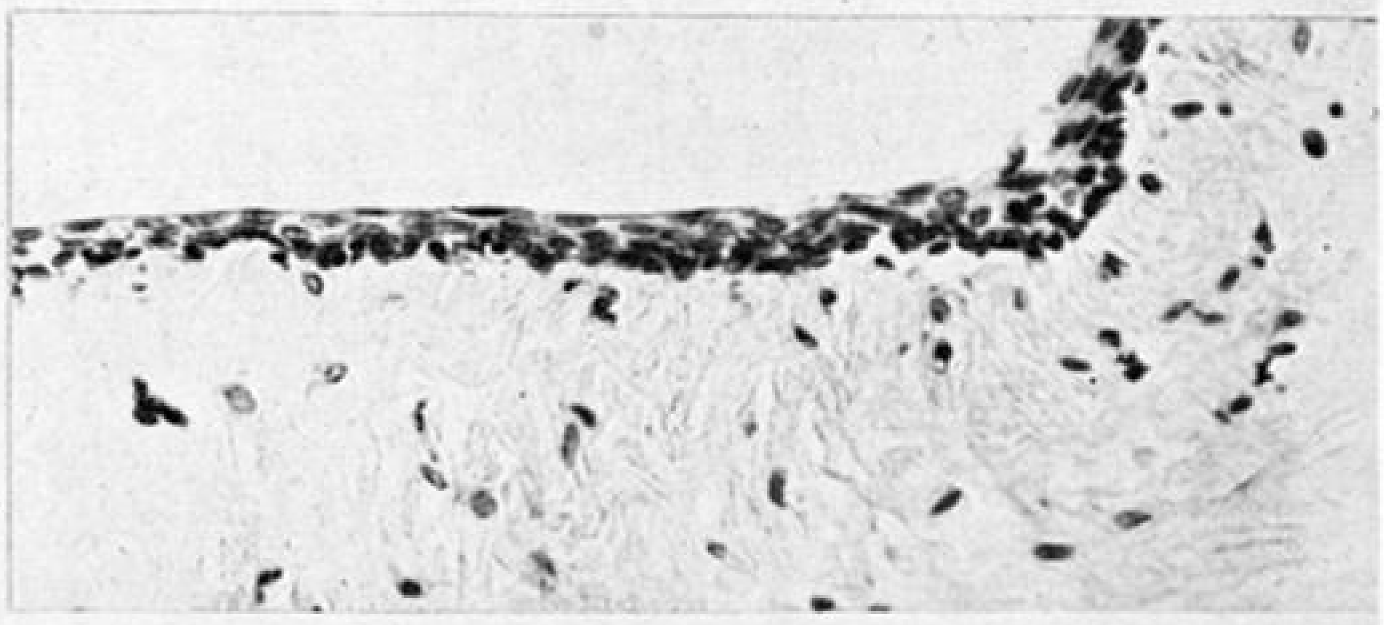

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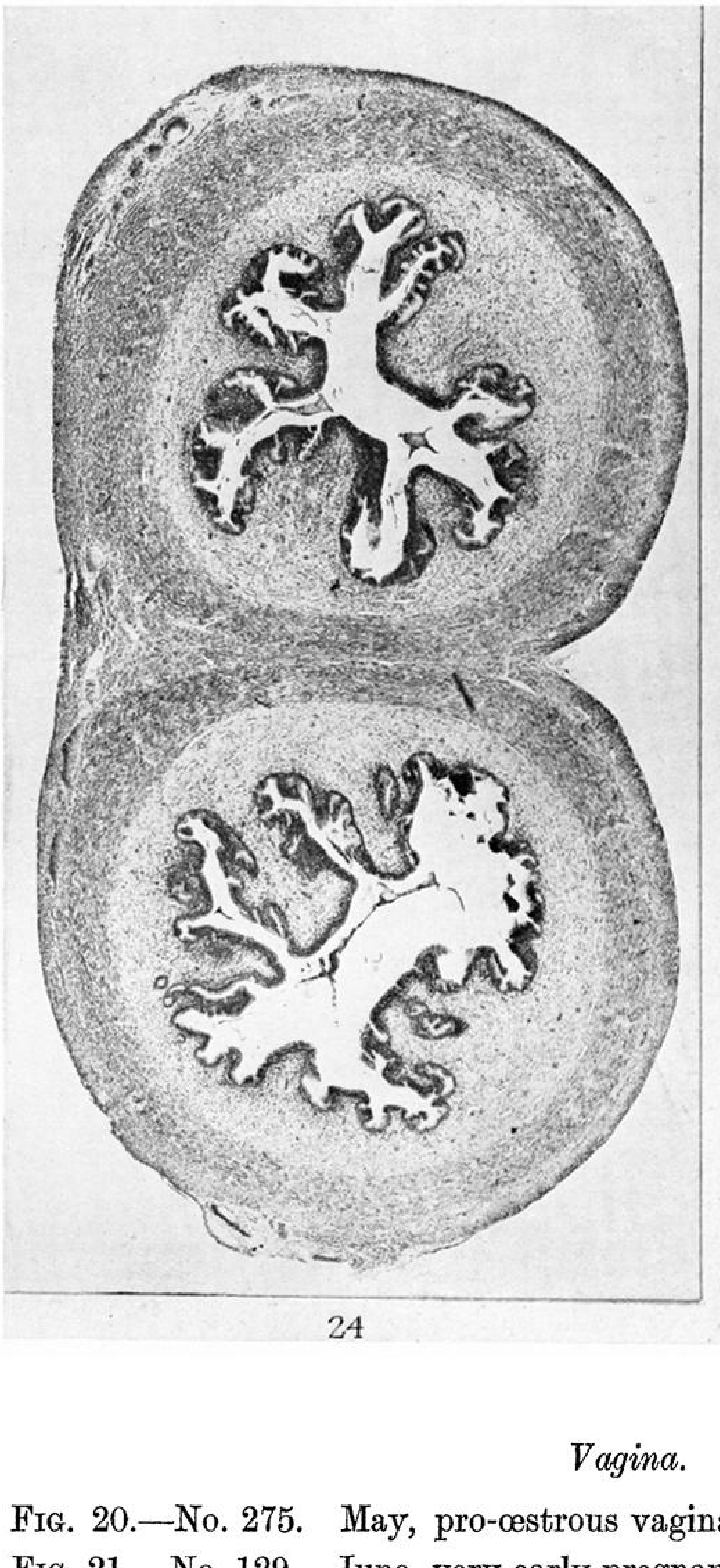

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PLATE 12.

Vagina. × 165. *Cervix.* × 17.

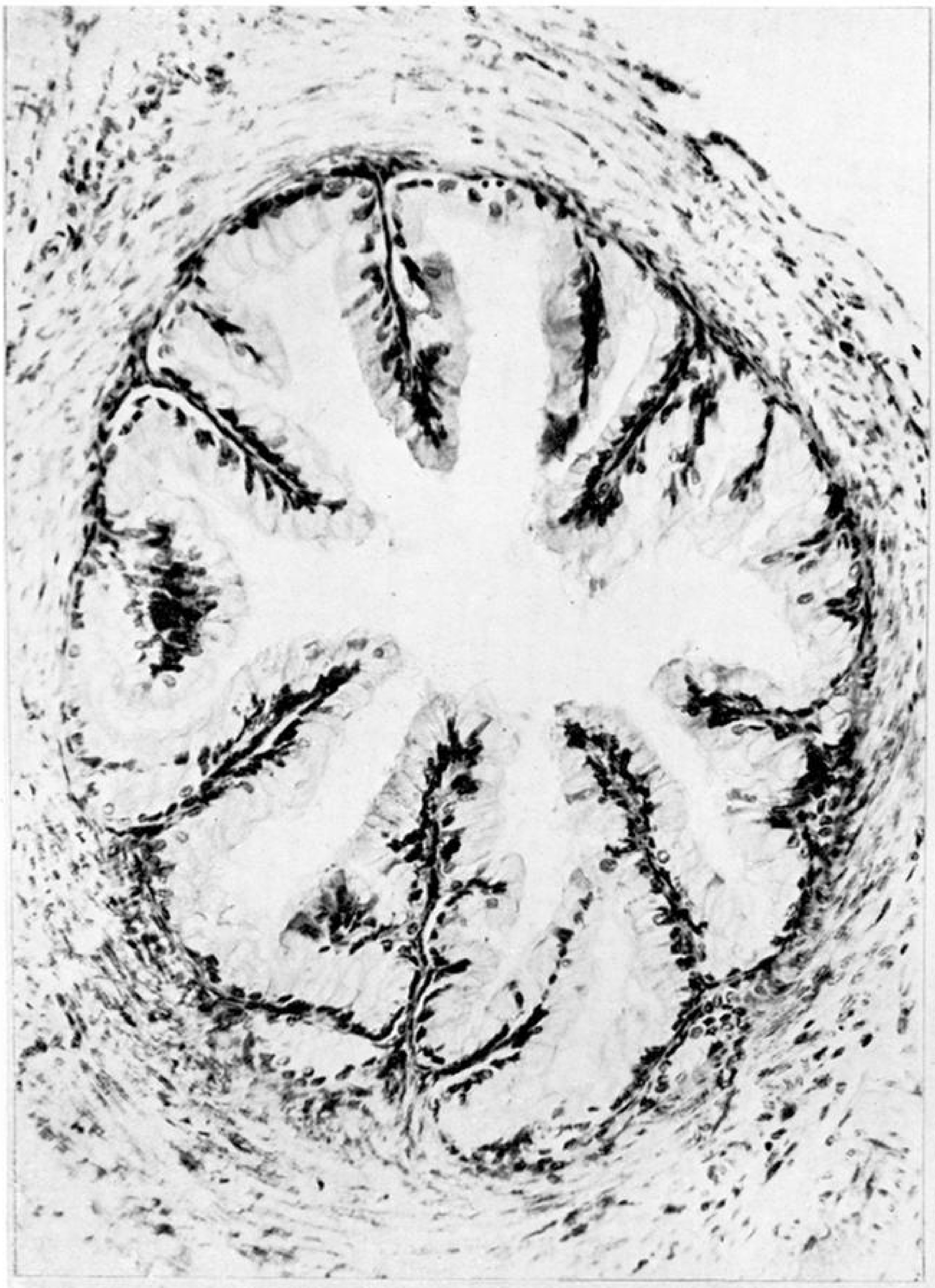
FIG. 20.—No. 275. May, pro-œstrous vagina, cornified cells sloughing into lumen.

FIG. 21.—No. 129. June, very early pregnancy, vaginal epithelium low.

FIG. 22.—No. 93. July, pregnancy, vaginal epithelium, showing development of layer of cuboid cells.

FIG. 23.—No. 102. July, late pregnancy, showing vaginal epithelium, irregular columnar cells.

FIG. 24.—No. 213. March, cervix showing early pro-œstrous growth of epithelium.



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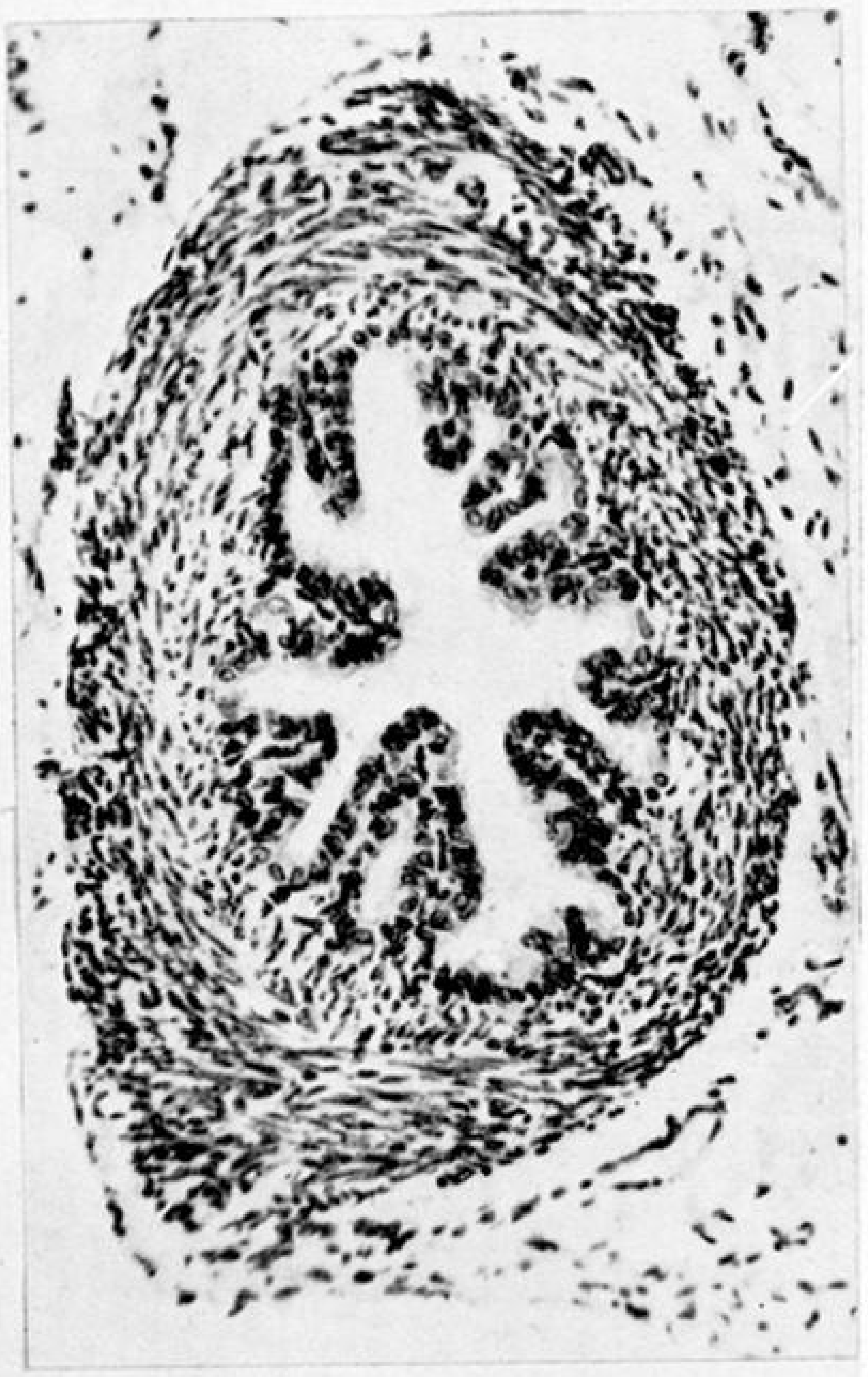

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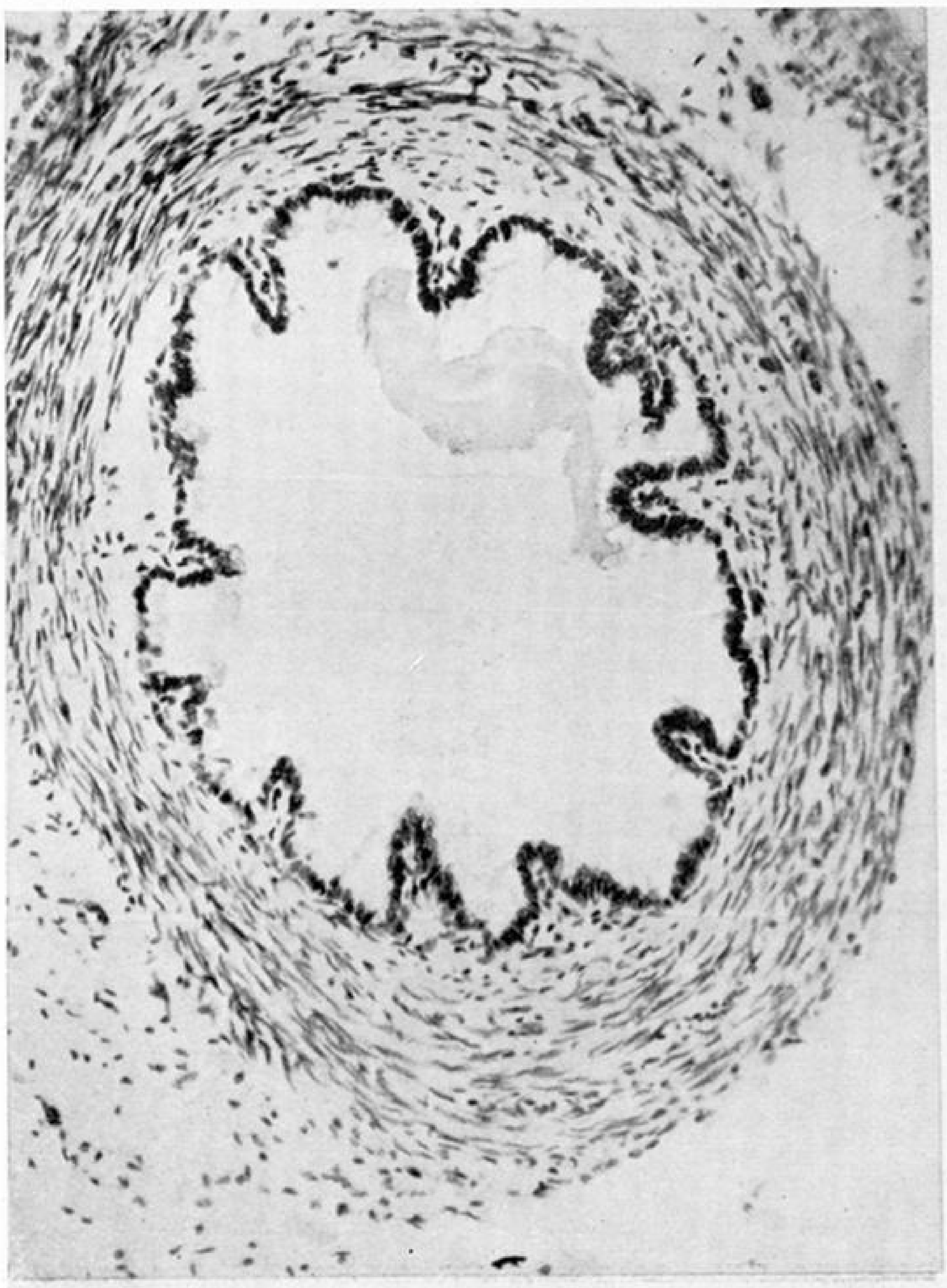

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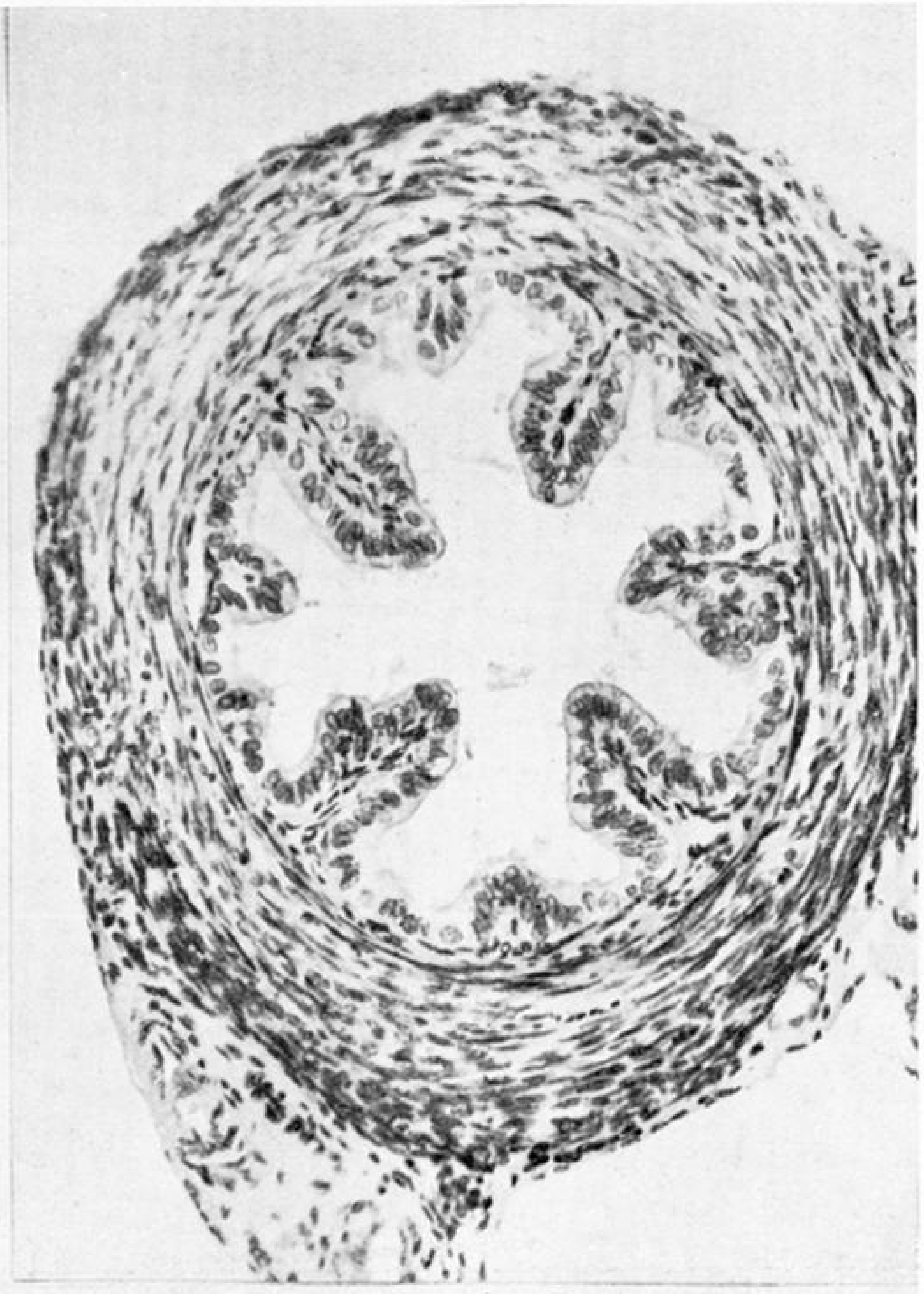

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PLATE 13.

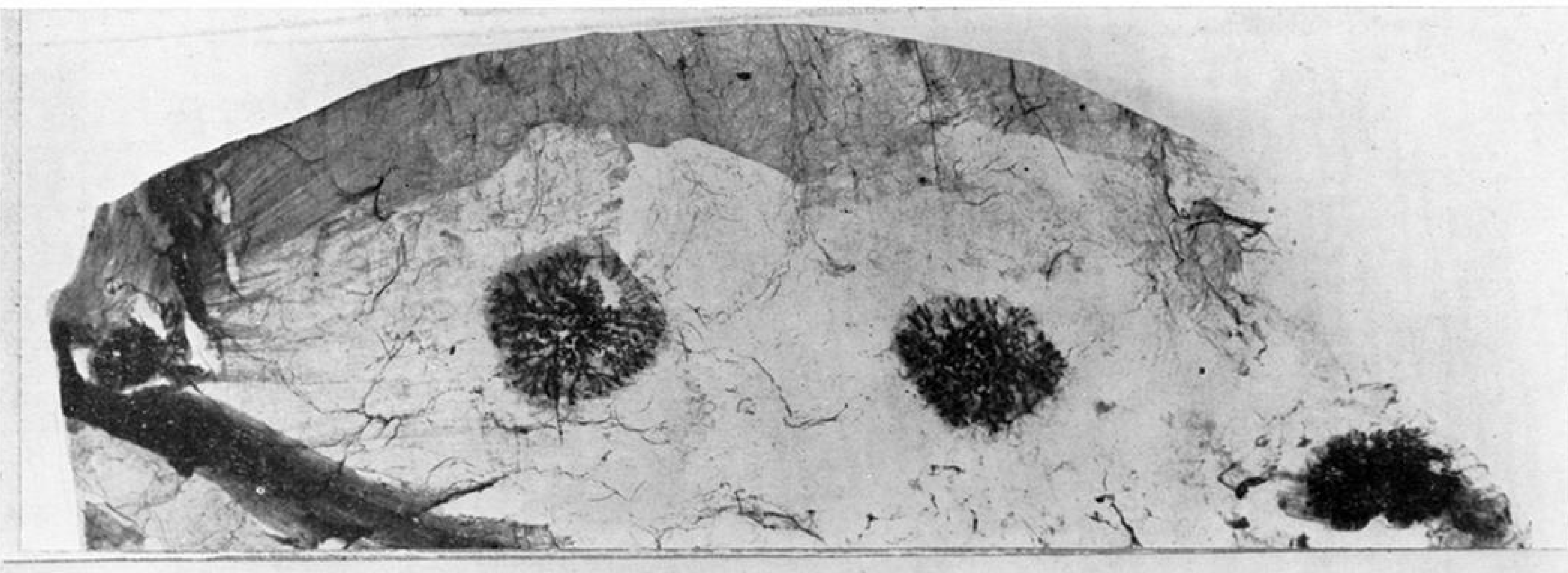
Fallopian Tube. × 154.

FIG. 25.—No. 148. December, anœstrous condition.

FIG. 26.—No. 271. May, early stage of œstrous growth of the epithelium. Tube increased in diameter.

FIG. 27.—No. 87. June, condition of tube just before œstrus. Further increase in size of tube ; epithelium high, with cilia.

FIG. 28.—No. 80. June, early pregnancy. Epithelium shrunk and disorganized, and traces of secretion in the lumen.



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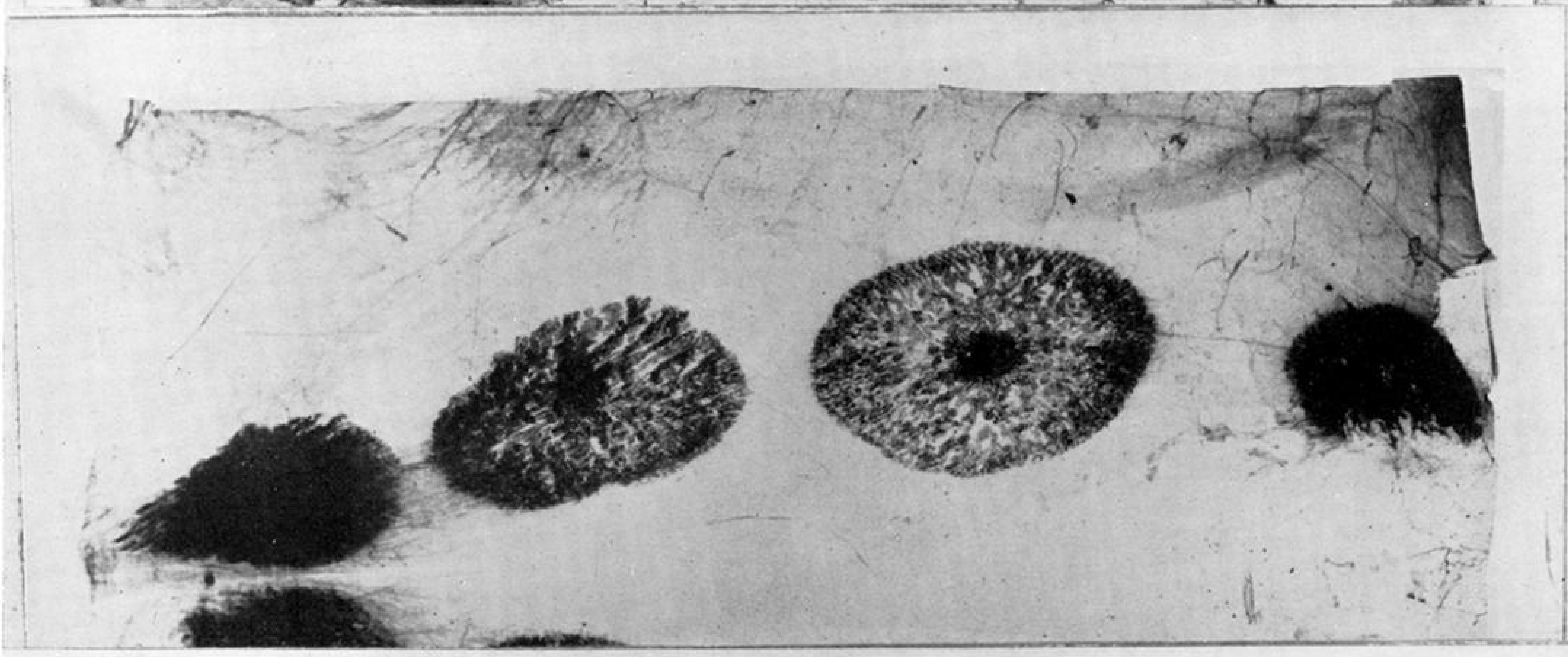

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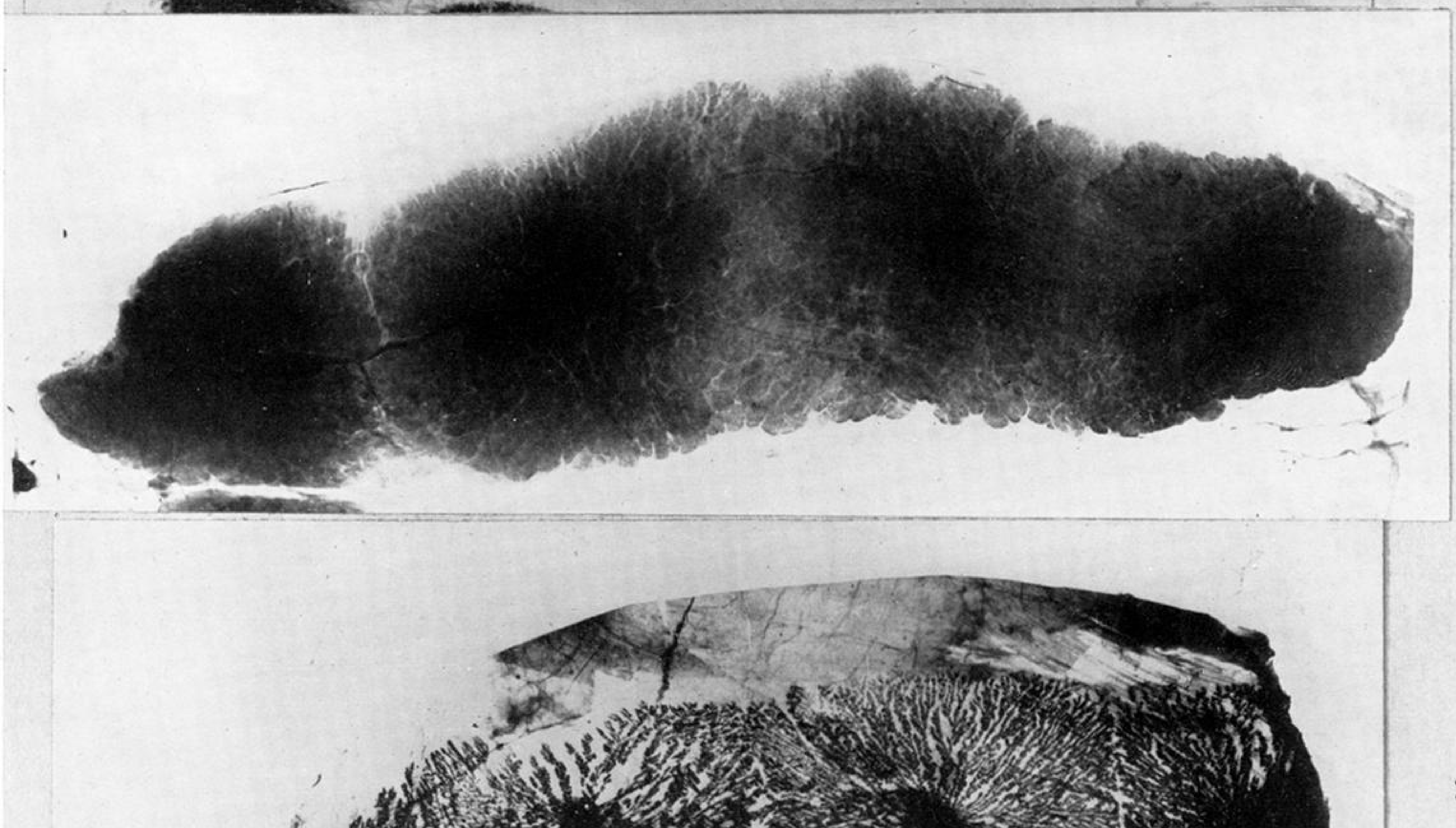

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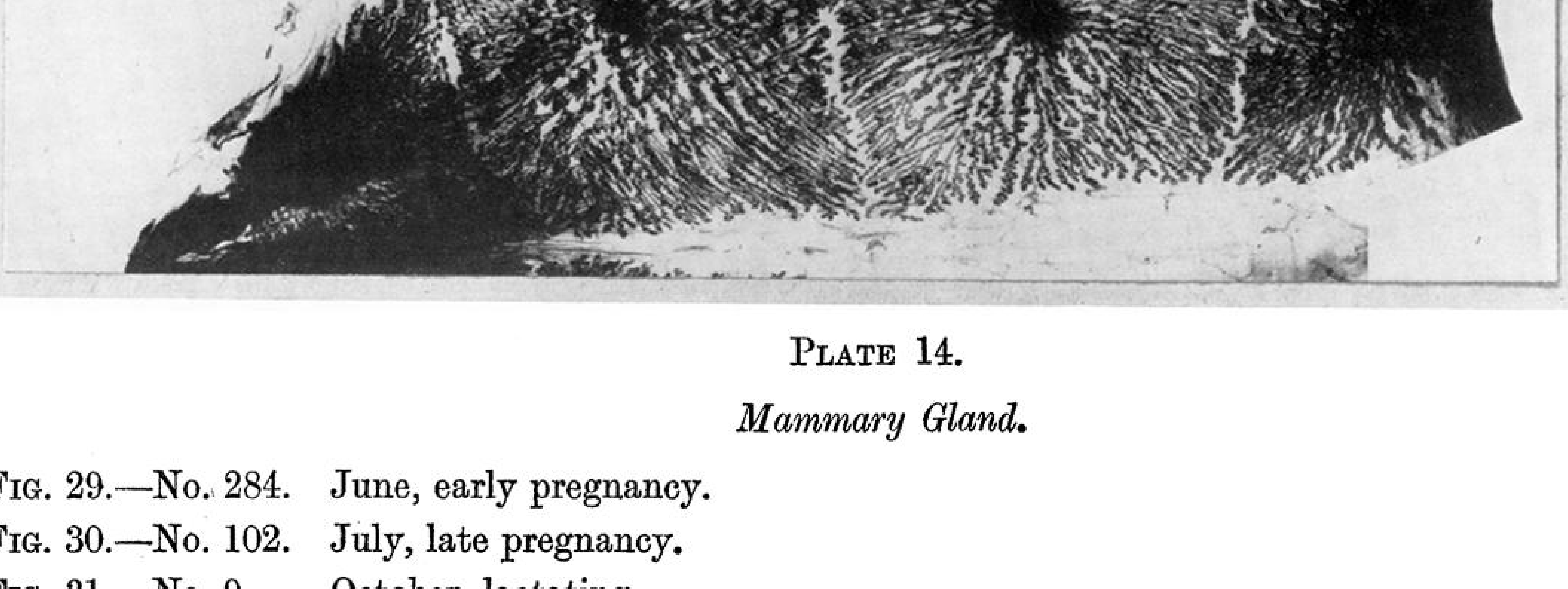

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PLATE 14.

Mammary Gland.

FIG. 29.—No. 284. June, early pregnancy.

FIG. 30.—No. 102. July, late pregnancy.

FIG. 31.—No. 9. October, lactating.

FIG. 32.—No. 280. June, early pregnancy superimposed on recent lactation.