MITOTIC ACTIVITY IN THE ADULT FEMALE MOUSE, *MUS MUSCULUS* L. A STUDY OF ITS RELATION TO THE OESTROUS CYCLE IN NORMAL AND ABNORMAL CONDITIONS

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A survey has been made of the mitotic activity of the reproductive system, the urinary system, the alimentary canal, various exocrine and endocrine glands, and other miscellaneous organs of the adult female mouse, both in relation to the normal oestrous cycle and to injections of oestrone. A similar survey has also been made of the mitotic activity associated with wound healing and with cancerous growth.

Evidence is recorded which indicates that, in all these cases, mitosis is stimulated by oestrogenic hormones.

In the ovary, the cells are highly resistant to the mitosis-stimulating effect of the oestrogen. Mitotic activity in this organ is induced mainly by the pools of follicular fluid which are particularly rich in oestrogenic hormone, and which stimulate the highest rate of division in any cells in direct contact with them. Thus, the follicular fluid causes the great mitotic activity associated with follicle growth, and after the follicle bursts, also induces the mitotic activity of the neighbouring germinal epithelium which it bathes. In the same way, some mitotic activity is stimulated in the developing corpus luteum before luteinization occurs.

In the other body organs there exists the widest range of sensitivity to oestrogenic hormones. In the brain no mitotic activity was seen; in the kidney there was some activity at full oestrus or after many injections of oestrone; in the epidermis there was a considerable reaction; and some of the most sensitive tissues of the body proved to be the lining epithelia of the Müllerian duct system and

the intestinal mucosa. The less active tissues responded slowly to oestrogen stimulation, while the more active tissues responded promptly and fully. The most active tissues examined proved to be the proliferating centres of the intestinal lymph nodules, which maintained a maximum rate of cell division even when, as on the first day of dioestrus, the oestrogen blood content was at a minimum.

The results obtained indicate the existence of mitosis inhibitors. First, there is the force, termed the cell inertia, which must be overcome before any tissue can react by cell division to the presence of an oestrogen. In the case of less active tissues the cell inertia is great, and is overcome only after the oestrogen has exerted its influence for several days, but in the more active tissues the cell inertia is slight, and is rapidly overcome by relatively small increases in the oestrogen blood content. The existence of a second mitosis inhibitor, termed the mitosis depressor, is shown by the fact that any sharp rise in mitotic activity is quickly brought under control, so that mitosis is again reduced to a minimum. With the continued presence of the stimulating oestrogen, there is, after a short interval, a second rise in mitotic activity which, like the first, is rapidly brought under control and in turn eliminated. Thus, even in the continued presence of oestrogen stimulation, mitotic activity proceeds in waves. However, an exception to the rule is furnished by the proliferating centres of the intestinal lymph nodules where it appears that both the mitosis-controlling forces are lacking, so that a maximum rate of cell division is always maintained.

It was discovered that, during wound healing, the mitosis rate rises sharply to an abnormally high maximum, and that it does this quite irrespective of the oestrous cycle and of injections of oestrone. It is evident, therefore, that the mitotic activity associated with wound healing is under the control of some special mechanism, and evidence is summarized which indicates that one of the functions of wound hormones is the elimination of the two mitosis-regulating forces in tissues closely adjacent to the wound. Because of this, the local cells react immediately and in an abnormal degree to the stimulation of the oestrogenic hormones, even if these are present only in small concentrations.

In cancerous growth it was found that great mitotic activity also proceeds without influence from the oestrous cycle or from injections of oestrone, and it is evident that in this case the two mitosis-regulating forces are permanently in abeyance, so that the maximum response to oestrogen stimulation is always possible.

It is concluded that in the adult female mouse, and probably in other animals, there exist three types of substances influencing mitotic activity. First, there are the oestrogenic hormones which act as general mitosis stimulators; second, there are the mitosis-inhibiting forces of cell inertia and mitosis depression, which prevent the rate of cell division from becoming excessive and unregulated; and third, there are the wound hormones which, by weakening the mitosis-inhibiting forces, allow an abnormally high reaction to oestrogen stimulation and a consequent rapid production of new tissue to meet the emergency.

This conclusion is discussed in relation to several biological problems, including ovarian function, pregnancy, embryonic growth, and cancer.

I. Introduction

It has been known for some time that one important function of the oestrogenic hormones, that, in fact, by which they are recognized, is to induce extreme mitotic activity in the female accessory sexual organs of the vertebrates, and it might be supposed that substances which are capable of inducing active mitosis in one type of tissue would also be capable of a similar action in others. As a preliminary test of this supposition it was decided to make a survey of cell division in the various body organs of the adult female mouse in an attempt to discover whether such division is normally under the control of these hormones. The results obtained in the course of the work suggested that a consideration of cell division in abnormal conditions would also prove interesting, especially by comparison with the normal. Further studies were therefore made of the relation between the oestrogenic hormones and the mitotic activity involved in wound healing and in cancerous growth.

II. Material and methods

(1) *Material*

Nearly five hundred female mice were examined in the course of this investigation, and they belonged, with only a few exceptions, to the following three strains:

(*a*) *Strong's CBA agouti.* These cancer-resistant black agoutis are prolific breeders, and during the many years in which they have been under observation in the Zoology Department of Leeds University, no spontaneous tumours have been recorded in either sex in spite of the fact that many have been allowed to live to extreme old age.

(*b*) *Kreyberg's white label albino.* Also prolific breeders, these albino mice, isolated by Kreyberg, have been preserved because of their susceptibility to spontaneous cancer. It was observed that about 80% of the females of this strain developed mammary carcinoma by the time when, at twelve or fourteen months of age, they became senile.

(*c*) *Strong's A albino.* Even more prone to develop mammary cancer than Kreyberg's strain, these mice proved to be poor breeders with a relatively high mortality at the time of birth in both the young and their mothers.

It soon became apparent that the mice of these three strains responded in an exactly similar manner to the treatment given, and they were therefore mixed indiscriminately in the various experimental groups. The results obtained were in no way affected by the fact that some of the mice used had previously been mated whereas others had not, but care was taken that only mice of about six months of age which exhibited normal steady oestrous cycles were included in the experiments.

All the animals were reared and kept at a temperature which varied only between 15 and 20° C, and they were exposed to a constant daily light period of 12 hr. The diet was as varied as possible, but it was uniform week by week. Oats, maize, dog biscuit, bread, milk with cod-liver oil, and lettuce were given, and with this treatment the stock of mice was maintained in good breeding condition. No diseases of any kind appeared in the colony, and with the exception already mentioned of some mortality at parturition among the Strong's *A* albinos, no deaths occurred.

(2) *Vaginal smear technique*

All the work recorded in this paper centres round the oestrous cycle, and to distinguish the phases of this cycle, a modification of the vaginal smear technique was used. Smears of loosened cells from the vaginal lining were taken in the usual way by means of a platinum wire loop, and before each mouse was killed or received its first injection of oestrone, they were studied twice daily for at least 10 days. The smears were dried and stained in Ehrlich's haematoxylin followed by Pasini's stain, this method having been found to permit a more precise subdivision of the oestrous cycle than normal staining methods. The following stages of the cycle were recognized although still further, but unnecessary, subdivision of these various stages was possible:

(*a*) Pro-oestrus: only nucleated epithelial cells, which stained blue throughout, were present.

(*b*) Pre-ovulation oestrus, early: some cells partly cornified with the cytoplasm staining red and the nuclei blue.

(*c*) Pre-ovulation oestrus, full: all cells fully cornified and staining bright red throughout.

(*d*) Post-ovulation oestrus: cornified cells in clumps and staining pale pink throughout.

(*e*) Metoestrus: leucocytes staining dark blue among the pale pink clumped cornified cells.

(*f*) Dioestrus, first day: first signs of nucleated epithelial cells staining blue throughout, leucocytes still more numerous, and traces of pale pink cornified cells usually still persisting.

(*g*) Dioestrus, second day: only blue staining nucleated epithelial cells and leucocytes present.

(*h*) Dioestrus, third day: as second day.

In the study of the effect of the oestrous cycle on normal mitotic activity, five mice were killed in each of the above eight stages.

(3) *Oestrone injection technique*

In the oestrone injection experiments five animals were killed in each experimental group. The groups were numbered from one to six, and they received respectively from one to six injections. The first injection was always given when the animal was in the first day of dioestrus, the time when the ovary was relatively quiescent and producing minimal quantities of oestrogen. The female sex-hormone oestrone (Menformon, Organon Laboratories) was introduced either into the abdominal coelomic cavity, or, in the majority of cases, into the subcutaneous space. The former method was used in studying the effects of this hormone on the cells of the germinal epithelium of the ovary, and for this purpose the solvent was sesame oil. In the case of the subcutaneous injections the oestrone was given in water solution. Both methods produced identical reactions in all the affected parts of the body except the germinal epithelium, and in both 250 i.u. (= 0·025 mg.) in 0·25 c.c. of solvent were introduced into each animal at each injection. The injections were repeated at 12 hr. intervals, and the last injection was given 12 hr. before death. Vaginal smears were taken at the time of each injection and at death.

(4) *Wound technique*

In animals specially chosen for their exceptionally steady oestrous cycles the hair was cropped from two mid-dorsal regions of the back, the one anterior between the shoulder blades and the other posterior above the pelvis, and with fine scissors a small transverse cut, approximately 3 mm. long, was made in each region. The cut penetrated both epidermis and dermis, and extended through the panniculus carnosus into the fatty connective tissue of the hypodermis so that the pink muscles of the back were clearly visible through the gap. Little or no bleeding occurred. In order to test the effect of the oestrous cycle on the process of healing, the wounds were made in some mice in early dioestrus and in others in early oestrus. In both series of mice, five animals were killed at daily intervals up to 7 days following the infliction of the wounds, and careful notes of the courses of the oestrous cycles were kept.

As a further check on the results obtained, other animals, wounded on the first day of dioestrus, received a series of oestrone injections given subcutaneously by the method

already described, and groups of five mice were again killed at daily intervals up to the maximum of 7 days.

In the great majority of the wounds inflicted healing proceeded rapidly and without complications, but two cases, in which there were considerable irritation and abnormally slow healing, had to be rejected.

(5) *Cancer implantation technique*

Spontaneous cancer occurs far more frequently at the onset of senility than during the normal reproductive life, and consequently, in order to test the effect of the oestrous cycle and of abnormal concentrations of oestrogen on such growth, it was necessary to make cancer transplants into young mice. A spontaneous and rapidly growing mammary carcinoma in a 12 months' old Kreyberg's white label female was excised and cut into small pieces in normal saline solution. The resulting mash was injected into 4 months old mice of various strains, the incision being made at the groin and the material deposited subcutaneously in the mid-ventral abdominal region. The grafts were successful in about 60% of cases, and the implanted cancers were large and rapidly growing about 2 months after the time of their injection. Groups of five mice each were then killed in the various stages of the oestrous cycle, and others, also of five mice each, were killed following from one to six injections of oestrone given in the manner already described.

(6) *Colchicine technique*

To make possible an accurate assessment of the mitotic activity of the cells of the various organs studied, the colchicine technique was used to prevent the completion of the cell divisions. The method adopted was that described by Allen (1937) in which 0·1 mg. of this alkaloid drug in 0·25 c.c. of water is injected subcutaneously $9\frac{1}{2}$ hr. before killing. By this means all the mitoses commencing during the final $9\frac{1}{2}$ hr. period were arrested in the metaphase. A clear picture of the rate of mitotic activity was thus obtained, and a direct comparison between different tissues, whose cell divisions might normally be completed at different speeds, was made possible.

(7) *Histological technique*

All the mice were anaesthetized with chloroform, and after the throat, thorax, and abdomen had been widely opened, they were dipped quickly into 70% alcohol as a wetting agent for the hair and fixed whole in Bouin's fluid for 2 days. After storage in 70% alcohol, the organs required were embedded in paraffin wax, and cut in serial sections 7μ in thickness. The standard staining method used for all organs was Ehrlich's haematoxylin and eosin, and the connective tissue was stained in saffron dissolved in absolute alcohol.

(8) *Measurements of mitotic activity*

The mitoses, being almost all in the metaphase and staining very darkly in contrast to a typically pale cytoplasm, were clearly visible, but different methods of counting them had to be devised for different organs. In the case of the ovary the methods used for assessing the mitotic activity of the various regions are described in the sections devoted to that organ. For the other parts of the body examined, two main techniques were used. In the

case of epithelia, simple, transitional, or stratified, the number of mitoses was estimated per unit length of 1 mm. In the case of solid homogeneous organs like the pancreatic and salivary glands, and in the case of tortuous masses of lobules or ducts like the thyroid and the growing region of the duodenal mucosa, the number of mitoses was estimated per unit area of section. Owing to the widely diverse mitotic rates of different organs of this latter type, two different areas were taken as standards. Thus in the case of the pancreas, which did not show great mitotic activity, the unit section area studied was 0·1 sq.mm., whereas in the case of the highly active proliferating zone of the duodenal mucosa it was 0·01 sq.mm. Whatever method was used, the numbers of cell divisions were counted in 20 unit lengths or areas in each animal. Thus for each group of five animals, 100 counts were made. The average number of mitoses per unit length or area for each group of animals was then calculated, and the standard deviation from the mean was estimated according to the formula $\sigma = \sqrt{(\Sigma f d^2/N)}$, where σ is the standard deviation, f is the frequency, d is the deviation from the mean, and N is the number in the sample.

III. Mitotic activity in normal body organs

(1) *Reproductive system*

(i) *Germinal epithelium*

Normal adult. This epithelium, one cell in thickness, entirely invests the ovary, and is continuous at the hilus with the peritoneum. It is based on a thin connective tissue layer, the tunica albuginea, and it varies locally in form from tall columnar to flattened squamous according to the degree of tension. Following the evidence of Allen (1923) that by the divisions of the germinal epithelial cells, which take place most actively during oestrus, the ovary is replenished with new stocks of oogonia, a study was made of the mitotic activity of the germinal epithelium throughout the oestrous cycle. All the mitoses in the germinal epithelium of one ovary from each animal were counted and classified according to their proximity to the following structures:

(*a*) Groups of very small follicles* or masses of stroma.
(*b*) Rapidly growing follicles* containing follicular fluid.
(*c*) Developing corpora lutea not yet luteinized.
(*d*) Fully developed corpora lutea of not more than one oestrous cycle old.
(*e*) Corpora lutea of more than one oestrous cycle old.

The separation of the last two groups was possible, as the cytoplasm of the older corpora lutea stained a deeper red with eosin than did that of the younger corpora lutea. The results obtained by the mitosis counts are shown in table 1 and in figure 1. As stated by Allen, the greatest mitotic activity of the germinal epithelial cells took place in the oestrous period. It is now clear, however, that the period of maximum activity is extremely short-lived, occurring as it does in the brief post-ovulation oestrous period. Further, it is clear from the classification of the mitoses that before ovulation the greatest numbers of cell divisions were situated in close proximity to the rapidly growing follicles, while during the post-ovulation oestrous period, an overwhelming majority were found by the new corpora lutea which were forming from the burst follicles (figure 13, plate 28). The connexion between the mitoses and

* For definitions of the types of follicles recognized see the classification on p. 461.

the growing or burst follicles is further illustrated in tables 2 and 3 and in figure 2. In general, those ovaries which, in the pre-ovulation oestrous periods, had abnormally low or abnormally high numbers of Graafian follicles, had produced respectively abnormally low or abnormally high numbers of germinal epithelial mitoses. Similarly, in the post-ovulation oestrous period there was a direct connexion between the numbers of follicles which succeeded in bursting and the numbers of mitoses present. The conclusion therefore seems justified that mitotic activity normally occurs in the germinal epithelium due to an influence exerted by the Graafian follicles and especially by the burst follicles. The most obvious influential factor produced by ovarian follicles is the follicular fluid rich in oestrogen, and it appears that the effect produced is greatest when, following the bursting of the mature follicles, this fluid actually bathes the germinal epithelial cells.

TABLE 1. AVERAGE NUMBERS OF MITOSES IN THE GERMINAL EPITHELIUM THROUGHOUT THE OESTROUS CYCLE

phase of oestrous cycle	numbers of mitoses: unrelated	by large follicles	by developing corpora lutea	by young corpora lutea	by old corpora lutea	total
dioestrus:						
1st day	9·8 ± 7·3	4·9 ± 5·4	—	4·6 ± 4·3	0·7 ± 1·1	20·1 ± 17·4
2nd day	3·6 ± 2·3	5·1 ± 2·0	—	8·9 ± 6·6	3·6 ± 2·6	21·0 ± 13·1
3rd day	5·7 ± 3·0	15·2 ± 5·7	—	7·7 ± 3·4	3·7 ± 1·8	32·2 ± 11·1
pro-oestrus	24·4 ± 6·1	36·3 ± 3·9	—	5·1 ± 2·8	7·2 ± 4·1	69·7 ± 14·7
oestrus:						
early	26·9 ± 6·6	39·4 ± 4·5	—	5·6 ± 3·0	6·7 ± 4·3	78·7 ± 16·5
full	28·7 ± 5·1	67·1 ± 11·3	—	5·0 ± 4·4	4·2 ± 3·5	104·8 ± 22·2
late	178·0 ± 46·8	10·8 ± 11·1	906·6 ± 267·5	—	13·7 ± 2·1	1109·2 ± 300·0
metoestrus	11·8 ± 5·8	14·9 ± 11·7	—	69·2 ± 19·4	5·8 ± 5·1	101·7 ± 34·6

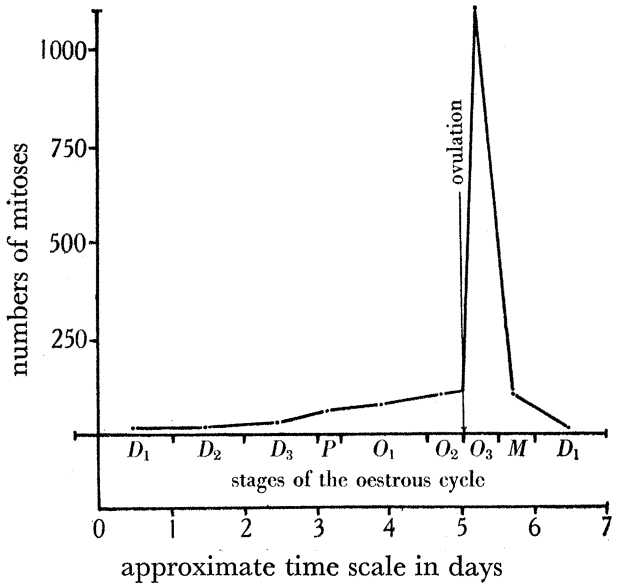

FIGURE 1. Graph showing the average numbers of mitoses present in the ovarian germinal epithelium at the various stages of the oestrous cycle. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

TABLE 2. THE NUMBERS OF MITOSES IN THE GERMINAL EPITHELIUM CORRELATED WITH THE NUMBERS OF LARGE FOLLICLES IN THE OVARIES OF MICE KILLED IN THE PRE-OVULATION OESTROUS PERIODS. EACH SET OF COUNTS REPRESENTS ONE OVARY

	numbers of mitoses					
number of mouse	unrelated	by large follicles	by young corpora lutea	by old corpora lutea	total	number of follicles
13	8	22	1	1	32	2
251	14	40	3	5	62	3
14	32	68	1	0	101	4
131	29	70	3	5	107	5
12	18	69	3	3	93	6
83	36	94	11	8	149	7
346	38	103	3	1	145	8
387	24	89	6	5	124	9
409	39	118	5	4	166	10
346	54	137	4	6	201	11
409	63	186	9	7	265	18

TABLE 3. THE NUMBERS OF MITOSES IN THE GERMINAL EPITHELIUM CORRELATED WITH THE NUMBERS OF LARGE FOLLICLES AND DEVELOPING CORPORA LUTEA IN THE OVARIES OF MICE KILLED IN THE LATE POST-OVULATION OESTROUS PERIOD. EACH SET OF COUNTS REPRESENTS ONE OVARY

	numbers of mitoses					numbers of	
number of mouse	unrelated	by large follicles	by young corpora lutea	by old corpora lutea	total	large follicles	developing corpora lutea
122	119	25	547	15	706	2	4
85	136	18	735	12	901	2	4
252	215	11	946	16	1188	1	5
351	203	—	1105	11	1319	0	7
96	217	—	1201	14	1432	0	7

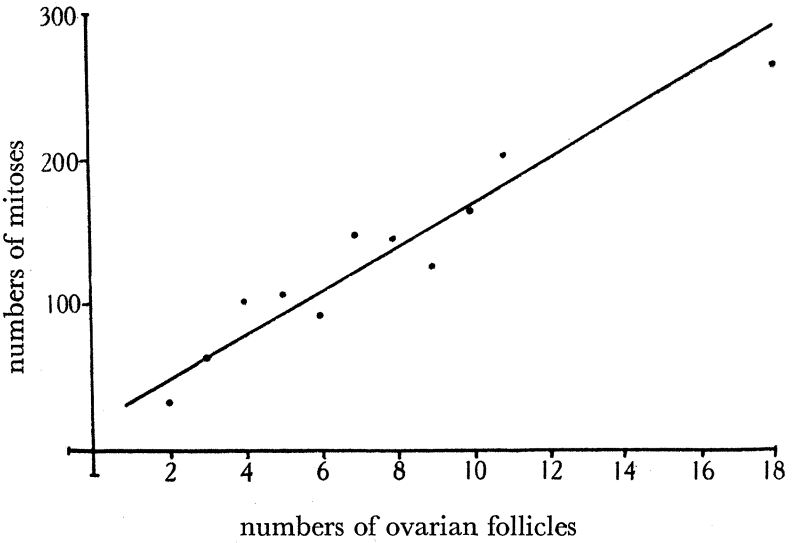

FIGURE 2. Graph showing the correlation between the numbers of mitoses present in the germinal epithelium during the pre-ovulation oestrous period and the numbers of large ovarian follicles.

Oestrone injection experiments. In order to discover whether the oestrogen produced by the ovary is the agent responsible for the waves of mitotic activity in the germinal epithelium, oestrone dissolved in sesame oil was injected abdominally into the region of the ovaries. This was done on the first day of dioestrus when the germinal epithelium is relatively quiescent. Following from one to six injections, counts were made of the total number of mitoses present in the germinal epithelium of one ovary from each animal, and the results so obtained are given in table 4. The variation in the response of the different mice was so great that it appeared unprofitable to work out the averages and standard deviations for each group. Consequently the results are recorded in full. In spite of the considerable variation, it will be noted that the lowest number of mitoses present in the ovaries of the injected animals was considerably higher than the highest in the control ovaries, and it is clear that, in all cases, some stimulation had occurred due to the presence of oestrone. Further, the highest count of all, 1818 mitoses noted after five injections, was considerably higher than the highest count obtained in any normal animal even in the post-ovulation oestrous period. It will also be noticed that the mitoses induced artificially bore little or no relation to the large ovarian follicles as they did in the normal animal in oestrus. The greatest numbers of the induced cell divisions were found, in fact, to be unrelated to any prominent ovarian structure. It seems probable that the great variation in the results obtained was due to the fact that, in those animals which gave relatively low mitosis counts, the connective tissue capsule surrounding the ovary had prevented the oily solution of oestrone from reaching that organ, whereas in the others which were highly stimulated the solution had actually penetrated into the capsule.

(ii) *Ovarian follicle*

Normal adult. The interior of the ovary is filled with masses of connective tissue, or stroma, in which are embedded the ovarian follicles and corpora lutea. The follicles, containing oogonia or primary oocytes, are found in all stages of development from a tiny newly formed group of cells, situated just beneath the germinal epithelium from which they arose, to a fully developed Graafian follicle with the oocyte floating free in the antrum. As many hundreds of times more oocytes and follicles are produced than ever reach maturity, great numbers are constantly to be seen undergoing atresia especially in the earlier stages of their development. For the purpose of studying their growth, the follicles which survived were classified into the following four types:

(*a*) Small primary follicles in which growth proceeds slowly and atresia commonly occurs. These follicles have a diameter of up to 100μ, and lack follicular fluid.

(*b*) Rapidly growing follicles with a diameter of over 175μ. Their cells show great mitotic activity, and there is a single antrum containing follicular fluid.

(*c*) Large follicles of about 575μ in diameter with thin walls to which the oocyte is still attached by the discus proligerus.

(*d*) Fully grown follicles of about 600μ in diameter with very thin walls, and with the oocyte, surrounded by the corona radiata, floating free in the follicular fluid.

The method of study adopted was as follows. A hundred median sections of each type of follicle were examined, and all the mitoses counted. Averages, together with standard

TABLE 4. THE NUMBERS OF MITOSES IN THE GERMINAL EPITHELIA OF CONTROL AND EXPERIMENTAL OVARIES

number of mouse	number of oestrone injections	numbers of mitoses				
		unrelated	by large follicles	by young corpora lutea	by old corpora lutea	total
11	0	7	0	2	0	9
10	0	6	2	2	0	10
174	0	5	4	3	0	12
123	0	4	7	2	1	14
176	0	3	4	5	2	14
184	0	4	12	2	1	19
145	0	3	9	9	5	26
187	0	6	17	8	4	35
186	0	10	21	7	5	43
173	0	21	13	12	3	49
273	1	26	18	8	13	65
276	1	29	12	22	13	76
274	1	49	21	27	10	107
275	1	66	25	19	18	128
277	1	141	39	27	26	233
95	4	114	69	31	60	274
247	4	156	74	32	41	303
248	4	269	44	30	51	394
93	4	695	179	92	127	1093
254	4	725	196	156	178	1255
258	5	128	56	34	43	261
250	5	197	62	28	35	322
259	5	249	111	83	145	588
257	5	938	426	170	284	1818
256	6	155	60	102	131	448

deviations, were then calculated to show the relative abundance of cell divisions in the following zones of the follicle wall:

(*a*) *Membrana granulosa. Oocyte zone*: In the small primary follicles this included all cells within a circle drawn with its centre at the centre of the oocyte and with a radius twice that of the oocyte. In the larger follicles it comprised the discus proligerus or the corona radiata. *Inner zone*: Absent from the small primary follicles, this included all cells within a line drawn through the membrana granulosa half-way between the inner edge, which is adjacent to the follicular fluid, and the outer edge, which is adjacent to the theca interna. *Outer zone*: Including all the other outer cells of the membrana granulosa.

(*b*) *Theca folliculi*, which in the larger follicles was subdivided into theca interna and theca externa.

The results obtained by this method are seen in table 5. The small primary follicles which were chosen for detailed study had walls three or four cells in thickness and an approximate diameter of 80μ. They showed only slow growth, and numbers of them were constantly becoming atretic. There were only slight differences between the mitotic activities of the various zones, but even allowing for the large standard deviations, a general tendency was apparent for the greatest number of cell divisions to be situated near the primary oocyte and the smallest number in the theca folliculi. When it is further remembered that the oocyte zone presented the smallest area, the conclusion that a gradient did in fact exist appears justified.

TABLE 5. AVERAGE NUMBERS OF MITOSES PER SECTION IN THE VARIOUS ZONES OF THE FOLLICLE WALL

diameter of follicle	numbers of mitoses					
	membrana granulosa					
	oocyte	inner	outer	folliculi	theca interna	theca externa
80μ	1·3 ± 1·1	—	0·9 ± 1·0	0·6 ± 0·7	—	—
350μ	28·4 ± 4·0	65·1 ± 6·6	7·8 ± 2·8	—	4·5 ± 2·1	1·2 ± 0·9
575μ	31·9 ± 4·5	7·3 ± 2·4	1·5 ± 1·6	—	1·6 ± 1·1	0·6 ± 0·7
600μ	1·5 ± 1·1	4·2 ± 2·1	0·3 ± 0·2	—	0·2 ± 0·2	0·1 ± 0·1

When the growing follicles reached an approximate diameter of 100μ growth was suddenly greatly accelerated, an occurrence which coincided exactly with the first appearance of pools of follicular fluid among the cells of the membrana granulosa. In a short time these pools of fluid ran together to form a single large reservoir, and growth proceeded rapidly. During this stage large numbers of follicles ceased to grow, and ultimately disorganized and disappeared. The next counts were therefore made on the few larger follicles which survived, and which were found to be especially common in late dioestrus and in pro-oestrus. Follicles were chosen with an approximate diameter of 350μ, and as can be seen from the table, all were undergoing extremely rapid growth. A steep gradient of mitotic activity was clearly present with its highest points in the discus proligerus and the inner zone of the membrana granulosa, and its lowest point in the theca externa (figure 11, plate 27).

The rate of follicle growth slackened considerably during the pre-ovulation oestrous period, and when the follicle diameter reached about 550μ, the mitotic activity was extremely reduced. At the same time the follicle wall became thinner, and in the inner zone of the membrana granulosa necrotic cells were common. Some cell debris was shed into the follicular fluid. Only the cells of the discus proligerus, or oocyte zone, remained as active as before, and it should be noted that almost all the mitoses recorded as present in the inner and outer zones of the membrana granulosa were in cells closely adjacent to the discus. As the follicle swelled to bursting point its wall became extremely thin, especially where it touched the outer surface of the ovary, and the oocyte, together with the surrounding cells of the discus proligerus, broke free to float in the antrum. By this time a diameter of about 600μ had been reached, and mitotic activity had almost ceased even among the cells of the old discus proligerus, now termed the corona radiata (figure 12, plate 27).

Oestrone injection experiments. Both the abdominal and the subcutaneous injections produced precisely similar results in the large follicles, but neither had any influence on the small primary follicles. These latter continued to grow at their normal slow rate or, in normal proportions, to undergo atresia. In the larger rapidly growing follicles, however, there were signs in some mice of a slackening of the mitotic activity of the membrana granulosa and the thecae after only one injection. After three or four injections this effect was pronounced, the reduction in mitotic activity being always greatest in the largest follicles. After five or six injections the growth of these follicles had often entirely ceased, but there was considerable variation in the response of different mice. In those ovaries most affected, mitoses were even absent in the discus proligerus which was always the

last part of the follicle to cease cell division. Here, as in all the regions where mitosis had previously been active, there were many necrotic nuclei, and masses of cell debris had been shed into the antrum to float in the follicular fluid.

(iii) *Corpus luteum*

Normal adult. Following the bursting of the Graafian follicle, a corpus luteum was rapidly formed from the torn remnants of the membrana granulosa and the thecae. So rapidly did this take place that no two animals in the post-ovulation oestrous period were found to show exactly the same stage of corpus luteum formation, and in consequence it did not prove possible to obtain a series of mitosis counts similar to those for the follicle. Instead, only a description is given of the changes which took place. Immediately after the egg had been shed, the cells of the old membrana granulosa protruded through the rent germinal epithelium and bulged from the ovary surface. At this stage mitotic activity, which had ceased in the follicle wall prior to ovulation, was resumed, and, as before, it bore a clear relation to the position of the follicular fluid. This fluid had escaped into the periovarian space, and, as already described, it stimulated mitoses in the neighbouring cells of the germinal epithelium. Similarly, most of the mitoses in the developing corpus luteum were in the extreme outer layer of cells still in contact with the extruded fluid. After a few hours the torn germinal epithelium healed over the new corpus luteum thus separating it from the follicular fluid, and immediately the mitotic activity of the outermost cells practically ceased. A new antrum containing follicular fluid now formed rapidly in the centre of the cell mass, and as in the growing follicle, mitoses appeared among the cells bordering this fluid. When the fluid was newly formed about fifteen mitoses per section were present in the inner zone of cells, while only two or three mitoses were found in the outer zone. However, the secretion of new follicular fluid quickly ceased, and in early metoestrus absorption commenced. With this the mitotic activity practically ceased throughout the whole corpus luteum, and the cells became luteinized. Divisions of luteinized cells were very rare, and the few that did occur appeared to be restricted to newly formed corpora lutea.

Oestrone injection experiments. Oestrone had no apparent effect on the corpora lutea, and neither abdominal nor subcutaneous injections caused any mitotic activity.

(iv) *Fallopian tube*

Normal adult. The inner layer of cells lining the cavity of the Fallopian tube forms an epithelium which varies from cubical to columnar in form. Thrown into simple folds it is based on a thin connective tissue layer and surrounded by muscle coats. Mitotic activity was low in all parts of the Fallopian tube in all phases of the oestrous cycle. However, as can be seen from table 6 and figure 3, a cycle of activity was evident in the cells of the inner lining epithelium. The counts were made on millimetre lengths of the epithelium, and following an almost entire absence of mitoses during the first and second days of dioestrus, mitotic activity rose slightly towards the end of that period. A peak of activity, in which there was about one mitosis in each 2 mm. length of epithelium, was reached in early pre-ovulation oestrus, and thereafter cell divisions became rarer until the lowest level was once again seen on the first day of dioestrus.

TABLE 6. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE INNER LINING EPITHELIUM OF THE FALLOPIAN TUBE

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·01 ± 0·09	0·01 ± 0·09	0·04 ± 0·19	0·30 ± 0·93	0·46 ± 0·74	0·27 ± 0·49	0·08 ± 0·27	0·04 ± 0·19

Oestrone injection experiments. Numbers of mitoses comparable to those seen in normal early oestrus were not induced by the injections of oestrone, but, nevertheless, mitotic activity was clearly stimulated (figure 3). Maximum numbers of cell divisions were obtained after two injections, and then, following a temporary reduction, a second lesser peak was reached after four injections. After five and six injections mitotic activity again declined.

TABLE 7. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE INNER LINING EPITHELIUM OF THE FALLOPIAN TUBE

numbers of oestrone injections					
1	2	3	4	5	6
0·14 ± 0·42	0·15 ± 0·55	0·03 ± 0·17	0·11 ± 0·31	0·08 ± 0·27	0·03 ± 0·17

(v) *Corpus uteri*

Normal adult. Between the fundus and the cervix the body of the uterus has a constant structure. There is an inner mucous membrane, the endometrium, based on a thick connective tissue layer, the lamina propria, which is in turn surrounded by two thick muscular sheaths, the inner circular and the outer longitudinal. The cyclical waves of mitotic activity were studied in the endometrium, the cells of which, together with those of the uterine glands, showed the highest mitotic rate of any of the uterine cell layers, although mitoses were commonly seen, especially during oestrus, both in the connective tissue cells (see p. 485) and in the muscle cells (see p. 486). The results of the mitosis counts for the endometrium are shown in table 8 and figure 3. Following only slight activity on the first day of dioestrus (figure 17, plate 29), the number of cell divisions rose rapidly to a maximum on the third day of dioestrus (figure 18, plate 29). Thereafter, an equally rapid fall took place (figure 19, plate 29), and the number of cell divisions was at its lowest for the whole cycle in late post-ovulation oestrus. A second but smaller burst of mitotic activity occurred in metoestrus (figure 20, plate 29), but activity again died down on the first day of dioestrus.

TABLE 8. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE ENDOMETRIUM OF THE CORPUS UTERI

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
3·6 ± 2·8	10·2 ± 7·1	21·4 ± 23·0	9·0 ± 11·1	5·9 ± 8·8	2·4 ± 2·3	0·6 ± 0·9	10·3 ± 9·2

Oestrone injection experiments. There was an extremely rapid and pronounced reaction to the injections of oestrone, and the highest rate of mitotic activity was reached after only one injection (figure 3). This induced activity was actually higher than that seen in any phase of

the normal oestrous cycle. There was, however, a rapid fall in the number of mitoses as the oestrone treatment proceeded, and only a few cell divisions were to be seen after four and five injections. A slight rise in mitotic activity was detected after the sixth injection.

TABLE 9. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE ENDOMETRIUM OF THE CORPUS UTERI

numbers of oestrone injections					
1	2	3	4	5	6
32·4 ± 21·9	21·1 ± 12·5	2·3 ± 3·8	0·1 ± 0·3	0·1 ± 0·2	0·2 ± 0·9

(vi) *Cervix uteri*

Normal adult. At its posterior end the cavity of the corpus uteri is suddenly reduced in size, and the single sheet of cells forming the endometrium ceases abruptly to be replaced by the stratified epithelium which lines the cervix uteri. Apart from this epithelium and the thin layer of connective tissue immediately beneath it, the greater part of the thick walls of the cervix uteri is made up of circular and oblique smooth muscle fibres. The mitotic activity was studied in the stratified epithelium. Counts were made on strips of this epithelium, 1 mm. long, immediately adjacent to the junction with the endometrium. As usual, the lowest mitotic activity was found in the first day of dioestrus. There was a slight rise during the second day, and the highest numbers of mitoses were present in the third day of dioestrus (figure 15, plate 28). This peak was followed by a rapid drop in mitotic activity during pro-oestrus (figure 16, plate 28), but a second, though smaller, peak of activity was observed in late oestrus (figure 3).

TABLE 10. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE CERVIX UTERI

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·6 ± 0·8	1·9 ± 1·6	43·1 ± 14·1	9·0 ± 5·2	12·9 ± 9·0	26·5 ± 10·9	24·5 ± 11·2	6·7 ± 7·6

Oestrone injection experiments. The effects of the oestrone injections were immediate and marked (figure 3). Abnormally large numbers of mitoses were found after only one injection, and a maximum was reached after two injections. A fall in the rate of cell division followed, but after five injections mitotic activity was again very pronounced.

TABLE 11. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE CERVIX UTERI

numbers of oestrone injections					
1	2	3	4	5	6
18·5 ± 12·9	26·7 ± 13·0	8·8 ± 7·3	14·1 ± 10·1	30·8 ± 16·8	18·3 ± 10·2

(vii) *Vagina*

Normal adult. The inner wall of this duct is thrown into deep longitudinal folds, and is lined with a stratified epithelium based on a relatively thick layer of connective tissue surrounded by a thin layer of smooth muscle fibres. All the cell layers of the vaginal wall

showed some mitotic activity, but attention was concentrated on the most active layer, the stratified lining epithelium. Mitoses were often present throughout the whole thickness of this stratified epithelium, but they were always most abundant in the basal layer of columnar cells. The numbers of mitoses per unit length of epithelium were counted in transverse sections cut at a point half-way down the vagina. Mitoses were least numerous during the first day of dioestrus, and there followed a rapid rise in mitotic activity which reached a maximum during the third day (figure 3). Relatively slight activity was found in

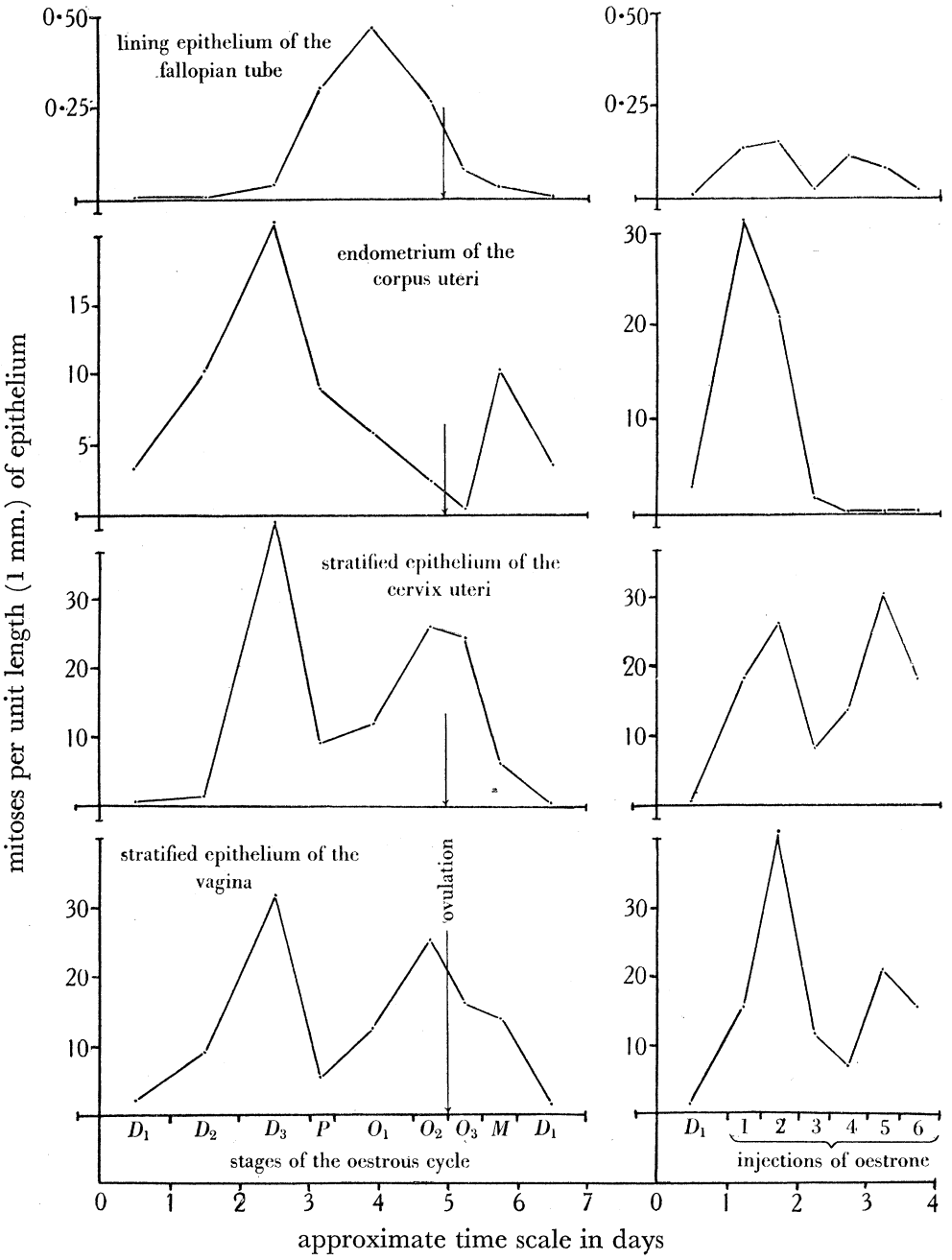

FIGURE 3. Graphs showing the average numbers of mitoses in various parts of the reproductive system during the normal oestrous cycle and following injections of oestrone. $D1$, first day of dioestrus; $D2$, second day of dioestrus; $D3$, third day of dioestrus; P, pro-oestrus; $O1$, early oestrus; $O2$, full oestrus; $O3$, late oestrus; M, metoestrus.

the pro-oestrous period, but large numbers of cell divisions were again present during full oestrus. The activity at this time was, however, not as great as that seen on the third day of dioestrus, and this second wave of mitotic activity died down during late oestrus and metoestrus.

TABLE 12. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE VAGINA

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
2·2 ± 1·5	9·7 ± 10·6	32·6 ± 14·8	6·5 ± 6·4	13·4 ± 10·0	26·7 ± 7·9	16·8 ± 13·9	14·7 ± 5·9

Oestrone injection experiments. Injections of oestrone caused a great rise in the mitotic activity of the stratified epithelium, the peak being reached after two injections (figure 3). As in the normal cycle, this great activity was immediately followed by a fall in the numbers of cell divisions, and although the oestrone treatment was continued, relatively few mitoses were present in animals which had received four injections. After five injections a second, though lesser, wave of activity was observed.

TABLE 13. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE VAGINA

numbers of oestrone injections					
1	2	3	4	5	6
15·6 ± 9·9	41·5 ± 23·4	12·1 ± 8·1	7·5 ± 6·1	21·9 ± 12·2	16·7 ± 10·2

(2) *Urinary system*

(i) *Kidney*

Normal adult. The cortex of the kidney is composed of Malpighian bodies embedded in masses of tortuous uriniferous tubules, the whole being permeated by a network of blood capillaries, while the medulla, leading into the pelvis, is composed of thousands of converging collecting ducts. Cell divisions proved to be extremely uncommon in all parts. Attention was concentrated on the cubical epithelial cells lining the uriniferous tubules of the cortex, but in spite of a prolonged search, mitoses were only discovered in these cells during full oestrus (figure 21, plate 30). Even in this phase of the oestrous cycle mitoses were rare, but careful and extended examinations disclosed their presence in all the five animals composing this group. It must be concluded that the length of life of kidney cells is so extended that their replacement is only a slow process.

TABLE 14. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE KIDNEY CORTEX

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0	0	0	0	0	0·02 ± 0·14	0	0

Oestrone injection experiments. No cell divisions were discovered in the uriniferous tubules of animals which only received up to four injections of oestrone. After five injections, however, the mitotic activity noted was about equal to that seen during full oestrus, and the highest activity of all was found after six injections (figure 4).

TABLE 15. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE KIDNEY CORTEX

numbers of oestrone injections					
1	2	3	4	5	6
0	0	0	0	0·03 ± 0·17	0·06 ± 0·24

(ii) *Ureter*

Normal adult. The ureter is lined internally by a transitional epithelium composed of about five layers of cells. The cells of the basal layers are small and either cubical or columnar in form, but the cells situated at the free surface are relatively very large and their long axes are parallel to that of the duct. Usually each of these large cells contains two, and even occasionally four, nuclei, a condition also common in similar cells in the transitional epithelium of the urinary bladder. The lining epithelium of the ureter is based on a thin layer of connective tissue which is in turn encased in smooth muscle layers. The transitional epithelium was studied for signs of mitotic activity, but although cell division was seen, such activity was never great. All the types of cells present in the epithelium were seen in division, but it appeared that when the large cells at the free surface underwent mitosis only the nucleus completed the process with the result noted above that two nuclei were commonly present in one cell. The numbers of cell divisions per millimetre length of the transitional epithelium throughout the oestrous cycle are shown in table 16 and figure 4. No mitoses at all were recorded in dioestrus, but a low peak of activity, which quickly died away, was found during full oestrus.

TABLE 16. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE TRANSITIONAL EPITHELIUM OF THE URETER

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0	0	0	0·01 ± 0·10	0·03 ± 0·17	0·18 ± 0·42	0·02 ± 0·14	0

Oestrone injection experiments. The epithelium showed no signs of reacting to the presence of oestrone until after three injections had been given. Mitoses were then still rare, but after five injections a peak of activity was reached which was considerably greater than the normal maximum seen in full oestrus. After six injections cell division was again uncommon (figure 4).

TABLE 17. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE TRANSITIONAL EPITHELIUM OF THE URETER

numbers of oestrone injections					
1	2	3	4	5	6
0	0	0·02 ± 0·14	0·13 ± 0·36	0·31 ± 0·52	0·03 ± 0·17

(iii) *Bladder*

Normal adult. On the inner posterior surface of the bladder there is a triangular area termed the trigone, which is described, together with the urethra, in the next section. The inner surface of the remainder of the bladder, when collapsed, is thrown into deep folds, and is lined by a transitional epithelium four or five cell layers thick. The basal two or three layers are composed of small cells with nuclei only about 3·5μ in diameter, but in the outermost layer, the cells, while far fewer in number, are relatively enormous in size with nuclei up to 20μ in diameter. The lining epithelium is based on a thick layer of connective tissue bounded by two prominent layers of smooth muscle, the inner circular or oblique and the outer longitudinal. Mitotic activity was very low in all layers of the bladder wall, and of those mitoses which were seen in the transitional epithelium, the majority were in the largest cells (figure 22, plate 30). In the normal mouse, even after a prolonged search, no mitoses were found in dioestrus, late oestrus, or metoestrus, and such mitotic activity as was noted in pro-oestrus, early oestrus, and full oestrus was only slight (figure 4).

TABLE 18. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE TRANSITIONAL EPITHELIUM OF THE BLADDER

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0	0	0	0·01 ± 0·10	0·01 ± 0·10	0·03 ± 0·17	0	0

Oestrone injection experiments. Mitoses were more common in the oestrone-injected than in the normal animals, although none was found after only one, two, or three injections. A very few were present after four injections, and the highest activity was reached after five injections (figure 4). This last group was the only one in which all animals showed some mitoses, but it should be noted that the relatively high average number recorded was largely due to the extreme activity shown by one animal. After six injections mitotic activity had again waned, although it still remained much higher than normal.

TABLE 19. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE TRANSITIONAL EPITHELIUM OF THE BLADDER

numbers of oestrone injections					
1	2	3	4	5	6
0	0	0	0·04 ± 0·19	2·19 ± 4·63	0·11 ± 0·31

(iv) *Urethra and trigone*

Normal adult. The inner lining of the urethra is a form of stratified epithelium which varies from three to ten cells in thickness, and which extends on to the inner surface of the bladder to cover most of the area known as the trigone. Beneath the stratified epithelium of the urethra is a prominent connective tissue layer which contains many large blood capillaries, and outside this are two smooth muscle layers, the inner and thinner being circular and the outer longitudinal. Counts of the cell divisions in unit lengths of the

stratified epithelium were made in the urethra at a point approximately half-way between the bladder and the clitoris. However, as the epithelia lining the urethra and the trigone are similar in appearance so also are they similar in mitotic activity, and the figures given in table 20 refer equally to the trigone. Cell divisions were always most common in the basal cells of the epithelium, which is a further point of difference between the stratified and transitional epithelia. It will be seen that the mitotic activity was lowest during the first and second days of dioestrus. It then rose slowly to reach a peak at full oestrus, and underwent a decline during late oestrus and metoestrus (figure 4).

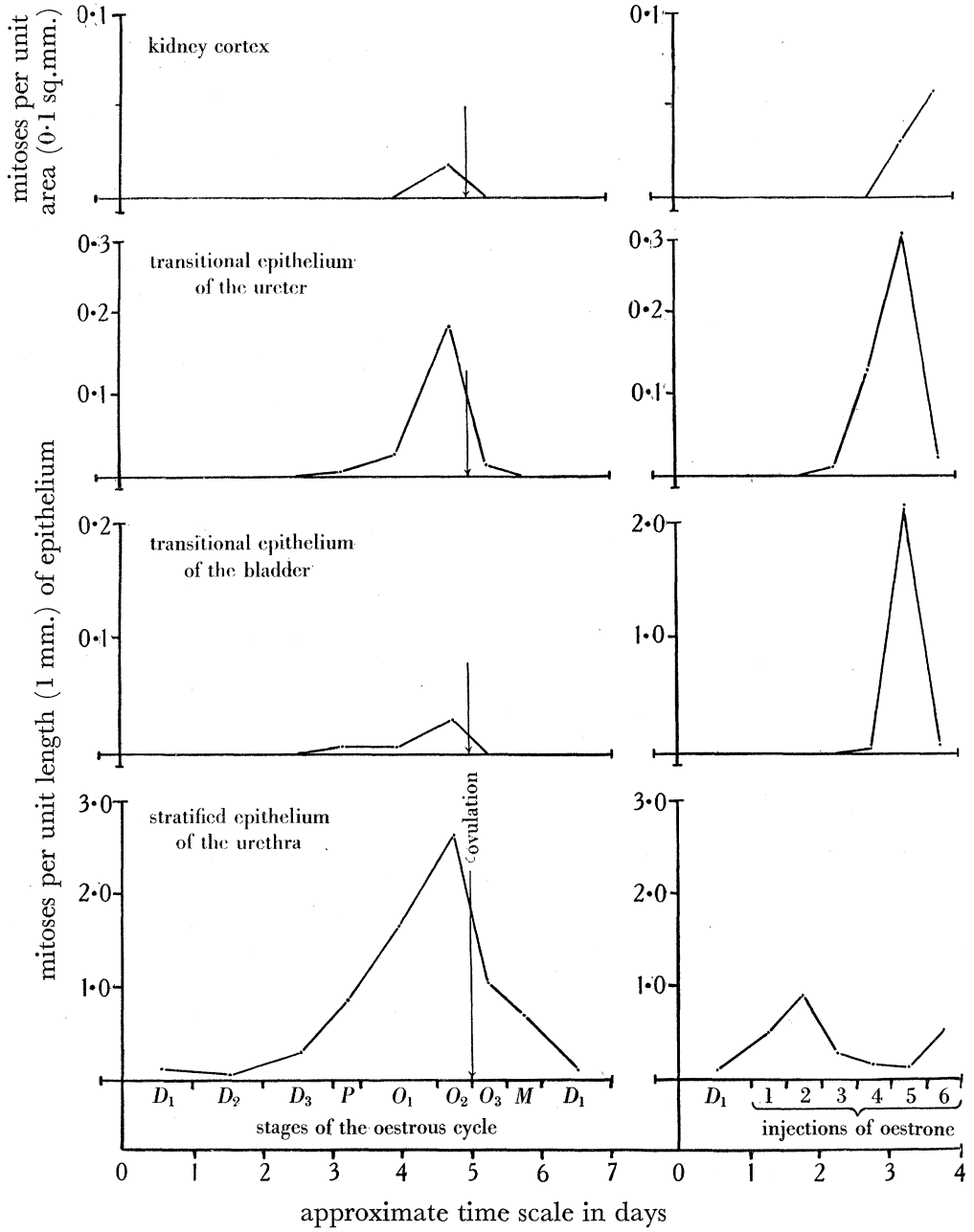

FIGURE 4. Graphs showing the average numbers of mitoses in various parts of the urinary system during the normal oestrous cycle and following injections of oestrone. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

TABLE 20. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE URETHRA

dioestrus			pro-	oestrus			met-
1st day	2nd day	3rd day	oestrus	early	full	late	oestrus
0·16 ± 0·42	0·08 ± 0·27	0·34 ± 0·68	0·87 ± 1·09	1·69 ± 1·48	2·62 ± 1·57	1·02 ± 1·25	0·72 ± 0·75

Oestrone injection experiments. The numbers of mitoses noted after artificial stimulation were never so high as those seen during the normal oestrous period, but nevertheless a clear peak of activity was apparent after only two injections of oestrone (figure 4). Thereafter, cell divisions became steadily rarer to reach a minimum after five injections, but after six injections there was a second rise in mitotic activity.

TABLE 21. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE URETHRA

numbers of oestrone injections					
1	2	3	4	5	6
0·48 ± 0·77	0·90 ± 0·95	0·27 ± 0·49	0·17 ± 0·46	0·12 ± 0·36	0·50 ± 0·79

(3) *Alimentary canal*

(i) *Oesophagus*

Normal adult. The posterior region of the oesophagus, which was the part examined in detail, is lined by a typical stratified epithelium, the thickness of which varies slightly according to the stage of the oestrous cycle. This epithelium is based on a connective tissue layer which is split by a very thin layer of longitudinal smooth muscle fibres and surrounded by muscle coats. Cell division was studied in the stratified lining epithelium where it was mainly confined to the cells of the basal layer. As can be seen from the table and from figure 5, such division was uncommon during dioestrus (figure 23, plate 30) and in pro-oestrus. Mitotic activity rose sharply during the early and full oestrous periods (figure 24, plate 30), but the maximum number of cell divisions was not found until the late post-ovulation oestrous period. In metoestrus mitoses were less common, and a minimum was again reached during the first day of dioestrus.

TABLE 22. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE OESOPHAGUS

dioestrus			pro-	oestrus			met-
1st day	2nd day	3rd day	oestrus	early	full	late	oestrus
1·7 ± 1·6	1·7 ± 2·3	1·9 ± 1·5	1·4 ± 1·1	5·6 ± 3·0	6·9 ± 3·8	10·2 ± 5·9	7·5 ± 5·2

Oestrone injection experiments. After only one injection of oestrone, the number of mitoses counted was significantly higher than normal, and a maximum was reached after two injections. A decline in mitotic activity followed, and after four injections the number of

cell divisions was even slightly lower than that seen in normal dioestrus. A second, though smaller, rise occurred after five injections, but again after six injections activity was low (figure 5).

TABLE 23. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE OESOPHAGUS

numbers of oestrone injections					
1	2	3	4	5	6
3·9 ± 3·0	5·0 ± 2·8	3·3 ± 2·5	1·5 ± 1·0	2·5 ± 1·5	1·8 ± 1·1

(ii) *Stomach*

Normal adult. The whole of the left half of the stomach as well as part of the right is lined by non-glandular stratified epithelium, and forms a storage organ. The remainder of the right half of the stomach is glandular, and is lined by a typical mucous membrane. The stratified epithelium lining the left part of the stomach was not closely studied for mitotic activity, but it was nevertheless clear that such activity did occur in a cyclical manner as in the oesophagus. In the mucosa of the glandular region mitotic activity was confined to the cubical cells lining the necks of the gastric glands close to the junction of these glands with the bases of the gastric pits. It appeared that, as a result of this activity, new cells were pushed outwards to replace those dying in the columnar lining epithelium, and also that others were passed into the tubular glands to replace any worn-out cells in that region. Thus there appeared a zone of mitotic activity about half-way through the thickness of the mucosa. In this zone the numbers of mitoses were counted in unit areas of 0·01 sq.mm., the results obtained being recorded in table 24 and figure 5. As elsewhere, a cycle of mitotic activity was immediately evident with the lowest numbers of mitoses present on the first day of dioestrus. From this point activity rose slowly through late dioestrus, pro-oestrus, and early oestrus to reach a sudden maximum during full oestrus. Cell divisions were still common in late oestrus, but during metoestrus they were rapidly reduced in numbers.

TABLE 24. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING ZONE OF THE STOMACH MUCOSA

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·88 ± 1·25	1·58 ± 1·55	3·00 ± 1·53	3·49 ± 3·18	3·47 ± 2·54	6·40 ± 2·65	4·73 ± 3·30	2·54 ± 1·76

Oestrone injection experiments. Following injections of the female sex hormone into mice in early dioestrus, there was a similar relatively slow rise in mitotic activity to that noted during normal pro-oestrus and early oestrus (figure 5). After the fifth injection a sudden maximum of activity was noted, and after the sixth injection cell division, though less frequent, was still commoner than at any time during normal dioestrus.

TABLE 25. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING ZONE OF THE STOMACH MUCOSA

numbers of oestrone injections					
1	2	3	4	5	6
2·64 ± 1·51	2·59 ± 1·60	1·98 ± 1·84	2·56 ± 2·13	4·89 ± 2·78	3·24 ± 1·83

(iii) *Duodenum*

Normal adult. Sections were cut at a point approximately 30 mm. from the pyloric sphincter. At this point, as is usual, the area of the inner surface is greatly increased by the presence of villi at whose bases open the short crypts of Lieberkühn lined by a simple cubical epithelium. In the region studied there is no muscularis mucosa, and no Brunner's glands were seen. Between the submucous connective tissue and the outer serous coat are two sheaths of smooth muscle fibres, the inner being circular and the outer longitudinal. No mitoses were seen in any of the cells of the villi, and it appeared that the columnar epithelium lining the inner wall of the duodenum was, in this respect, entirely inert. Cell divisions, however, were common in the cubical cells lining the crypts of Lieberkühn, and the columnar epithelium of the villi, which was constantly being worn away, was probably replenished from this source. Mitotic activity was therefore studied in the cubical cells, but as the crypts were short and tortuous, it was impossible, as in the stomach, to assess the numbers of cell divisions per unit length of epithelium. Instead, counts were made of the mitoses present in unit section areas of 0·01 sq.mm. of the proliferating zone. The results obtained in this way are shown in table 26 and in figure 5. Minimum mitotic activity was found on the first day of dioestrus (figure 25, plate 31). A slight increase was evident on the second day, and on the third day great numbers of cell divisions were present (figure 26, plate 31). Activity then decreased during pro-oestrus and early oestrus, and it was relatively low at full oestrus. A second rise began in post-ovulation oestrus, and numbers of mitoses, exceeding even those in the third day of dioestrus, were present during metoestrus.

TABLE 26. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING ZONE OF THE DUODENAL MUCOSA

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
4·9 ± 3·7	5·9 ± 4·4	14·1 ± 4·3	12·8 ± 5·4	12·0 ± 5·5	8·6 ± 4·7	10·6 ± 4·9	14·5 ± 5·7

Oestrone injection experiments. The reaction to the abnormal presence of oestrone was immediate, and great activity, equal to that in normal metoestrus, was seen after only one injection. As the treatment proceeded the numbers of cell divisions fell rapidly until after four injections they approached those normally seen on the first day of dioestrus. However, a second rise in mitotic activity followed, and considerable numbers of cell divisions were again counted after six injections (figure 5).

TABLE 27. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING ZONE OF THE DUODENAL MUCOSA

numbers of oestrone injections					
1	2	3	4	5	6
14·9 ± 4·4	11·8 ± 4·6	6·4 ± 4·5	5·5 ± 3·1	10·1 ± 5·4	12·1 ± 5·5

(iv) *Large intestine*

Normal adult. The inner surface of the large intestine is covered by a columnar epithelium, while the crypts of Lieberkühn, which make up the thickness of the mucosa, are lined by cubical cells. The mucosa and submucosa are sheathed by a thick layer of circular

smooth muscle fibres and a thin layer of longitudinal fibres, the whole being bounded by the serous coat. As in the stomach and duodenum the cells of the inner lining epithelium were never seen to undergo division. The replacement of these cells, as well as that of the glandular cells of the crypts of Lieberkühn, was effected by the great mitotic activity of the cubical cells lining the crypts. The same method of assessing this activity was adopted as was used in the case of the stomach and duodenum, all the cell divisions within unit

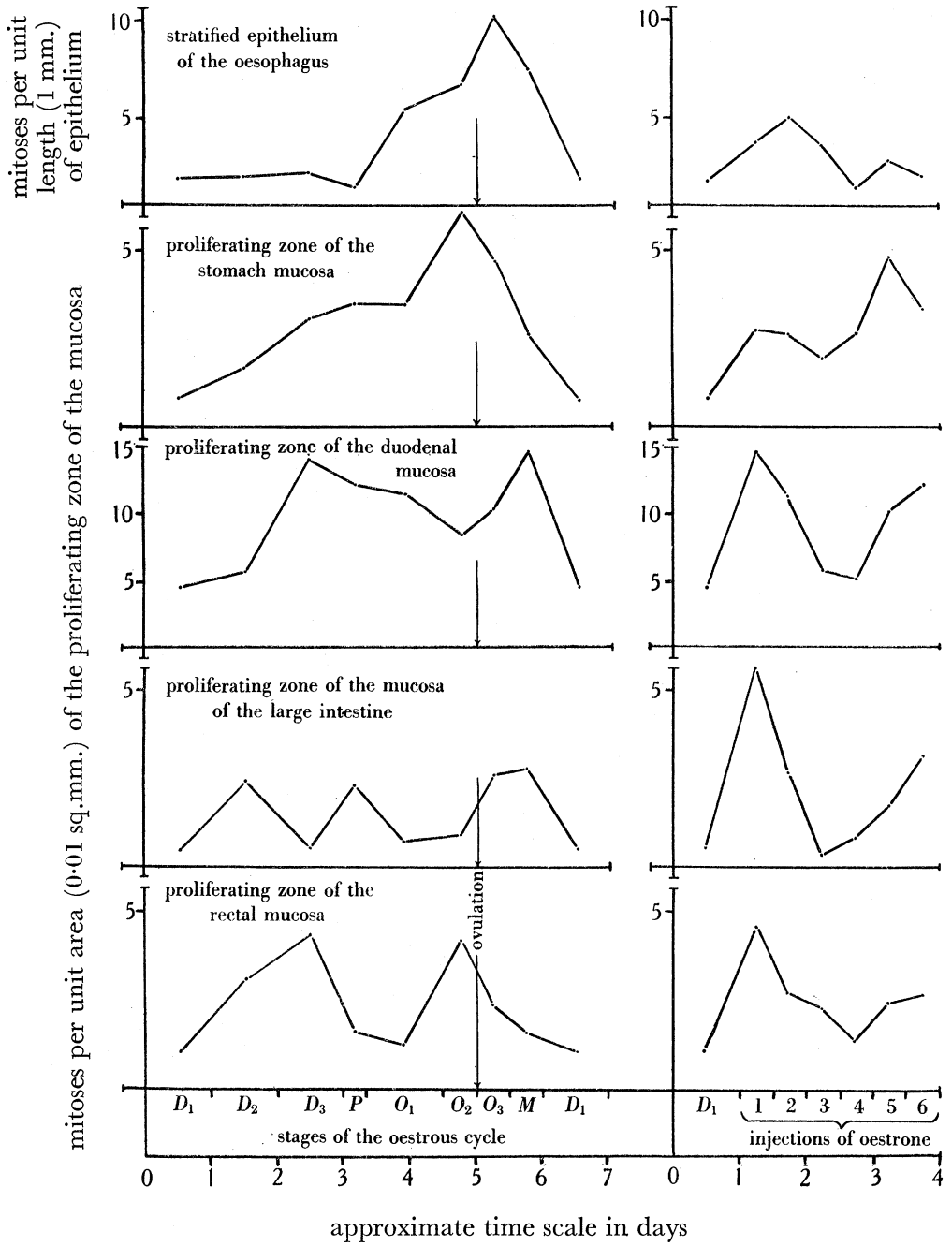

FIGURE 5. Graphs showing the average numbers of mitoses in various parts of the alimentary canal during the normal oestrous cycle and following injections of oestrone. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

areas of 0·01 sq.mm. being counted. After remaining low during the first day of dioestrus, mitotic activity rose sharply to a maximum on the second day. On the third day of dioestrus mitoses were again relatively infrequent, but activity was high once more during pro-oestrus. Throughout early oestrus cell division was reduced, and after a slight increase at full oestrus, another peak of activity was noted during late oestrus and metoestrus. Thus three waves of mitotic activity were evident in the large intestine during one oestrous cycle (figure 5).

TABLE 28. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF THE PROLIFERATING ZONE IN THE MUCOSA OF THE LARGE INTESTINE

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·49 ± 0·81	2·44 ± 2·90	0·57 ± 0·79	2·33 ± 2·41	0·79 ± 0·97	0·96 ± 1·12	2·69 ± 2·89	2·87 ± 2·81

Oestrone injection experiments. There was a very rapid and pronounced response to the injected oestrone solution, and after only one injection the average number of mitoses present per unit area was approximately twice that found at the most active periods of the oestrous cycle. Large numbers of mitoses were also found after two injections of oestrone, but the activity at that time was reduced and approximated to that found in normal metoestrus. After three injections the lowest mitosis count of all was recorded, but this was followed by a rise to a second abnormally high peak after six injections (figure 5).

TABLE 29. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF THE PROLIFERATING ZONE IN THE MUCOSA OF THE LARGE INTESTINE

numbers of oestrone injections					
1	2	3	4	5	6
5·76 ± 3·48	2·78 ± 2·47	0·30 ± 0·56	0·86 ± 1·68	1·72 ± 1·71	3·10 ± 4·58

(v) *Rectum*

Normal adult. The part of the rectum studied was that enclosed by the pelvic girdle and tightly bound by connective tissue to the vagina and the surrounding tissues. The detailed structure of the wall is similar to that of the large intestine, except for the presence of a prominent muscularis mucosa. Near the anus the lining mucosa ceases abruptly to be replaced by a thick stratified epithelium which is continuous through the anus with the epidermis. The mitotic activity was studied in the proliferating zone of the lining mucosa. As in the other regions of the intestine, division was seen only in the cells lining the crypts of Lieberkühn, and, as usual, the numbers of mitoses present in unit areas (0·01 sq.mm.) of the sections were counted. The results are shown in table 30 and in figure 5. Relatively few cell divisions were to be found during the first day of dioestrus (figure 27, plate 31), but mitotic activity rose rapidly during the second day to a maximum on the third day (figure 28, plate 31). This was followed by a marked reduction in the numbers of cell divisions present in pro-oestrus, and an even further reduction in early oestrus. During full oestrus a second wave of mitotic activity reached its peak only to die away during late oestrus and metoestrus.

TABLE 30. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING ZONE OF THE RECTAL MUCOSA

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
1·06 ± 1·16	3·17 ± 2·32	4·41 ± 2·05	1·69 ± 2·12	1·37 ± 1·13	4·26 ± 2·29	2·45 ± 2·30	1·69 ± 1·41

Oestrone injection experiments. A rapid response to the injected oestrone resulted in the presence, after only one injection, of large numbers of mitoses similar to those normally seen on the third day of dioestrus and during full oestrus. Activity then died away to a minimum after four injections. After five and six injections a second increase in mitotic activity took place, but this was not so considerable as the first (figure 5).

TABLE 31. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING ZONE OF THE RECTAL MUCOSA

numbers of oestrone injections					
1	2	3	4	5	6
4·61 ± 2·94	2·85 ± 1·54	2·35 ± 1·49	1·42 ± 1·58	2·46 ± 2·34	2·68 ± 2·58

(4) *Exocrine glands*

(i) *Introduction*

A series of four of the principal exocrine glands, not including the mammary gland which is described in detail on p. 496, was studied in order to discover the rate of replacement growth normally taking place within such organs. For convenience, the liver is included in this section.

(ii) *Salivary gland*

Normal adult. The main salivary gland of the mouse is a large and prominent structure situated ventrally across the base of the neck and the anterior region of the thorax. The gland cells are grouped into acini which are drained by large numbers of ramifying ducts. Cell division was never frequent, but was seen both in the glandular cells of the acini and in the duct cells. The mitotic activity of these two regions was of the same order and showed the same variations, and as in the sections the two appeared inextricably mixed, the whole tissue was treated as though it was homogeneous. The total numbers of mitoses were counted in unit section areas of 0·1 sq.mm., and the variations throughout the oestrous cycle are shown in table 32 and in figure 6. No mitoses were discovered during the first and second days of dioestrus, but slight activity was noted on the third day. Thereafter, mitoses became more common until a maximum was reached during full oestrus. A rapid decline in the numbers of cell divisions was then recorded during late oestrus and metoestrus.

TABLE 32. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE SALIVARY GLAND

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0	0	0·03 ± 0·17	0·05 ± 0·22	0·15 ± 0·43	0·34 ± 0·53	0·11 ± 0·34	0·06 ± 0·24

Oestrone injection experiments. The reaction of the gland cells to the presence of oestrogens was slow during the normal cycle, and the artificial induction of cell division by means of oestrone injections also proved difficult. No mitoses were discovered after the first injection, and only slight mitotic activity, such as is normally found during late dioestrus and in pro-oestrus, was present after two, three, four, and five injections. However, after six injections, the response was similar to that normally seen during early oestrus, and had the injections been continued longer a more marked reaction might possibly have been obtained (figure 6).

TABLE 33. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE SALIVARY GLAND

	numbers of oestrone injections				
1	2	3	4	5	6
0	0·02 ± 0·14	0·05 ± 0·22	0·03 ± 0·17	0·06 ± 0·24	0·14 ± 0·34

(iii) *Pancreas*

Normal adult. Apart from the endocrine islets of Langerhans, this gland is composed of compact acini separated from each other by thin strands of connective tissue along which run many blood capillaries. The pancreatic cells have prominent spherical nuclei, and commonly two, and occasionally four, nuclei are to be seen within the one cell. The mitotic activity of the acinous cells was studied throughout the oestrous cycle with the results shown in table 34 and in figure 6. As in the case of the salivary gland, a relatively large unit area of 0·1 sq.mm. was adopted as standard because of the infrequency of the cell divisions. Mitoses were very rare throughout the whole dioestrous period, but their numbers rose slowly during pro-oestrus and early oestrus. Relatively large numbers were present during full oestrus (figure 33, plate 33), the peak of activity being reached in late oestrus. In metoestrus the activity had begun to decline.

TABLE 34. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE PANCREATIC ACINOUS CELLS

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·03 ± 0·15	0·01 ± 0·07	0·01 ± 0·07	0·02 ± 0·09	0·03 ± 0·15	0·08 ± 0·19	0·11 ± 0·24	0·09 ± 0·21

Oestrone injection experiments. The acinous cells reacted only slowly to the presence of injected oestrone, and no significant rise in mitotic activity was noted until after four injections had been given. Then, the activity was greater than at any time during the normal oestrous cycle (figure 6). After five injections the highest number of mitoses was found, although, as indicated by the large standard deviation, there was considerable variation in the degree of reaction shown by the various individuals within the group. All the mice showed considerable stimulation, but two gave especially high mitosis counts and so greatly raised the average for the group. After the sixth injection there was a considerable fall in the incidence of cell division, and the mitotic activity was similar to that normally seen in metoestrus.

TABLE 35. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE PANCREATIC ACINOUS CELLS

numbers of oestrone injections					
1	2	3	4	5	6
0·03 ± 0·15	0·04 ± 0·16	0·05 ± 0·16	0·43 ± 0·54	1·08 ± 1·24	0·09 ± 0·21

(iv) *Liver*

Normal adult. Sections were cut of portions of liver taken from the posterior edge of the large ventral lobe, and it was found that the mitotic activity of the hepatic cells was extremely slight. A prolonged search disclosed no cell divisions at any stage of the oestrous cycle except full oestrus, and even at this time mitoses were very rare. In no animal was there a sufficient number present to allow counts to be made with reasonable accuracy and ease, and consequently no table has been compiled.

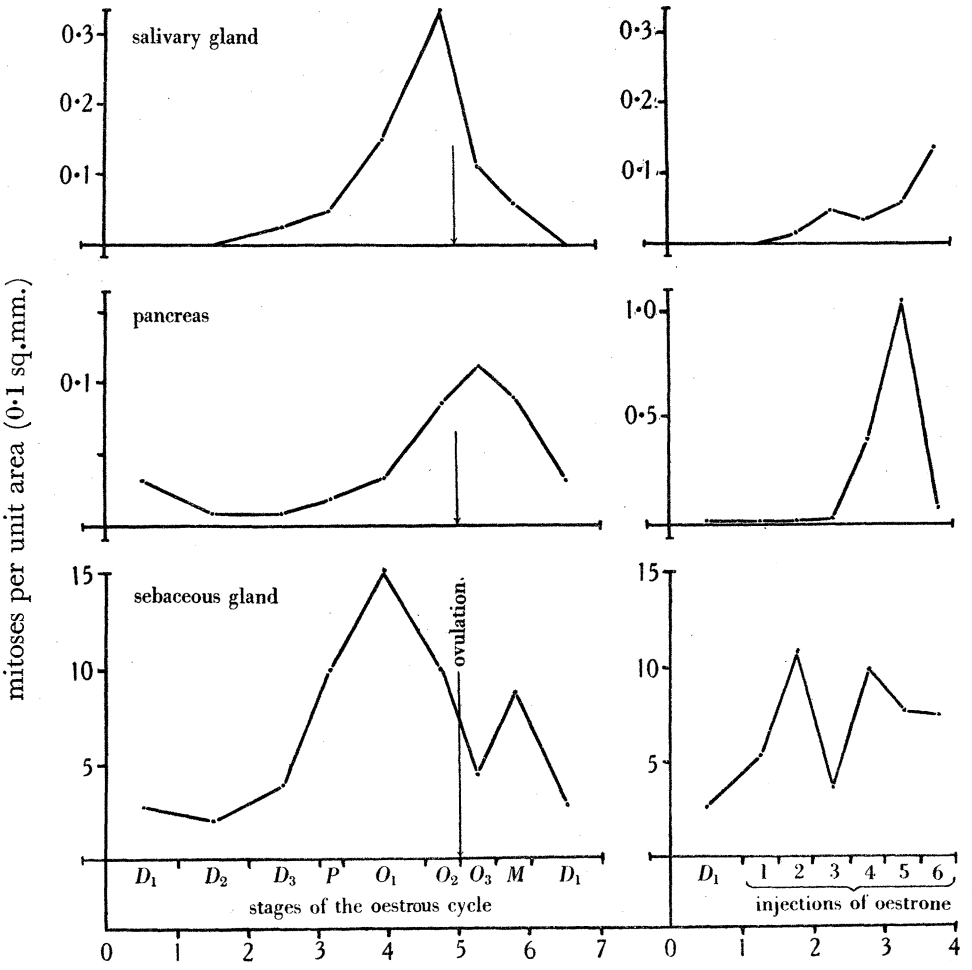

FIGURE 6. Graphs showing the average numbers of mitoses in various exocrine glands during the normal oestrous cycle and following injections of oestrone. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

Oestrone injection experiments. Here also the mitotic activity of the hepatic cells remained at an extremely low level, and no cell divisions were found following the first four injections. After an extended examination, a very few were found after five injections and perhaps slightly more after six injections, but again it proved difficult to make accurate counts.

(v) *Sebaceous gland*

Normal adult. The sebaceous glands, attached by their short ducts to the hair follicles contain large secretory cells with characteristically clear cytoplasm. Each gland is bounded by a thin layer of connective tissue which is continuous with the connective tissue sheath of the follicle. The sebaceous gland differs from the other exocrine glands which have been considered in that it is of the holocrine type. That is to say, not only do the cells produce a secretory fluid, but in releasing this fluid they break down. It was therefore to be expected that the rate of cell replacement would be relatively high, and reference to table 36 shows that this is in fact the case. Mitoses were generally seen in cells close to the sides or the base of the glands (figure 34, plate 33), and the new cells formed were apparently pushed upwards to disintegrate, after the disappearance of the nucleus, near the apex of the gland. It should be noted that this is contrary to the usual accounts of cell replacement in the sebaceous gland, and that Maximow & Bloom (1940), for instance, state that the new cells are derived from the walls of the duct. The numbers of mitoses were counted in unit section areas of 0·1 sq.mm., but due to the small size of the glands it was necessary to examine several sections to make up the total count for one of these unit areas. Only low mitotic activity was found during dioestrus, the lowest point of all being noted on the second day. On the third day of dioestrus the numbers of mitoses were rising, and a peak of great activity was discovered during early oestrus (figure 34, plate 33). Then followed a depression, and only small numbers of cell divisions were counted in late oestrus. In metoestrus, however, there was a second rise to a smaller maximum before the final slackening of mitosis as dioestrus was again reached (figure 6).

TABLE 36. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE SEBACEOUS GLAND

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
2·7 ± 2·5	2·0 ± 1·9	4·1 ± 3·0	9·9 ± 4·3	15·2 ± 5·8	10·0 ± 4·6	4·4 ± 3·4	8·4 ± 4·2

Oestrone injection experiments. There was an immediate response to the influence of injected oestrone so that an increase in the mitosis rate was evident after only one injection. The first wave of mitotic activity reached its peak after two injections (figure 6). After three injections the number of cell divisions had fallen to a figure more typical of normal

TABLE 37. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE SEBACEOUS GLAND

numbers of oestrone injections					
1	2	3	4	5	6
5·2 ± 3·1	10·8 ± 4·2	3·6 ± 2·7	10·0 ± 4·0	7·6 ± 3·9	7·5 ± 3·4

dioestrus, but a second wave of activity rapidly mounted to a maximum after four injections. The decline in cell division which followed was not so marked as before, and even after six injections activity remained relatively high.

(5) *Endocrine glands*

(i) *Pituitary**

Normal adult. The partes nervosa and tuberalis of the pituitary proved to be virtually lacking in cell division, and attention was directed mainly to the anterior lobe where the greatest mitotic activity was found. This lobe consists of the normal chromophobe, acidophil, and basophil cells which show well-defined patterns in their distribution. The chromophobes are the smallest cells and by far the most numerous, while the basophils are the largest and the least numerous. Due to the unequal distribution of the three cell types and to their varying reactions and restricted mitotic activity, it proved impossible to assess their rates of cell division by the methods used in the case of the other glands. Only a general account of the variations in mitotic activity is therefore given. Throughout the first and second days of the dioestrous period no mitotic activity was discovered in the acidophils and basophils, although rarely mitoses were found in the chromophobes. During the third day of dioestrus and in pro-oestrus the chromophobes showed increased activity, and acidophils and even basophils were also found in division. All this activity was reduced during the early oestrous period, but in full and late oestrus there was a return of general activity which died away in metoestrus. The cells of the pars intermedia also showed similar variations in their mitoses rate.

Oestrone injection experiments. The chromophobe cells showed considerable mitotic activity after one and two injections of oestrone, and then, after a period of depression following three and four injections, there was a marked recurrence of activity after five and six injections. In the case of the basophils and especially of the acidophils there was also a wave of cell division after only one injection. The rate of division was then greater than at any time during the normal oestrous cycle, but it died rapidly and almost completely away to return, however, in some degree after five and six injections. Again the cells of the pars intermedia showed similar responses.

(ii) *Thyroid*

Normal adult. The follicles of the thyroid gland vary greatly in size and are lined by a glandular epithelium which may be cubical or squamous in form according to the degree of tension. The interfollicular spaces are filled with a connective tissue, and with many cells more closely resembling those of the glandular epithelium than of normal connective tissue. It was found that mitotic activity was extremely slight. Both the glandular epithelial cells and the cells of the interfollicular spaces were only rarely seen in division, and, in fact, the only animals in which mitoses were seen at all were those killed in full oestrus. Even within this group there was some discrepancy, as in two of the five individuals, even after long searches, no cell divisions were found. In the other three individuals occasional

* This account of the mitotic activity of the pituitary is based on the same material as was used elsewhere in this paper, but the examination of the gland was made by Mr T. Kerr, who has published a detailed account of his results in 'Mitotic Activity in the Female Mouse Pituitary'. *J. Exp. Biol.* **20** (1943) 74-78.

mitoses were discovered in the glandular as well as in the interfollicular cells, but it was not considered worth while to attempt detailed counts.

Oestrone injection experiments. There was no immediate reaction to the presence of injected oestrone, and no mitoses were found until after the fifth injection. Then the mitotic activity discovered was perhaps similar to that noted in normal full oestrus. After the sixth injection a considerably greater response was induced, although there was great individual variation. This was the only group in which counts could easily be made, and these varied between one individual with apparently no mitoses at all to another with an average of 2·1 mitoses per unit section area of 0·1 sq.mm. Part of the thyroid of this last individual is shown in figure 29, plate 32.

(iii) *Parathyroid*

Normal adult. These glands are composed of packed masses of small cells which, in section, appear to be arranged in groups or cysts separated from each other by strands of connective tissue and by blood capillaries. These small cells were far more active mitotically than the larger cells of the thyroid, and it was easily possible to make comparative counts of the cell divisions. This was done, as usual, for unit areas of 0·1 sq.mm., and the results are shown in table 38 and in figure 7. It will be seen that mitotic activity was low during the first and second days of dioestrus, and that it began to increase during the third day. Considerable numbers of mitoses were present in pro-oestrus, but the numbers were again relatively low in early oestrus. Then followed a slow increase in the rate of cell division during full and late oestrus to a second peak of activity in metoestrus.

TABLE 38. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE PARATHYROID GLAND

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·04 ± 0·19	0·06 ± 0·23	0·23 ± 0·50	0·96 ± 0·77	0·19 ± 0·41	0·40 ± 0·72	0·43 ± 0·55	0·94 ± 0·90

Oestrone injection experiments. The response to the injections of oestrone was immediate so that after only one injection almost as many cell divisions were counted as in normal pro-oestrus or metoestrus. The usual fall in the rate of division followed, and a minimum was reached after four injections. A second wave of mitosis began to develop after five injections, and had increased still further after the sixth and last injection (figure 7).

TABLE 39. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE PARATHYROID GLAND

numbers of oestrone injections					
1	2	3	4	5	6
0·89 ± 0·78	0·55 ± 0·68	0·24 ± 0·44	0·10 ± 0·30	0·36 ± 0·61	0·53 ± 0·67

(iv) *Islets of Langerhans*

Normal adult. The islet cells have characteristically pale staining cytoplasm with clearly defined cell limits. Several instances of binucleate cells were observed, but it was found that

cell division was rare. Indeed, no signs of mitotic activity were discovered in any of the mice except those in full oestrus and one in late oestrus (figure 30, plate 32). Even in full oestrus only four mitoses were found in three out of the five animals, while in late oestrus only one mitosis was discovered. It was clear that the rate of cell replacement was extremely slow, and in these circumstances comparative counts were not attempted.

Oestrone injection experiments. No cell divisions were discovered in any of the groups receiving from one to four injections of oestrone, but several mitoses were discovered in the five injection group. Sample sections from one of the animals in this last group yielded seven mitoses, and even greater activity was found in some of the animals receiving six injections. However, it should be noted that in both of these last groups there were individuals whose islets showed no cell divisions, and that in neither did the induced activity approach such proportions that it could easily be counted.

(v) *Adrenal*

Normal adult. The adrenal glands are divided into cortex and medulla, the former being further subdivided into three zones, a narrow outer zona glomerulosa, a prominent middle zona fasciculata, and an inner zona reticularis. The rate of replacement growth was studied both in the cortex and the medulla. The cortex was regarded as homogeneous for the purpose of the mitosis counts which were made on unit section areas of 0·1 sq.mm. The activity in this region was found to be higher than that in the medulla, and the results of the counts are shown in table 40 and in figure 7. Few cell divisions were found in dioestrus and in pro-oestrus, but the mitotic activity increased during early oestrus to reach a maximum in full oestrus (figure 31, plate 32). Thereafter it fell quickly to another low level in late oestrus and metoestrus. Throughout the whole cycle the rate of division in the cells of the medulla was very much lower than that in the cortex, and in fact, no mitoses at all were discovered in this region during dioestrus, pro-oestrus, and early oestrus. In full oestrus several cell divisions were found in the medulla, but they were never so numerous as to make comparative counting easily possible. Two or three mitoses were also found in late oestrus, but they did not appear to be so numerous at this stage as they were during full oestrus. None was discovered during metoestrus.

TABLE 40. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE CORTEX OF THE ADRENAL GLAND

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·03 ± 0·17	0·01 ± 0·09	0·01 ± 0·09	0·02 ± 0·14	0·11 ± 0·31	0·12 ± 0·33	0·02 ± 0·14	0·04 ± 0·19

Oestrone injection experiments. Following only small numbers of cell divisions in the adrenal cortex after one injection, mitotic activity in this outer region rose rapidly (figure 7). After two injections it reached an intensity similar to that seen in normal pre-ovulation oestrus, but then instead of becoming rarer, the cell divisions continued to increase in frequency as the injections proceeded. After the fifth injection of oestrone, the number of mitoses was about six times that seen in normal oestrus, and after the sixth injection the number was again doubled. This great stimulation of cell division was not equalled in the adrenal

medulla, although an increase in the mitotic activity of this region was induced. Occasional rare mitoses were discovered after protracted examinations in individual mice receiving one, two, and three injections, but it was not until after four injections that mitoses were discovered in as many as three out of the five animals comprising the group. After five and six injections, one or two mitoses were found in four out of the five animals in each group, and one of these divisions is shown in figure 32, plate 32. This induced activity appeared to be considerably greater than that normally seen during pre-ovulation oestrus, but in the absence of precise counts no definite conclusion on this point could be reached.

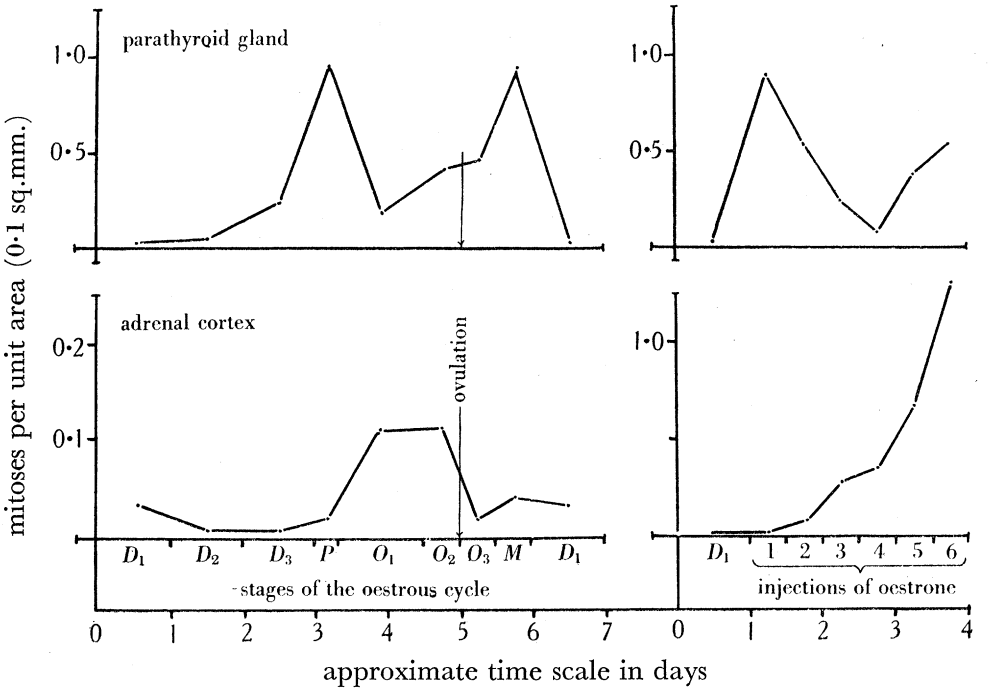

FIGURE 7. Graphs showing the average numbers of mitoses in the parathyroid and the adrenal cortex during the normal oestrous cycle and following injections of oestrone. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

TABLE 41. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·1 SQ.MM.) OF SECTIONS OF THE CORTEX OF THE ADRENAL GLAND

numbers of oestrone injections					
1	2	3	4	5	6
0·04 ± 0·19	0·11 ± 0·31	0·28 ± 0·53	0·36 ± 0·61	0·68 ± 0·97	1·29 ± 1·51

(6) *Miscellaneous tissues*

(i) *Introduction*

The results recorded above are mainly concerned with epithelia as these are most easily studied and generally show the most active mitosis. However, other types of tissue were examined incidentally during this work, and some of these, which appeared to be especially interesting, are described below.

(ii) *Uterine connective tissue*

Normal adult. Of the types of connective tissue examined, that situated immediately beneath the endometrium of the corpus uteri, and known as the lamina propria, was found to be especially suitable for a study of cell division. It contains an abundance of cells in the network of reticular fibres, and these cells appear to be much more active mitotically than those, for instance, of the submucous connective tissue of the alimentary canal and of the dermis, although mitoses were seen in both of these last situations. The mitoses observed in the lamina propria were entirely typical. The cells became swollen with non-staining cytoplasm, and showed the normal metaphase figure arrested by the action of colchicine. It should be recorded, however, that this change in the size and appearance of the cell when in division made it difficult in most cases to be quite certain that the cell involved was in fact a connective tissue cell and not a wandering lymphocyte. However, as lymphocytes, once fully formed, were not seen to divide in other localities, such as the wall of the duodenum where they are common, it was presumed that most, if not all, of the mitoses observed were in cells belonging to the connective tissue. Counts of mitoses were made in unit areas of 0·01 sq.mm. in sections of the uterus, and the type of variation to be seen during a normal oestrous cycle is indicated in table 42 and in figure 8. Cell division was rare during the first and second days of dioestrus, but it became much more common on the third day of dioestrus and in pro-oestrus. The wave of activity reached its height during early oestrus, and was followed by a period of quiescence which lasted into dioestrus. A slight, and perhaps insignificant, rise in the numbers of mitoses was observed in metoestrus.

TABLE 42. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE LAMINA PROPRIA OF THE CORPUS UTERI

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·03 ± 0·19	0·04 ± 0·19	0·51 ± 0·75	0·53 ± 0·67	0·74 ± 0·73	0·07 ± 0·25	0·05 ± 0·22	0·08 ± 0·27

Oestrone injection experiments. There was an immediate reaction to the injected oestrone so that an increase in the numbers of mitoses was observed after only one injection, while after two injections a considerable number was present (figure 8). However, this artificial stimulation never succeeded in producing mitotic activity as great as that normally seen in early oestrus. After the third injection a reaction set in so that only a few cell divisions were to be seen, but after the fourth injection the number again began to rise. A peak was reached after five injections to be followed once more by much reduced activity after six injections.

TABLE 43. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE LAMINA PROPRIA OF THE CORPUS UTERI

numbers of oestrone injections					
1	2	3	4	5	6
0·22 ± 0·44	0·25 ± 0·49	0·05 ± 0·22	0·18 ± 0·37	0·39 ± 0·56	0·12 ± 0·35

(iii) *Uterine smooth muscle*

Normal adult. A search was made for signs of mitotic activity in the smooth muscle coats of the alimentary canal, but cell division in this region proved to be rare. It was found that of all the types of smooth muscle sectioned with the various organs, that in the uterus was by far the most active mitotically, and consequently counts were made in this region. There are two sharply defined layers binding the corpus uteri, the inner being circular in direction and the outer longitudinal. Mitotic activity was studied in the inner layer, and the numbers of cell divisions were counted in unit areas of 0·01 sq.mm. in sections which were cut along the length of the muscle fibres. It was found that, following very low activity throughout the dioestrous period, mitoses were relatively common in pro-oestrus and most numerous in early oestrus. Then followed a rapid slackening of mitotic activity, and during full oestrus, late oestrus, and metoestrus, cell division was as rare as during dioestrus (figure 8).

TABLE 44. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF UTERINE SMOOTH MUSCLE

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
0·02 ± 0·14	0·01 ± 0·10	0·04 ± 0·19	0·15 ± 0·38	0·26 ± 0·52	0·03 ± 0·17	0·02 ± 0·14	0·03 ± 0·17

Oestrone injection experiments. Although the mitosis counts from animals receiving one, two, and three injections of oestrone were higher than those from the dioestrous controls, the maximum reaction was not seen until after the fourth injection (figure 8). At that time, even more cell divisions were counted than were normally present in early oestrus, but in the following groups, as in normal, full, and late oestrus, activity was again low.

TABLE 45. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF UTERINE SMOOTH MUSCLE

numbers of oestrone injections					
1	2	3	4	5	6
0·06 ± 0·24	0·05 ± 0·22	0·09 ± 0·32	0·32 ± 0·56	0·08 ± 0·30	0·06 ± 0·23

(iv) *Hypodermal striped muscle*

Following the discovery of mitotic activity, noted above, in various types of smooth muscle, a search was made for similar activity in striped muscle. Longitudinal sections were cut of the panniculus carnosus, the striped muscle layer which is present at the base of the dermis, but at no stage of the oestrous cycle and in none of the oestrone-injected animals was there any sign of nuclear division present in the sections examined. It was concluded that if mitotic division does occur in this type of muscle, it is extremely rare.

(v) *Cerebrum*

One of the cerebral hemispheres from each animal was separated and cut into transverse serial sections. An extensive search failed to show mitotic activity in any part of this region, either in the normal mice or in those receiving injections of oestrone.

(vi) *Lymph nodules*

Normal adult. Lymphatic tissue is especially common in the intestine wall, small nodules of this tissue being present in the mucous membrane, and larger nodules between this membrane and the submucous connective tissue. Each lymph nodule has one or more proliferating regions towards its centre, and these are characterized by the presence of large cells and of masses of cell debris. The large cells, whose limits are often obscure, have nuclei of up to 13μ in diameter, and they are frequently seen in mitosis (figure 35, plate 33). In this way they give rise to a broad outer zone of small lymphocytes, with nuclear diameters of only about 5μ, which ultimately migrate into the body. As the small lymphocytes, once formed, were never seen to undergo mitosis, attention was focused on the divisions of the large cells of the proliferating centres, and the numbers of cell divisions were counted in unit section areas of 0·01 sq.mm. The degrees of activity occurring during the oestrous cycle are shown in table 46 and in figure 8. It is seen that very little variation

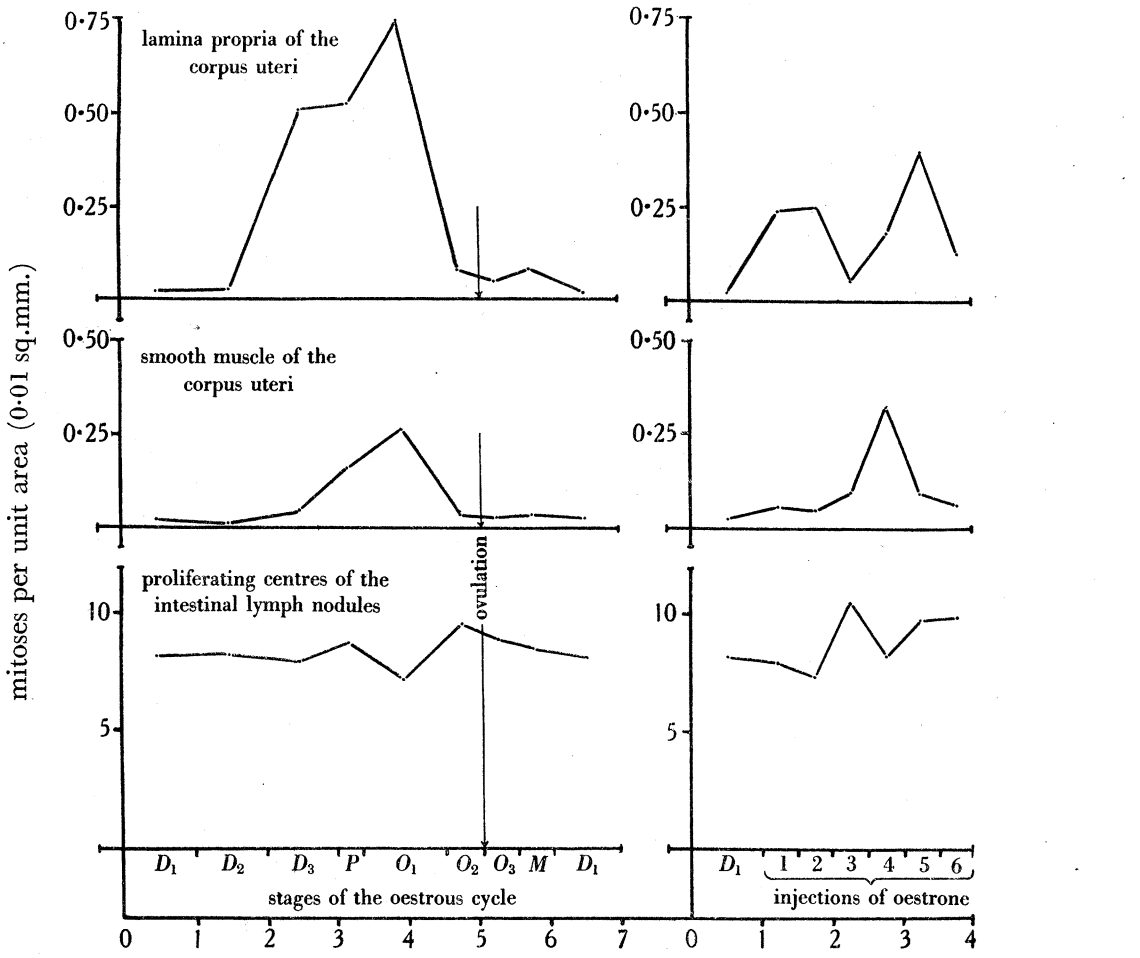

FIGURE 8. Graphs showing the average numbers of mitoses in the connective tissue and smooth muscle coats of the corpus uteri and in the proliferating centres of the intestinal lymph nodules during the normal oestrous cycle and following injections of oestrone. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

in the high rate of cell division is evident, and that the activity noted on the first day of dioestrus was about the same as that found during full oestrus. The average for early oestrus is slightly lower, and that for full oestrus slightly higher than usual, but when the standard deviations are taken into account, it appears that little of importance can be deduced from these variations.

TABLE 46. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING CENTRES OF THE INTESTINAL LYMPH NODULES

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
8·09 ± 3·67	8·17 ± 3·56	7·98 ± 3·26	8·78 ± 3·03	7·07 ± 2·59	9·64 ± 5·84	8·93 ± 4·68	8·45 ± 4·60

Oestrone injection experiments. Similar results are evident from a study of the effects of oestrone injections. As during the normal oestrous cycle, slightly irregular average figures were obtained, and although these variations were greater than those recorded during the normal cycle, they were still not large (figure 8). It appears that the mitotic activity of the proliferating centres of the lymph nodules remained unaffected by the presence of injected oestrone.

TABLE 47. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF THE PROLIFERATING CENTRES OF THE INTESTINAL LYMPH NODULES

numbers of oestrone injections					
1	2	3	4	5	6
7·96 ± 3·53	7·28 ± 3·90	10·67 ± 2·63	8·11 ± 3·79	9·80 ± 4·47	9·96 ± 4·96

(7) *Comparisons and conclusions*

(i) *The ovary*

Starting early on the second day of dioestrus, a new crop of follicles, varying in number between 1 and 18 per ovary, begins to enlarge rapidly. In these growing follicles there is a gradient of mitotic activity with the highest point in that part of the membrana granulosa adjacent to the follicular fluid, and the lowest point in the theca externa. This appears to be due to the great mitosis stimulating powers of the oestrogen dissolved in the follicular fluid, the power of which is felt most acutely in those cells with which it actually comes in contact. Before ovulation mitotic activity almost ceases in all layers of the follicle wall, but because of the continued secretion of fluid into the antrum, the follicle continues to enlarge. In consequence the wall, no longer growing, becomes progressively thinner until it ruptures.

Those regions of the germinal epithelium which overlie rapidly growing follicles are also stimulated to cell division, but this division does not reach its maximum rate until, at ovulation, the follicles burst. Then such parts of the epithelium as come into direct contact with the extruded follicular fluid are stimulated to high mitotic activity, an effect which can be produced experimentally by bathing the ovary with an injected solution of oestrone. This wave of cell division, which quickly dies down, results in the production of new cells

of the germinal epithelium, and of a new crop of oogonia (figure 14, plate 28) together with follicle cells.

Following ovulation, cell division recommences in the remnants of the burst follicle. At first this activity is confined to those cells of the old membrana granulosa which, protruding from the ovary surface, are still in contact with the extruded follicular fluid. Then as a new antrum containing follicular fluid is formed in the centre of the cell mass, mitosis begins in the adjacent cells, but as the secretion slackens and the follicular fluid is absorbed, all activity quickly ceases. Finally, the cells of the old follicle become luteinized to form a typical corpus luteum.

Thus in the follicles, in the germinal epithelium, and in the corpora lutea high mitotic activity is invariably related to the presence of local concentrations of oestrogenic substances dissolved in follicular fluid. However, except in the case of the germinal epithelium with which it is able to come into direct contact, injected oestrone depresses and finally inhibits ovarian mitosis. The significance of this apparently anomalous result is discussed later.

(ii) *Cell divisions*

As all growth in the ovary which is due to mitosis appears to be related to the presence of oestrogenic substances, so also, with the apparent exception of the proliferating centres of the lymph nodules, similar growth in the body organs is shown to be related to the same substances. When the ovary is producing a new crop of follicles, oestrogenic hormones, in increasing concentrations, are passed into the blood, and with the possible exceptions of striped muscle and of the brain, all parts of the body so far examined sooner or later respond by cell division. One or more waves of mitotic activity occur during the period of active oestrogen secretion in all organs of the reproductive, urinary, and alimentary systems, and the same is true in all glands examined of the exocrine and endocrine systems as well as in connective tissue and smooth muscle. Dedifferentiation of the cells involved is not necessary. There is great variation in the degree of response shown by the different tissues, some being characterized by extremely high activity and others by extremely low activity. Thus the proliferating zone of the duodenal mucosa responds to the presence of oestrogens by the most active cell division, whereas, for example, the cells of the islets of Langerhans show only the slightest response. All conditions intermediate between these two extremes are described, the differences observed appearing to depend on the rate of cell replacement required. In this way the sebaceous gland, being of the holocrine type, requires a far higher rate of replacement growth than do the salivary and pancreatic glands, and epithelia, which as a group are more exposed to wear and tear, are far more active mitotically than the connective tissue and muscles which they protect.

It is also possible to produce cell division artificially by means of injections of oestrone, and those cells which are observed to divide most actively during the normal oestrous cycle respond most actively to the abnormal stimulation.

(iii) *Cell inertia*

The organs and tissues which were observed to show a high rate of mitotic activity in the presence of an oestrogen were also found to react most quickly, and, conversely, those organs which show a low mitosis rate react more slowly. Thus the wall of the ovarian follicle and

the lining epithelia of the uterus and vagina, as well as many of the highly active proliferating regions of the alimentary canal, reach a maximum rate of cell division before the end of dioestrus, while less active tissues, such as the stratified epithelium of the urethra, do not develop their maximum mitotic activity before the full oestrous period. Further, certain regions, such as the transitional epithelium lining the urinary bladder and the cells of the thyroid gland, develop only the slightest activity by the time when, in full oestrus, the oestrogen concentration is at its highest. It appears that such cells do not normally react to their full capacity owing to the relative shortness of the oestrous period, but it usually proved possible to force them to an abnormally high response by continued injections of oestrone.

It may be concluded that cells possess an inherent inertia which must be overcome before they can react by division. In tissues in which a high rate of replacement growth is necessary this cell inertia is low, and an immediate and full response is therefore possible even to relatively low concentrations of oestrogen. Conversely, in organs which require only a low rate of replacement growth the cell inertia is high, and is not overcome except by the prolonged presence of oestrogen in high concentrations. Between these two extremes many intermediates have been found.

This conception allows an explanation to be given for the apparently anomalous condition of the proliferating centres of the lymph nodules. It appears that, unlike any other tissue so far examined, there is always sufficient oestrogen present in the normal adult female to cause maximum stimulation of cell division in these regions. In other words, the cell inertia is extremely low, or even entirely lacking, so that almost as high a rate of mitosis may be present on the first day of dioestrus as during full oestrus. Similarly, as mitotic activity is always at a maximum, injections of oestrone have no power to induce any further increase.

(iv) *Mitosis depression*

Besides the limitation imposed on cell division by the existence of a cell inertia, mitosis is also kept within reasonable limits by some force which may be termed a mitosis depressor. With the apparent peculiar exception of the adrenal cortex, this force is rapidly built up within any tissue which undergoes an increase in the rate of cell division, and results in the almost complete cessation of that division. It is therefore necessary, even in the presence of a high and steady concentration of oestrogen as in the oestrone injection experiments, to speak of tissues as undergoing waves of mitotic activity. Thus, for instance, cell division becoming active in the proliferating zone of the rectal mucosa on the second day of dioestrus and reaching a maximum on the third day, dies down abruptly in pro-oestrus. Then, after a pause, there follows a second wave of activity reaching its peak during full oestrus, and like the first wave dying rapidly away. The same principle is apparent in the rapidly growing ovarian follicle, and in this case the second wave of mitosis contributes to the production of the corpus luteum.

As will be seen later in the section on growth in pathological conditions, a rapid decline in the rate of cell division following high mitotic activity does not appear to be due to any exhaustion of the cells concerned. Rather, it seems that as soon as the rapidly accelerating cell division exceeds a certain rate some factor is produced which inhibits mitotic activity.

Each tissue is thus provided with an automatic braking mechanism which prevents cell division becoming excessive and unregulated. Once developed, the depressor takes some time to be eliminated, but as soon as this happens, if sufficient oestrogen is still present, another wave of mitosis, reaching as high a peak as the first, follows. It is common, in organs with a high mitosis rate, for there to be two waves of cell division within each oestrous cycle, but the large intestine is unusual in showing three such waves, the first reaching its maximum on the second day of dioestrus, the second in pro-oestrus, and the third in metoestrus.

(v) *General conclusion*

It may be concluded that those substances which have come to be called oestrogenic or female sex hormones are in fact general mitosis stimulators, that the degree of response of a tissue to this general stimulation depends on its own inherent cell inertia, and that the prevention of excessive mitosis is assured by the development of a mitosis depressor which is also peculiar to the tissue involved.

IV. Mitotic activity in abnormal conditions

(1) *Wound healing*

(i) *Introduction*

A preliminary survey was made of the normal cycle of mitotic activity occurring in the epidermis and dermis, as this region proved to be the most convenient for a study of the processes involved in wound healing. It is well known that the healing of skin wounds is the result of several different processes, but attention was focused on the abnormally active cell division which contributes to the closing of such wounds, and an attempt was made to discover any relation which may exist between this activity and the oestrogenic hormones.

(ii) *Cell division in the skin**

Normal adult. The skin of the mouse consists of an outer epidermis which is a stratified squamous epithelium, and an inner dermis and hypodermis composed of connective tissue. The epidermis varies considerably in thickness in different regions of the body and in different stages of the oestrous cycle, but usually it may be subdivided into the basal stratum germinativum, the stratum spinosum, the stratum granulosum, and the outer stratum corneum. The dermis is composed of dense connective tissue, and it merges into a broader zone of loose connective tissue, the hypodermis, through which, in the regions sectioned, passes the thin sheet of striped muscle fibres, the panniculus carnosus. It was found that all the cell types present in the dermis and hypodermis rarely showed any mitotic activity, and indeed, as already described, the nuclei of the striped muscle of the panniculus carnosus appeared to be entirely inert. Attention was consequently directed mainly to the epidermis.

* This description of the normal mitotic activity of the epidermis is largely a summary of the results obtained by Dr Helena F. Bullough (1943), who also studied these same mice. Her results are, however, presented here in a slightly different manner to make them uniform with those for the various organs already described.

The mitotic activity of the epidermis was found to be almost entirely confined to the basal stratum germinativum, although very occasionally cells of the stratum spinosum were also seen in normal division. To assess this activity the numbers of cell divisions were counted in unit lengths of 1 mm. of sections of skin taken from the mid-dorsal line in the region of the scapulae. The results are shown in table 48 and in figure 9. As usual, the lowest numbers of mitoses were found on the first day of dioestrus, but after the second day, mitotic activity rose rapidly to a maximum in pro-oestrus. The rate of cell division then declined during the oestrous period so that in metoestrus it once again approached a minimum.

TABLE 48. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE EPIDERMIS

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
1·65 ± 1·13	3·22 ± 1·62	3·80 ± 1·47	7·61 ± 4·45	5·23 ± 3·23	4·94 ± 2·49	2·72 ± 1·34	2·36 ± 1·11

Oestrone injection experiments. The first wave of mitotic activity in the epidermis, caused by the abnormal presence of quantities of injected oestrone, reached its maximum after the second injection, but this induced activity was not so high as that seen during the normal pro-oestrous period (figure 9). Cell division was much reduced after the third and fourth injections, but after five injections a second wave of mitotic activity was noted. This wave, however, was not so great as the first, and after the sixth injection activity was again reduced.

TABLE 49. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE STRATIFIED EPITHELIUM OF THE EPIDERMIS

numbers of oestrone injections					
1	2	3	4	5	6
2·26 ± 2·12	5·12 ± 3·63	1·14 ± 1·45	1·41 ± 1·43	3·02 ± 2·76	1·64 ± 1·60

(iii) *Wound healing in the skin*

Normal adult. As already described, a simple incised wound was made in the skin extending through the dermis into the hypodermis and the panniculus carnosus. It was found that healing, by the method known as primary union, was rapid and similar in both the regions of the back which were studied, and that it took the following course. Immediately after wounding the edges of the cut retracted to leave a gap of about 0·5 mm. in the centre of the wound. Bleeding was always negligible, but it was sufficient to form a small clot. The first sections made after 12 hr. showed that the edges of the cut epidermis had rolled slightly into the wound, and come to lie partly beneath the scab. The only other development was the appearance of large numbers of leucocytes densely packed into a narrow zone stretching from the cut epidermis down through the dermis and hypodermis beneath the wound. This zone of leucocytes was separated from the scab by a narrow zone of uninvaded wound tissue. These same conditions persisted after 24 hr., but after 36 hr. further changes were apparent. In the epidermis close to the wound, the cells of the

strata germinativum, spinosum, and to a lesser extent the granulosum began to swell, and due partly to this and partly to the retraction of the cut edges, this region of the epidermis became thicker. The effect was seen for a distance of about 0·5 mm. from the edge of the wound, and in the same region cell division was accelerated. At the same time, cells, derived partly from the strata germinativum and granulosum but mostly from the stratum spinosum, had begun to migrate across the wound immediately beneath the zone of leucocytes (figure 37, plate 34). These migrating cells, much larger than normal epidermal cells, were generally elongated in the direction of their movement, and they never showed any mitotic activity.

Between two and three days after wounding, the healing processes reached a maximum. The migration of the epidermal cells across the wound was completed, and the epidermis, both in the old wound edges and in the new epithelium, attained a thickness of about four or five times the normal (figure 38, plate 34). All the cells were much enlarged, the volumes of both cytoplasm and nucleus being increased many times, and the cell limits were clearly distinct even in the stratum granulosum. This clarity of the cell limits resulted in the abnormal prominence of the spiny cell connexions in the stratum spinosum. Mitotic activity increased to a rate far in excess of the normal pro-oestrous maximum. In the old wound edges of the epidermis it affected the stratum germinativum and, in a lesser degree, the stratum spinosum, and in the new epidermis beneath the scab, once continuity was established, it affected all the cells. Cell division was now also active in the underlying dermis and hypodermis in which, as has been shown, it is normally rare. Mitoses were commonly seen in the connective tissue cells and in the endothelial cells lining the newly forming capillaries, and they were even present, although less frequently, in the striped muscle of the panniculus carnosus, a region which, in this respect, is normally entirely inert. In addition, an unusually high rate of cell division was present in any closely adjacent hair follicles and sebaceous glands, and, in fact, with the apparent exception of the leucocytes, all cell types lying within a radius of about 0·5 mm. of the wound were stimulated to divide, the effect dying away very rapidly at greater distances.

After the fourth day, with healing almost complete, the scab began to peel off, and when this had happened the line of the wound on the skin was rapidly obscured. In section, however, it was seen that the epidermis was still about four or five times thicker than normal, partly due to the cells remaining large in size and partly to their greatly increased numbers following the abnormally high rate of division. Mitotic activity was now rapidly waning, but occasionally cells in division were still to be found in the dermis and hypodermis. This same general picture was seen after five, six, and seven days.

A detailed study was made, in relation to the oestrous cycle, of the mitotic activity occurring in the wounded epidermis. Counts of the numbers of cell divisions were made as usual for unit lengths of 1 mm. of epithelium, but in this case they represent the sum of the numbers present in the two strips of epidermis, each 0·5 mm. long, adjacent to the two edges of the wound. The counts were made on mice killed from one to seven days after the infliction of the wounds. The average figures are shown in the following tables, in which the approximate stages of the oestrous cycle are also recorded. First, in table 50 and in figure 9, are the results obtained when the mice were wounded in early dioestrus. Following a slight fall in the numbers of cell divisions during the first 24 hr., mitotic activity rose to an abnormal

height during the second and third days after wounding. This activity, although more than twice as great, approximately coincided with the normal pro-oestrous peak of cell division, and as in the normal cycle, it died down during the late oestrous period so that after 6 days it was reduced approximately to normal. This apparent connexion with the oestrous cycle is, however, discounted by the results obtained from the series of mice wounded in early pre-ovulation oestrus, results which are recorded in table 51 and in figure 9.

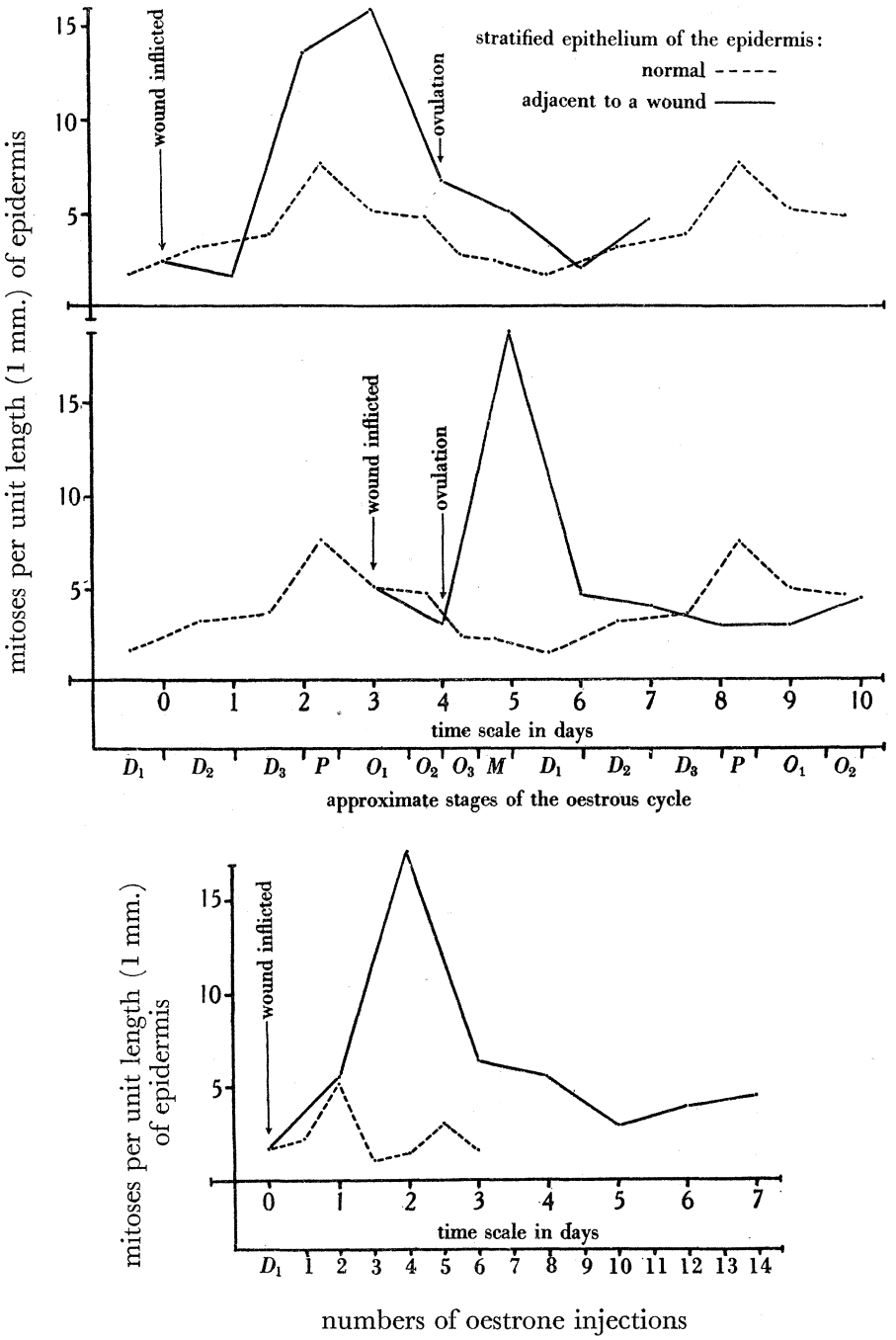

FIGURE 9. Graphs showing the average numbers of mitoses in regions of the epidermis adjacent to healing wounds. For comparison, the mitotic activity of normal epidermis is also indicated. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

In this series, as in the last, the numbers of cell divisions present in the first 24 hr. after wounding were below normal, but the peak of the mitotic activity was reached after only 2 days when all the mice forming the group were in early dioestrus. After the third day the rate of cell division approached normal, and throughout the following oestrous period, 5 and 6 days after wounding, it actually remained subnormal.

TABLE 50. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE EPIDERMIS ADJACENT TO SKIN WOUNDS INFLICTED IN EARLY DIOESTRUS

dioestrus		pro-	oestrus		met-	dioestrus		
2nd day	3rd day	oestrus	early	full late	oestrus	1st day	2nd day	3rd day

days after wounding

1	2	3	4	5	6	7
1·6 ± 1·8	13·6 ± 11·0	15·9 ± 11·9	6·7 ± 4·2	5·1 ± 2·9	2·0 ± 2·2	4·6 ± 3·6

TABLE 51. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE EPIDERMIS ADJACENT TO SKIN WOUNDS INFLICTED IN EARLY OESTRUS

oestrus		met-	dioestrus			pro-	oestrus		
full	late	oestrus	1st day	2nd day	3rd day	oestrus	early	full	late

days after wounding

1	2	3	4	5	6	7
3·3 ± 2·0	18·6 ± 9·1	4·8 ± 2·9	4·2 ± 3·3	3·2 ± 2·0	3·2 ± 2·3	4·6 ± 4·1

Oestrone injection experiments. The conclusion that the abnormal mitotic activity associated with wound healing is independent of the oestrous cycle is further strengthened by the results of the oestrone injection experiments (table 52 and figure 9). In spite of the high concentrations of oestrone maintained within the body by the repeated injections, the course of the healing processes, as reflected by the rate of cell division, was not greatly affected. Unlike the uninjected animals, there was a sharp rise in the rate of cell division during the first 24 hr. after wounding, and it may be noted that this rise was almost exactly similar to that seen after 24 hr. in the unwounded epidermis of oestrone-injected animals. The mitotic activity continued to increase until a high maximum was reached after 2 days, and then after 3, 4, and 5 days activity was progressively reduced. During the sixth and seventh days the rate of cell division was again slightly increased.

TABLE 52. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE EPIDERMIS ADJACENT TO SKIN WOUNDS INFLICTED IN EARLY DIOESTRUS

numbers of oestrone injections

2	4	6	8	10	12	14

days after wounding

1	2	3	4	5	6	7
5·5 ± 2·1	17·5 ± 6·1	6·5 ± 2·7	5·6 ± 3·6	3·0 ± 1·2	4·1 ± 1·6	4·6 ± 2·1

(2) *Cancerous growth*

(i) *Introduction*

For studying the growth of cancer in relation to oestrogenic substances, mammary carcinoma was chosen because of its common occurrence in laboratory mice. Unlike the conditions observed in many other animals, including man, it has been found that spontaneous cancer in the mouse is usually situated in the mammary glands, a phenomenon for which some explanation is offered by Bittner's (1937) discovery of a carcinogenetic virus-like 'milk influence' distributed throughout the body and infecting this tissue. In the present experiments a mammary cancer occurring in a 12 months' old Kreyberg's white label female, no doubt due to the action of the 'milk influence', was cut into small fragments and transplanted into young mice as already described. Examination of pieces of the original growth confirmed that it was a healthy carcinoma with large numbers of mitoses in its dedifferentiated cells, and it retained this character in the animals into which it was introduced.

(ii) *Cell division in the mammary gland*

Normal adult. Before examining the mitotic activity in mammary cancers, an examination of the normal activity of the mammary gland was made. It was found, however, that the tiny groups of secretory cells at the ends of the long collecting ducts were too small to allow accurate measurements of the varying mitotic activity within them. Therefore, unlike the other exocrine glands described earlier, attention had to be focused on the ducts, but it should be noted that the impression was gained that the gland cells undergo cycles of mitotic activity which are similar to, if not identical with, those of the ducts. In all cases the particular gland sectioned was that in the left axilla. Here the nipple opens into a cavity in the hypodermis from which the collecting ducts radiate in all directions. Counts of the numbers of cell divisions were made on unit lengths of 1 mm. of the cuboidal epithelium lining these ducts. The mitosis rate of the epithelia lining the cavity of the nipple and of the space which lies beneath it was not studied in detail, but it was noticed that in both of these positions this rate was usually higher than that of the collecting ducts. During the first 2 days of dioestrus the mitotic activity of the duct walls was only slight, but on the third day it increased greatly so that an average of more than three mitoses per millimetre length was recorded. In pro-oestrus the frequency of cell division declined, and a minimum was reached in early oestrus. A second wave of mitotic activity started during full oestrus to reach a maximum immediately after ovulation. This maximum was similar to that seen on the third day of dioestrus, and again the high rate of cell division was not maintained but died away during the metoestrous period (figure 10).

TABLE 53. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE EPITHELIUM LINING THE COLLECTING DUCTS OF THE MAMMARY GLAND

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
$0{\cdot}25 \pm 0{\cdot}50$	$0{\cdot}16 \pm 0{\cdot}44$	$3{\cdot}16 \pm 2{\cdot}88$	$1{\cdot}50 \pm 1{\cdot}47$	$0{\cdot}66 \pm 0{\cdot}83$	$1{\cdot}48 \pm 1{\cdot}56$	$3{\cdot}06 \pm 2{\cdot}58$	$1{\cdot}57 \pm 1{\cdot}20$

Oestrone injection experiments. Considerable mitotic activity was present in the lining epithelium of the ducts after only one injection of oestrone, but this activity was not nearly so great as that normally seen, for instance, in late oestrus. After two and three injections the rate of cell division was found to decline, but after four injections it again increased. Large numbers of cell divisions were present after five injections, when the second wave of mitotic activity reached its height, and a sharp decline was apparent after the sixth injection (figure 10).

TABLE 54. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT LENGTH (1 MM.) OF SECTIONS OF THE EPITHELIUM LINING THE COLLECTING DUCTS OF THE MAMMARY GLAND

numbers of oestrone injections					
1	2	3	4	5	6
$0{\cdot}83 \pm 0{\cdot}85$	$0{\cdot}58 \pm 0{\cdot}75$	$0{\cdot}54 \pm 0{\cdot}75$	$0{\cdot}98 \pm 0{\cdot}90$	$2{\cdot}70 \pm 2{\cdot}46$	$0{\cdot}91 \pm 0{\cdot}94$

(iii) *Cell division in mammary cancer*

Normal adult. The fragments of mammary cancer, injected subcutaneously into the mid-ventral abdominal region, developed within 2 months to a volume of about 2 c.c., and on sectioning they were found to be embedded in the fatty connective tissue of the hypodermis so that the panniculus carnosus, dermis, and epidermis were displaced outwards. The malignant cells had multiplied to form twisted lobules and strands of cancerous tissue between which thin sheets of connective tissue were present. This connective tissue stroma was usually more prominent close to the edges of the cancer where it was continuous with the thin connective tissue capsule surrounding the whole growth. Only occasionally was the cancerous mass healthy throughout, and it was normal in growths of this size for the centres to be composed of degenerated tissue which often flowed out as a thick liquid when the cancer was opened. In extreme cases this liquid made up almost the entire volume of the cancer, leaving only a thin rind of carcinomatous cells, and occasionally, during widespread necrosis, the capillaries broke down to add their blood contents to the degenerating central pool. Both cell necrosis and cell division were always common throughout the whole cell mass, but it was a general rule for mitotic activity to be greatest in the outermost zone of healthy cells (figure 36, plate 33). It was noticed that in the fatty hypodermal connective tissue immediately surrounding the growth there were occasional signs of mitotic activity. This activity, however, was very slight, and in no way approached that seen after wounding.

As the structure of each cancer varied so greatly from healthy to dead tissue, it followed that there were also marked local variations in the rate of cell division. The difficulties which this fact presented to a comparative study of mitotic activity were overcome by surveying the whole structure of each cancer sectioned, and choosing the most healthy and mitotically active regions within it. The mitosis counts, recorded below, were then made exclusively in these active regions on unit section areas of 0·01 sq.mm. The figures obtained in this way show that the rate of cell division bore no relationship to the oestrous cycle. Within the limits of individual variation and experimental error, mitotic activity remained constant and high (figure 10).

TABLE 55. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF ACTIVELY GROWING REGIONS OF IMPLANTED MAMMARY CARCINOMATA

dioestrus				oestrus			
1st day	2nd day	3rd day	pro-oestrus	early	full	late	met-oestrus
11·1 ± 2·9	10·2 ± 3·2	10·6 ± 3·5	10·4 ± 2·6	11·7 ± 3·5	11·3 ± 2·4	10·7 ± 2·9	9·1 ± 1·5

Oestrone injection experiments. As shown in table 56 and in figure 10, similar results were obtained from a study of the mammary cancers of mice receiving injections of oestrone. As during the normal oestrous cycle, an almost steady average of about ten mitoses per unit section area was recorded, and it was evident that the injections had in no way affected the rate of cell division.

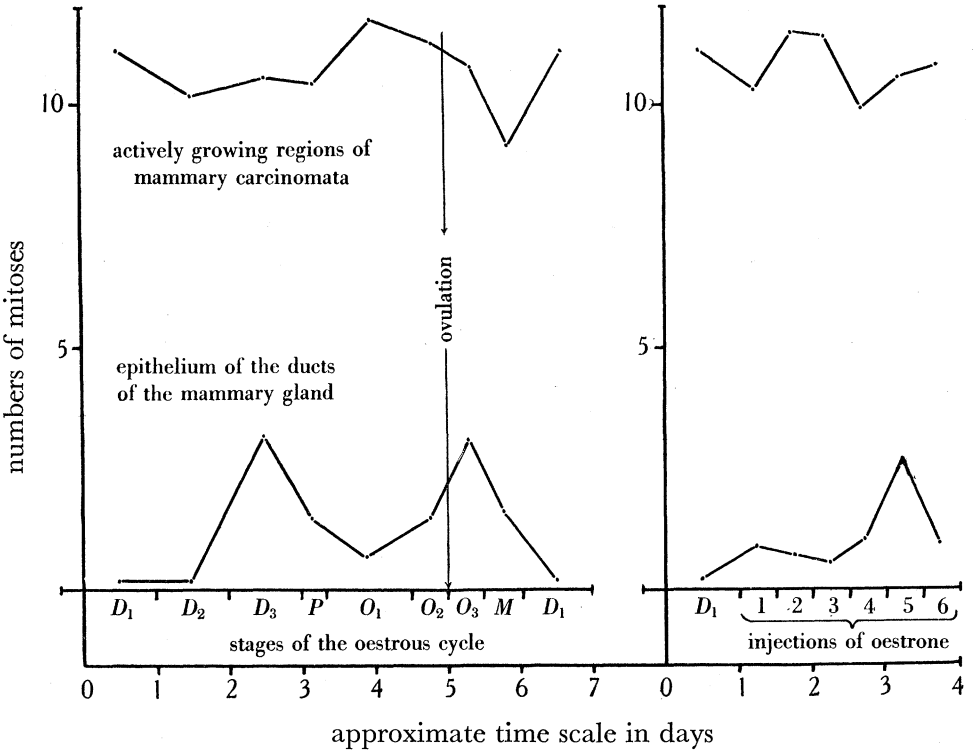

FIGURE 10. Graphs showing the average numbers of mitoses in the ducts of normal mammary glands (unit length of epithelium = 1 mm.) and in mammary carcinomata (unit section area = 0·01 sq.mm.) during the normal oestrous cycle and following injections of oestrone. *D*1, first day of dioestrus; *D*2, second day of dioestrus; *D*3, third day of dioestrus; *P*, pro-oestrus; *O*1, early oestrus; *O*2, full oestrus; *O*3, late oestrus; *M*, metoestrus.

TABLE 56. AVERAGE NUMBERS OF MITOSES PRESENT PER UNIT AREA (0·01 SQ.MM.) OF SECTIONS OF ACTIVELY GROWING REGIONS OF IMPLANTED MAMMARY CARCINOMATA

numbers of oestrone injections					
1	2	3	4	5	6
10·3 ± 1·7	11·5 ± 2·9	11·4 ± 2·2	9·9 ± 2·1	10·6 ± 3·0	10·8 ± 3·4

(3) *Comparisons and conclusions*

(i) *Wound healing in the skin*

Mitotic activity in the mouse skin is normally considerable in the epidermis, and negligible in the dermis and hypodermis. In the epidermis it is cyclic with a single maximum during the pro-oestrous period, and a minimum on the first day of dioestrus. After wounding, however, there is a great increase in the rate of cell division, which is unconnected with the oestrous cycle, and not influenced by repeated injections of oestrone. It affects especially the epidermal cells, but in the dermis and hypodermis cell division is also common in the connective tissue cells, the capillary endothelial cells, the cells of the hair follicles and sebaceous glands, as well as occasionally occurring in the striped muscle of the panniculus carnosus. In fact, it is apparent that, with the exception of the leucocytes, all cells within a radius of about 0·5 mm. of the wound are stimulated to abnormal mitotic activity, and that beyond this radius the effect rapidly disappears. Cell migration, together with this abnormal cell division, ultimately closes the wound, and once this has been achieved, mitotic activity is rapidly reduced.

It is evident that the cell division associated with wound healing is not caused solely by a general mitosis stimulator such as oestrone, for this abnormal activity is unrelated to the oestrous cycle, it affects cells which are normally almost, if not entirely, inert, and it is strictly local. Its action is not prevented or hindered by cell inertia because all cells, however variable they may normally be in their reactions to oestrogenic hormones, react simultaneously and fully when in the vicinity of a wound, and neither is it subject to the restriction of a mitosis depressor because the mitotic activity continues until after the wound is closed. This last point was well illustrated by two mice which, owing to constant scratching, were slow in wound closure. In these mice, even after a week, mitotic activity continued to be abnormally high in all cells.

The conclusions are reached that the agent responsible for the mitotic activity comes from the wound itself, and that it does not exert its effect in the same way as an oestrogen. The facts are more easily explained by a theory that the wounded tissue emits not a mitosis stimulator, but something which eliminates cell inertia from all the neighbouring cells. Thus, whatever may be their normal reactions to the oestrogens, all cells are now able to react fully and simultaneously to the presence of even minimal concentrations of these hormones, and in the same way the action of any mitosis depressor is prevented for as long as the wound remains open.

(ii) *Mammary cancer*

Mitotic activity is cyclic in the ducts, as well as in the secretory cells, of the normal mammary gland, a maximum rate of cell division occurring on the third day of dioestrus and again in late oestrus, while in a mammary carcinoma the rate of division of the malignant cells is rapid and sustained. In the normal gland, mitosis is kept under control by the forces of cell inertia and mitosis depression, but in the cancerous gland these controls are lacking. As the malignant cells show no additional reaction to the presence of high concentrations of oestrogen, the impression is given that cell division is always proceeding at the maximum possible rate. These conditions may be compared with those already described for the proliferating centres of the intestinal lymph nodules where the

mitotic activity is also constantly high. It is interesting to note that the rate of the uncontrolled cell division, both in the mammary cancer and in the lymph nodule centres, approximates to an average figure of ten mitoses per unit section area of 0·01 sq.mm., so that even in detail the process of cell division in these two regions is similar.

It may be concluded that, in the absence of any effective controlling forces within a mammary carcinoma, there is always sufficient oestrogen present in the body to maintain the mitotic activity of the cancer cells at a maximum.

(iii) *General conclusion*

It is evident that wounded and cancerous tissues differ from most normal tissues in that the restraining influences of cell inertia and mitosis depression are removed, allowing maximum mitotic activity at all times irrespective of normal and abnormal fluctuations in oestrogen blood content. In the case of wound tissues, this lack of restraint persists only until the wound is closed, but in the case of mammary carcinomata, as in the intestinal lymph nodules, the absence of control is permanent.

V. Discussion

(1) *Mitotic activity in the mouse ovary*

The results reported in previous publications (Bullough & Gibbs 1941; Bullough 1942*c*,*d*,*e*, 1943), and their amplification in the present paper, make possible a fuller understanding of the endocrine mechanism behind the normal functioning of the mammalian ovary. It is generally considered that the ultimate control of ovarian function is centred in the anterior pituitary gland (see reviews by Smith 1939; Fevold 1939), and that in this connexion one of the most important substances produced by the pituitary is the follicle-stimulating hormone which initiates the final rapid growth of successive groups of ovarian follicles. It now appears probable that this hormone exerts its influence by inducing within the ovary the active production of an oestrogenic hormone, some of which, secreted in concentrated solution into the follicular antra, is the actual cause of the rapid follicle growth, and some of which, passed into the blood, reaches all parts of the body. The precise place of origin of the ovarian oestrogens, if indeed one exists, is still unknown. Mossman (1937), from a study of the peculiar ovarian follicles of the pocket gopher, *Geomys bursarius* (Shaw), and Corner (1938) consider that most, if not all, is produced in the follicle wall by the cells of the theca interna, but it has been shown by Parkes (1927) that the total destruction of the oocytes and follicles in the adult mouse ovary by means of X-rays does not inhibit oestrogen production. Since the total elimination of the ovaries is known to stop the oestrous cycle (see review by Allen 1932), it must be admitted that the non-germinal and non-follicular ovarian cells are capable of producing this hormone. Possibly it may prove that the oestrogen passed into the antrum is formed mainly in the follicle wall, whereas that sent out in the blood stream to affect the body is produced mainly by cells in the ovarian stroma.

Whatever may be the source of the oestrogen in the ovary, it is clear that the ovarian cells themselves are highly resistant to its influence, so that, with the probable exception of the cells of the primary follicles discussed later, they only react by division when they come

into close contact with the highest concentrations of this hormone. Thus, although the close proximity of an antrum containing follicular fluid will stimulate some mitotic activity in the germinal epithelium, the full mitotic potentialities of this epithelium are not developed until it is actually bathed by the follicular fluid. Then as many as fifteen hundred, or even two thousand (Allen & Creadick 1937), mitoses may occur in one ovary within a $9\frac{1}{2}$ hr. period. The significance of this great mitotic activity has been the subject of much dispute (see reviews by Heys 1931; Swezy 1933) between those who consider it to be the mechanism for the production of new oogonia, and those who consider, sometimes only on the theoretical grounds of Weissmann's theory of the continuity of the germ plasm, that new germ cells are never produced during adult life. After a detailed study of the mouse ovary, Allen (1923) has described how, at each oestrous period, the mitotic activity of the germinal epithelium does result in the formation of a new crop of oogonia which passes into the ovary through the tunica albuginea. His conclusions have been supported in the case of the eutherian mammals by many authors (see review by Swezy 1933), and in the marsupials by Everett (1942). In the birds, Bullough & Gibbs (1941) and Bullough (1942*a*) have described the mitotic activity of the germinal epithelium of the starling, *Sturnus vulgaris* L., which develops during the egg-laying period and results in the production of many new oogonia; in the Amphibia, Gatenby (1916) has shown that new oogonia arise from the germinal epithelium of the frog, *Rana temporaria* L., each spring; and in the fish, Bullough (1942*b*) has described the post-spawning period of mitotic activity in the germinal epithelium of the minnow, *Phoxinus laevis* L., resulting in the formation of new oogonia, and Mendoza (1943), working with the viviparous teleost *Neotoca bilineata*, also concludes that some at least of the germ cells formed during adult life arise from the germinal epithelium. However, these results, indicating the cyclic production of new oogonia throughout adult life, have been doubted for several reasons. One criticism (Cowperthwaite 1925) is that while the newly formed oocytes of the embryo show early meiotic phases, the youngest oocytes in the adult do not. Duke (1941) has shown that in the rabbit these early meiotic phases cease to be apparent at the age of about 50 days, but he considers that oocytes do continue to be formed thereafter. It is perhaps more logical to doubt the potentialities of the embryo oocytes, with their uncompleted reduction division, than to doubt those of the oocytes formed in the adult which undergo meiosis when they are shed from the mature Graafian follicles. A more important criticism comes from the work of Parkes (1929), who showed that, following the complete destruction by X-rays of the oocytes and follicles in the adult mouse ovary, there is no new formation of germ cells from the germinal epithelium. However, even if the X-rays do not in themselves destroy some vital potentiality in the germinal epithelial cells, the absence of ovarian follicles containing high concentrations of oestrogen may mean the absence of a sufficient stimulus for the necessary mitotic activity. Such an absence of stimulus may also account for the lack of ovarian regeneration from the peritoneum of the hilus following complete ovariectomy (Heys 1931), as well as for the reported failure (Martinovitch 1938) of the germinal epithelium to form new oogonia when ovaries are grown *in vitro*, for apart from the fact that this latter experiment lasted only about a month, normal Graafian follicles were not produced. The observations made in the course of the present work fully support Allen's thesis, and it is concluded that the function of the mitotic activity of the mam-

malian germinal epithelium is partly the production of new epithelial cells, and partly that of new oogonia, perhaps together with their follicle cells. In the mouse, newly formed oogonia may be seen in hundreds immediately beneath the germinal epithelium, especially at the end of each oestrous period. Their continued production in such numbers is necessary in order to replace the constant great loss especially by atresia. It is probable that these conclusions apply equally to the lower vertebrates, where such evidence as there is available suggests that it may be normal for the cells of the germinal epithelium to divide actively, and so to replenish the ovary with new oogonia, about the time when the eggs are shed.

When atresia is avoided, the growth in the mouse of the small primary follicles is steady and continuous. It has not been proved that the mitotic activity which is the basis of this early follicle growth is induced by oestrogenic hormones, but if it is, as seems most probable in view of the evidence accumulated with regard to the other parts of the body, then the growth appears to be of the non-cyclic type seen, for instance, in the proliferating centres of the intestinal lymph nodules. However, a striking change takes place when the follicle cells come into contact with the oestrogens in the follicular fluid of the newly forming antrum. Then the follicle wall reacts by the most active cell division, the effect being greatest, as in the germinal epithelium, in those cells in actual contact with the fluid. Thus the cells adjacent to the poles of liquor folliculi are at the high point of a steep gradient of mitotic activity which extends into, and dies away in, the surrounding cell mass. This mitotic activity of the follicular cells allows the rate of growth of the follicle to keep pace with its rate of expansion due to the increase in volume of the fluid in the antrum, and the most rapid follicle growth is the result of these two interrelated processes. When the pre-ovulation oestrous period is reached, however, the mitotic activity of the follicle wall begins to wane, and finally, in full oestrus, it ceases altogether. It has been shown, in the various normal tissues studied, that any sharp increase in mitotic activity is followed by an equally sharp decrease even in the continued presence of the stimulating oestrogen, and therefore the follicle cells appear to follow the general rule. However, although the follicle wall ceases to grow by cell division, the volume of the liquor folliculi, and with it that of the entire follicle, continues to increase. In consequence, the follicle wall becomes rapidly thinner until it finally bursts, and the liquor folliculi, carrying with it the oocyte together with the corona radiata, oozes into the periovarian space. This appears to be the basis of the follicle bursting mechanism, but that it does not represent the complete picture is evident from the review by Hartman (1939).

As is normal in other tissues, the depression of mitotic activity in the follicle wall at the time of ovulation is followed, due to the continued presence of the oestrogen, by a second wave of mitoses. The new activity occurs especially in those cells of the old membrana granulosa which are either in contact with the follicular fluid extruded into the peri-ovarian space or with the new follicular fluid forming in the centre of the cell mass. As the oestrogen secretion rapidly wanes this activity is only short-lived, and is followed by the luteinization of the cells to form a typical corpus luteum. Once luteinization has been completed, division of the cells of the corpus luteum is rare.

To this theory of the endocrine control of normal ovarian function must be related the results of the oestrone injection experiments. With regard to the germinal epithelium these results are straightforward. As would be expected from the observations on the normal

animal, raising the oestrone concentration in the blood by means of subcutaneous injections has no effect on the mitotic activity of this epithelium (Bullough, unpublished), but abdominal injections of oestrone, administered in such a way that the solution introduced actually bathes the ovary, cause a great reaction. The extreme mitotic activity which may be induced in this way was first reported in the case of the mouse by Stein & Allen (1942) and by Bullough (1942*d*), and in the case of the minnow by Bullough (1942*b*). However, the results of the oestrone injections on follicle growth are at first sight anomalous. In the small primary follicles the injections have no discernible effect, but in the larger rapidly growing follicles they are followed by a cessation of follicular fluid secretion and by the inhibition of mitosis. The lack of any effect on the growth rate of the primary folliciles may be explained, as in the case of the lymph-node centres, by the theory that, as cell division in these regions is already proceeding unchecked, no further stimulation is possible by means of an increase in the oestrogen blood content, but clearly an entirely different explanation is necessary in the case of the larger follicles. In this latter connexion the theory put forward by Moore & Price (1932), and strikingly supported by the experiments of Meyer, Leonard, Hisaw & Martin (1930, 1932), appears to be relevant. It seems that oestrogen depresses the secretion by the anterior pituitary gland of the follicle-stimulating hormone. Thus excessive quantities of injected oestrone probably inhibit the pituitary action and with it the normal oestrogen secretion into the antra. This does not affect the primary follicles, as the injected oestrone exerts a sufficient stimulus for their continued growth, but the mitotic activity of the larger follicles, which is normally stimulated by the oestrogen in the liquor folliculi, ceases. The injected oestrone, permeating the body via the blood stream, is powerless to replace this action of the natural oestrogen as it is not passed into the antrum as follicular fluid.

The conclusion, therefore, seems justifiable that in the mouse, and possibly in the vertebrates as a group, cell division within the ovary is normally induced by the oestrogenic hormones there produced, and it is possible that the final maturation divisions, which occur when the egg is shed, may also be related to these hormones. In view of this conclusion it is of interest to consider what evidence there is available that the cell division, which is so characteristic of the testis activity, is related to androgenic hormones. It has frequently been reported in the case of the mouse that these hormones retard or inhibit spermatogenesis (see review by Moore 1939), a result which appears to be comparable with the inhibition of ovarian mitosis by injections of oestrogenic hormones. Much, however, appears to depend on the dose given, for Selye & Friedman (1941) have shown that although small amounts of androgenic substances inhibit testis growth, large amounts do not. This they consider due to the fact that, while in both cases there is an inhibition of pituitary function and so of natural androgen secretion, only with heavy dosage is sufficient androgen introduced to maintain spermatogenesis. Further, Shay, Gershon-Cohen, Paschkis & Fels (1941) report that large doses of androgens stimulate spermatogenesis in immature rats, while Cutuly & Cutuly (1940), using testosterone propionate, have induced and maintained spermatogenesis in hypophysectomized rats. The experiments with mice and rats have usually been complicated by the existence in a functioning state of the natural mechanism for testis stimulation, but in annually breeding mammals experiment is easier. Thus, in the ground squirrel, *Citellus tridecemlineatus*, Wells & Moore

(1936), using testis extracts, and Wells & Gomez (1937) and Wells (1942), using androsterone and testosterone propionate, have caused the precocious development of spermatozoa in young animals, their unseasonable development in adults, and active spermatogenesis in hypophysectomized adults. Similarly, in fish, the rapid development of spermatozoa in the minnow in autumn has been induced by injections of testosterone propionate (Spaul & Bullough, unpublished), and this result has even been obtained in the exhausted post-spawning testis (Bullough 1942*b*). Also in fish, androgenic hormones have been found to cause the precocious maturation of the testes in *Lebistes reticulatus* (Eversole 1941). It may, therefore, be concluded that, in the absence of the normal stimulus, the production of spermatozoa in the testis can be induced by androgenic hormones, and inferred that these hormones, formed in response to a stimulus from the anterior pituitary gland, normally induce spermatogenesis.

(2) *Mitotic activity in the mouse body*

Having considered the effects exerted within the ovary by that part of the oestrogenic hormone which is passed with the follicular fluid into the antrum, it is now necessary to consider the effect on the rest of the body of that part of the hormone which enters the blood stream. It is generally considered that the principal function of the oestrogenic hormone secreted by the ovary is the maintenance in a suitable condition of the accessory sexual organs and the secondary sexual characters. In the mouse, these hormones, by means of induced mitosis, cause enlargement of the Fallopian tube, uterus, and vagina, as well as of the mammary gland. This is indicated by the well-known fact that the removal of the ovaries results in the atrophy of these organs, an effect which may be reversed by means of oestrone injections (see review by Allen, Hisaw & Gardner 1939). Certainly the accessory and secondary sexual organs are especially sensitive to the influence of the oestrogens, and the study of their responses has monopolized this field of research for many years. The result has been that these hormones have come to be regarded as almost exclusively concerned with oestrous phenomena in the female sex so that they are referred to as oestrogenic or female sex hormones, and recognized and standardized by means of the response of the lining epithelium of the vagina. However, it is also known that in many vertebrates unusual tissues and organs have become involved, for various reasons, in the mechanism for sexual reproduction. Thus, in some monkeys, the skin around the anus and external genitalia becomes thickened under the influence of oestrogenic and androgenic substances, just as in the cock, *Gallus gallus* L., the skin on the head is greatly developed into comb and wattles. In the male frog, *Rana temporaria* L., thumb pads are present, and in the male stickleback, *Gasterosteus aculeatus* L., the posterior region of the kidney is enlarged and modified during the breeding season, as also is the musculature of the pectoral fins (Craig-Bennett 1930). The diversity of the organs which may be involved in this way suggests that these specializations are merely extreme modifications of some normal and universal phenomenon. For instance, the specialized development of a region of sexual skin in the monkey or of the thumb pad in the frog is probably merely an accentuation of that response which is normally shown in some degree by all the skin. It has recently been realized that the growth of organs other than those directly related to the sex function is influenced by the sex hormones. Hyperplasia and hypertrophy of the kidney (Ludden, Krueger & Wright

1941; Pfeiffer, Emmel & Gardner 1940) and of the larger biliary ducts (Gardner, Allen & Smith 1941) have been described in mice which received injections of oestrogens and androgens; oestrone injections into cockerels induce weight increases in the alimentary canal, liver, pancreas, and adrenal (Breneman 1942); and oestrogen therapy has been used successfully for the stimulation of growth in the buccal mucous membrane of menopausal women (Richman & Abarbanel 1942). The results reported in the present paper bring a uniformity to these seemingly diverse observations, for it has been shown in the mouse that, with the apparent exceptions of striped muscle and the brain, all the tissues and organs examined sooner or later react by mitosis to the presence of oestrogenic substances, and it cannot be doubted that these substances normally stimulate the necessary cell replacement in the whole body. It follows incidentally that all organs have the potentiality of enlargement in relation to the sexual processes as secondary sexual characters or even as accessory sexual organs. It must have been in such a manner that the mammary gland was evolved.

It is evident from the study of the mouse that the degrees of response of the various body organs to oestrogen stimulation vary widely. Thus, while the connective tissues show only a slight reaction to the presence of oestrogenic hormones, tissue such as the epidermis show a marked response. Even higher responses are seen in the linings of the uterus, vagina, and alimentary canal, and the highest of all in the lymph nodule centres. The degree of reaction appears to depend on the needs of the various tissues for cell replacement rather than on the degree of their differentiation. In this way, although most glands have only a low rate of cell replacement, the sebaceous gland shows relatively great mitotic activity due to the fact that, being of the holocrine type, its cells are destroyed when releasing their secretory products and must, therefore, be constantly renewed. The general conclusion may be reached that the muscles, the connective tissues, and the protected body organs need relatively little cell replacement and so react only slightly to oestrogen stimulation, while the epithelia covering the body surfaces, whether external on the skin or internal throughout the alimentary canal and the Müllerian duct system, require frequent cell replacement and consequently show a rapid and pronounced reaction to any increase in oestrogen blood content. The extreme mitotic activity of the proliferating centres of the intestinal lymph nodules is apparently due to the necessity for a constant supply of large numbers of lymphocytes.

The results obtained show clearly the existence of mitosis inhibitors, and indicate the fact that there is a constantly varying interaction between them and the stimulation exerted by the oestrogens. First, there is the force, termed the cell inertia, which must be overcome before any tissue can react by mitosis to the presence of an oestrogen. In some organs, such as the brain, this inertia is apparently so great that cell division is never able to occur, while in others, such as the ureter, it is only overcome at full oestrus after the influence of the oestrogen has been strongly exerted for some days. On the other hand, in those organs which require a high rate of cell replacement, inertia is low, and this enables them to respond immediately and fully to any slight rise in the oestrogen blood content. In the extreme case of the constantly active proliferating centres of the intestinal lymph nodules it appears that cell inertia is entirely lacking, and consequently cell division continues unchecked even during periods of low oestrogen blood content. Further, it is seen that the degree of cell inertia may vary greatly within the one organ or even within the one tissue.

Thus the duodenal mucosa has a proliferating zone the cell products of which show no mitotic activity at all, while the stratum germinativum of the epidermis produces cells of the stratum spinosum which are practically inert.

The second mitosis regulator is that force which limits, and then temporarily damps down, any burst of mitotic activity, and which, in consequence, has been termed the mitosis depressor. Because of the action of this depressor, mitotic activity proceeds in waves even when, as in the oestrone-injection experiments, the concentration of oestrogen within the body remains constantly high. Again an exception to the rule is provided by the proliferating centres of the intestinal lymph nodules which, with their constant high rate of cell division, are apparently not subject to this control.

These two mitosis regulating forces are clearly so powerful in the normal adult that, with the usual concentrations of oestrogen, hyperplasia and hypertrophy cannot occur. Unfortunately, it is at present difficult to gain any clear impression of their nature. If it were not for the observations on growth in pathological conditions, especially those on wound healing, it could be argued that the effects observed were merely due to some exhaustion following cell division, so that the most active tissues were those which recovered most rapidly after each wave of mitotic activity. However, in the proximity of wounds it has been found, first, that cell inertia is eliminated even in such normally inactive tissues as dermis and striped muscle, and secondly, that the unusually high rate of cell division which ensues continues without the usual check from the mitosis depressor. It follows that cells are capable of a considerably higher rate of cell division than they normally develop, and also that they are capable of maintaining this great activity unchecked over long periods. Indeed, in cancerous growths it appears that the highest rate of mitotic activity may be continued indefinitely. Exhaustion, therefore, cannot be considered the basis of regulation, and, in fact, it would appear peculiar if such a potentially dangerous process as cell division was not subject to some rigid control of a more positive nature. It is possible that mitosis regulation is achieved by the production of antihormone substances for it has been suggested that every hormone may have a controlling inhibitor. Already, for instance, antigonadotrophic, antithyrotrophic, and antithyroid hormones are known (see reviews by Collip, Selye & Thomson 1940; Thompson 1941). It has been shown that continued treatment with oestrogens inhibits growth in such organs as the skin (Hooker & Pfeiffer 1943), and even the mammary gland (Gardner 1941), as would be expected if an anti-hormone had been produced in quantity, but it has so far proved impossible to isolate such a substance. However, the greatest difficulty in accepting this view of the nature of the normal mitosis regulating forces is that the various body tissues react to oestrogen stimulation quite independently of each other, and it is difficult to believe that one antihormone substance could be capable of producing such a complex effect. It is also important to notice in this connexion how frequently the immediate products of cell division in tissues showing high mitotic activity are themselves entirely inert. This is particularly obvious in the case of the inert lymphocytes produced by the highly active proliferating centres of the intestinal lymph nodules.

An understanding of the problem of mitosis control is clearly desirable, for the subject relates closely to those of wound healing and cancerous growth. It is generally considered that damaged tissues, both in animals (see review by Arey 1936) and plants (see reviews by

Swingle 1940; Schopfer 1943), produce wound hormones one of whose functions is the stimulation of abnormally active cell division. The precise nature of these hormones is not yet understood. Possibly they are breakdown products of protein formed in the dead cells lining the wound, but there is evidence, for instance, from the study of the production of growth-promoting substances in yeast injured by ultra-violet radiation (see review by Needham 1942), that they may be produced as a physiological response to injury rather than as cell disintegration products. The extremely local areas which these substances are able to influence, and the fact that they are apparently not distributed to the body via the blood stream, suggests either that they are unstable or that some mechanism exists for their rapid destruction. Whatever their method of formation and destruction, and whatever their chemical structure, the effect of these wound hormones appears definite. Diffusing into the tissues immediately adjacent to the wound, they greatly increase the rate of mitosis by the elimination of the two forces of cell inertia and mitosis depression. This elimination appears to be almost, if not entirely, complete, and it takes place in all the types of cells examined except the peculiarly inert leucocytes. As a consequence, cell division is observed to occur simultaneously and at an abnormally high rate in tissues of such diverse character as the epidermis, the sebaceous gland, the dermal and hypodermal connective tissue, and even striped muscle, and to continue in these tissues for as long as the wound hormone is secreted. For the duration of the healing process, the tissues upon which these hormones act are similar in their method of growth to the proliferating centres of the intestinal lymph nodules in that it appears there is always sufficient oestrogen present in the body to stimulate their mitotic activity to a maximum degree. They are apparently limited in their rate of cell division only by the availability of their food supply, and consequently they cannot be stimulated to any higher activity by increases, normal or artificial, in the oestrogen blood content. It is in this way that wound tissues resemble cancerous growth, in which, however, control is permanently lacking both in the original cells and in those to which they give rise.

It may be concluded that in the tissues of the normal adult female mouse there is an interaction, constantly varying, between the stimulation to mitosis of the oestrogenic substances and the inhibition of mitosis by the two regulating forces. An increase in the stimulation increases the mitosis rate temporarily and within limits, but a removal of the regulating forces increases the mitosis rate permanently and to an abnormal degree.

(3) *Mitotic activity in animal and plant tissues*

The results and conclusions recorded above relate closely to several fields of zoological, and even botanical, research, for if an oestrogen has been adopted by the various organs and tissues of the adult female mouse for stimulating that mitotic activity which is necessary for cell replacement, it can hardly be doubted that related hormones also function as mitosis stimulators in many other situations in many other organisms. Oestrogenic substances are known to be present and to stimulate the growth of the Müllerian duct system and the secondary sexual characters in representative species of the various classes of vertebrates. It now appears probable that they will also be found to stimulate general mitotic activity in these animals, although considerable variation in the details of mitosis control may be expected, especially in annually breeding species and in groups like the

teleosts which continue to grow in body size throughout adult life. Considerably less information is available regarding the distribution and action of oestrogenic substances in the invertebrate phyla. However, the presence of these substances, as determined by the vaginal reaction of adult ovariectomized mammals, has been recorded in protozoans, coelenterates, worms, echinoderms, molluscs, crustaceans, arachnids, and insects (see review by Scharrer 1941). The diversity of the species which have given these positive results strongly suggests that oestrogens are widely and commonly distributed among invertebrate, as among vertebrate, animals, and although there do not appear to be any records of their effects on the invertebrate Metazoa, oestrogens have been found to exert a stimulating effect on cell division in cultures of the protozoan *Colpidium* sp. (Calcutt 1942) and of colon bacilli (Portes, Lantz & Krajevitch 1939).

Oestrogenic substances are also known to be present in many plant tissues including yeast, potatoes, female willow catkins, palm kernels, and rape seed (see reviews by Doisy 1939; Schopfer 1943). There is also considerable evidence (Schopfer 1943) that these hormones stimulate growth and increase the dry weight of many flowering plants, and it is of great interest to find that a gonadotrophic substance, similar to that which stimulates oestrogen production in the mammalian ovary, has been extracted from oat plants (Borasky & Bradbury 1942).

Returning to the female mammal, it is now possible to suggest a reason for at least part of the peculiar endocrine mechanism associated with pregnancy. It has long been a source of comment (see review by Needham 1942) that during this period large quantities of oestrogen are excreted in the urine, having been formed apparently not in the ovary, which is quiescent, but in the placenta. It must now be considered possible that this hormone is the activating force behind the high mitotic activity of the embryo and placenta, and that during pregnancy the mother is sufficiently protected from the stimulation of the oestrogen by mitosis-inhibiting forces. The placenta apparently also produces a gonadotrophic hormone, and it seems probable that this is the factor which induces the oestrogen production.

As the period of growth of the mammalian embryo is so closely associated with the presence of high concentrations of mitosis-stimulating oestrogens, it is of interest to notice the evidence which is available regarding the conditions in which the embryos of the lower vertebrates develop. In the birds, it has been found that, while the concentrations are never high, oestrone is present in the undeveloped hen's egg (Riboulleau 1938), and that oestrogen synthesis accelerates as development proceeds (Serono, Montezemolo & Balboni 1936). Similarly, oestrogenic substances have been detected in amphibian eggs (Loewe, Lange & Kaer 1929) as well as in those of teleost fishes (Sereni, Ashbel & Rabinowitz 1929). In all these instances the quantities of oestrogen present are not large, but as has been found in the present work, the most active cell division may be stimulated by only small quantities of oestrogen providing that the mitosis regulators are sufficiently weak.

It is therefore possible, at least in the vertebrates, that the great mitotic activity associated with embryonic growth is normally stimulated by oestrogenic substances, a conclusion which makes necessary some consideration of the subject of embryo organizers and evocators. Unfortunately, the chemical nature of these substances is as yet unknown,

although Needham (1942), reviewing the available evidence, concludes that the primary evocator, which increases the rate of cell division and induces neurulation, may, like the oestrogens, be of a steroid nature. However, as has been shown in the present paper, mitotic activity may be increased either by the direct stimulation of an oestrogenic hormone or by the weakening of mitosis control brought about by wound hormones, and it has actually been found that an evocator action is exerted by small, that is by wounded, pieces of adult tissue (see review by Needham 1942).* However, Waddington (1938) has not only shown that a whole series of oestrogenic and carcinogenic steroids, including oestrone, will act on the embryo in a manner similar to that of the primary evocator, but he (1940) has measured the acceleration of the mitotic activity which accompanies neural tube formation, and has found that the increase induced by the steroids is similar to that induced by the natural evocator. Further, while those steroids which were tested induced essentially normal neurulation, the implanted fragments of adult tissues, and even of dead organizers, tended to induce the formation of excessive numbers of irregularly placed structures. In other words, the action of the steroids, like that of the natural evocator, appears to take place in a relatively normal manner within the limits imposed by the embryo individuation field, that set of morphogenetic forces which determines the relative positions, numbers, and shapes of the developing organs. The action of the adult tissue, on the other hand, partially overcomes the control of the individuation field so that organ growth is multiple and disoriented. This latter effect would be expected of substances such as the wound hormones which weaken the mitosis-controlling forces and so allow a rapid and unregulated response to the oestrogens present. A similar breakdown in the control exerted by the individuation field may afford an explanation for the interesting results of Witschi (1930) on the effects on development of the overripeness of amphibian eggs. Briefly, these results indicate that the greater the age of frog's eggs at the time of fertilization, the more abnormal is their subsequent development, and that, following extreme overripeness, growth is chaotic and the resultant cell mass may be transplanted into a normal animal where it grows like a malignant tumour. It has been suggested that this is due to the formation of dangerous carcinogenic steroids (Needham 1936), but, on the other hand, it may be that, with overripeness and approaching death, physiological changes occur which are similar to those in cells injured and dying from other causes. Substances related to wound hormones may then be produced to prevent the development of a controlling individuation field and so to produce rapid and unregulated growth.

The idea of the embryo individuation field, and of the possibility of breaking it by the methods indicated above, are interesting because of their apparent relation to growth control in the adult animal. The persistence of an individuation field into the adult condition, controlling relative growth and so maintaining the correct body form, is indicated, among other things, by regeneration phenomena, and it may well be that such a field forms the basis of the mitosis-controlling forces which have been described in the present work. Just as it appears that the stimulus to cell division within the embryo individuation field may be an oestrogenic hormone, so also the stimulus to mitosis within the system of controls in the adult body is provided by a similar substance, and just as it is possible that

* Berrill (1943) states that Holtfreter's recent unpublished work shows that 'the embryonic inductor responsible for neurulation is liberated from damaged cells'.

the control of the embryo individuation field is weakened by the implantation of pieces of dead or wounded tissue, so also control in the adult is broken by the process of wounding. In the embryo, the total destruction of the individuation field leads to the production of a disorganized growth which may be transplanted as a tumour, and in the adult the elimination of control also leads to malignant growth. In exactly the same way the separation of a tissue from the control of the individuation field, as in tissue culture, enables rapid cell division to occur.

These conclusions are particularly interesting because it is known that cancerous growth frequently begins in association with some region of constant irritation or with wounds which are unable to heal properly. For instance (Muir 1936), the irritation set up in the bladder by the parasite *Schistosoma haematobium* Bilharz causes thickening of the lining epithelium and of the connective tissue, followed by nodular growths, ulcers, and in many cases, cancer. Similarly, a renal calculus in the kidney pelvis may result in the production of an epithelioma, and an ulcer in the stomach or intestine frequently develops into a carcinoma. These are obvious examples, but many other irritating and wounding agents, such as X-rays, ultra-violet rays, radium, and viruses, may also cause the formation of a malignant growth. In all such cases there is irritation or injury, and mitosis is stimulated in the immediate neighbourhood. It can hardly be doubted that wound hormones are responsible for this effect, and it is very probable that cancerous growth results from the prolonged elimination of the normal mitosis-regulating forces. In these abnormal conditions a full response to the stimulating presence of oestrogenic substances is possible, even if these substances are present only in small quantities. So the normal strengthening and repair processes of the adult body, the ability to grow in response to irritation and the ability to close a wound, become dangerous when the irritation is continued to excess or when the wound is prevented from closing.

It may also be noticed here that it is possible, although very difficult, to break the control of the mitosis-inhibiting forces, and to initiate the development of cancerous growth, by the stimulating action of the oestrogenic substances alone (see review by Allen 1942). The prolonged treatment with high concentrations of these substances which is required to achieve this result provides further evidence of the powerful nature of the mitosis inhibitors.

Summing up, it is evident that oestrogenic substances are of widespread occurrence in both animal and plant kingdoms, and although experimental observations are still few, these substances are already known to stimulate cell division and growth in such widely divergent groups as the vertebrates, the Protozoa, the *Bacteria*, and the flowering plants. They are also known to be associated with such regions of high mitotic activity as vertebrate embryos, germinating seeds, and cancerous growth. In the adult female mouse it is probable that ovarian oestrogen formation is induced by the follicle-stimulating hormone of the anterior pituitary gland, and it is interesting to find that a similar follicle-stimulating hormone is associated with the high oestrogen production of the placenta, and that yet another of these hormones has been extracted from plants. With regard to those forces inhibiting mitosis, speculation is limited by an almost complete ignorance of their nature, but it is possible that they are a component part of an individuation field persisting into the adult state. It is a function of the wound hormones to break the mitosis control and

allow a full response to the stimulation of the oestrogenic hormones so that, if necessary, extra tissues may be rapidly provided for wound closure. If this action is continued unduly, excessive and finally malignant growth ensues.

(4) *General conclusions*

The synthesis of oestrogenic substances has been shown to occur in many living organisms of the most diverse nature. In the lower forms of life the ability to manufacture these substances may be a general cell property, but in the vertebrates the ability has become almost entirely restricted to the ovary, testis, and placenta. It is particularly suitable, in these higher animals, that mitosis-stimulating hormones should be produced by organs which must themselves indulge in a high rate of mitotic activity, but it is possible that other tissues may also retain in some degree the power of production, for it has been shown that oestrogens, as well as androgens, are still formed in small quantities after castration (see review by Robson 1940).

Of the large numbers of steroid compounds which have been found to stimulate cell division, only a certain few appear to have been utilized by living organisms. Among those apparently not utilized must be included the carcinogens, substances which induce cell division but for which the normal mitosis-regulating forces are not entirely adequate.

Evidence has been accumulated to show that the normal action of the naturally occurring oestrogenic hormones is related to the stimulation of mitosis, not only in the ovary and in organs and tissues related to the female sex function, but in all body cells. The peculiar sensitivity to oestrogens developed by the female reproductive organs of the vertebrates, and to androgens by the male reproductive organs, is, in all probability, a specialized relationship which may have been evolved only in this branch of the animal kingdom. Certainly very different conditions exist in insects for, although oestrogens are present in these animals, perhaps as general mitosis stimulators, the reproductive organs and the secondary sexual characters are genetically, and not hormonally, controlled.

Although the terms oestrogen, androgen, and carcinogen may be convenient, especially in medical practice, it would appear necessary to introduce a special name to cover this whole group of substances and to denote the basic reaction which they all induce. This would appear to be especially necessary in relation to lower organisms where the distinction between oestrogen and androgen may be lost unless artificial reference is made to the action of these substances on mammalian vaginal epithelium. It is therefore suggested that the term 'mitogen' be used to cover the whole group of mitosis-stimulating substances which exert their influence directly and not through an induced weakening of mitosis control. The naturally occurring mitogenic hormones may all be steroids, but the term cannot be restricted to this chemical group because many unrelated synthetic substances, such as stilboestrol, exert a similar effect.

It may be concluded that there are at least three classes of naturally occurring substances which affect mitotic activity in the mouse:

(*a*) *Mitogenic hormones.* Steroids which act as general mitosis stimulators, and which, according to their influence on vaginal growth or on the growth of the combs of capons, are divided into oestrogens and androgens.

(*b*) *Mitosis inhibitors.* These prevent cell division from becoming excessive and unregulated, and as each type of tissue is controlled independently of its neighbours, it is evident that they are local in their effects. Their nature is almost entirely unknown.

(*c*) *Wound hormones.* Substances, released from damaged cells, which weaken the action of the mitosis inhibitors and so permit the development of an abnormally high rate of cell division in response to the stimulation of the mitogenic hormones. Normally used to assist in wound closure, they may induce the development of cancerous growth if they continue to act for an excessive length of time.

Although there appears to be every justification for the recognition of these substances in the adult mouse, and although it can hardly be doubted that they also exist elsewhere, it is evident that a considerable amount of research remains to be done before similar generalizations can be made regarding other species. Work along these lines may produce results of fundamental importance which may also bear closely on the practical problems of oestrogen therapy, wound healing, and cancerous growth.

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Description of plates

Plate 27

Figure 11. Rapidly growing ovarian follicle showing the gradient of mitotic activity in the membrana granulosa. Large numbers of mitoses are present in the discus proligerus (*d.p.*) and the inner zone (*i.z.*) of the membrana granulosa adjacent to the follicular fluid, but elsewhere in the outer zone (*o.z.*) activity is only slight. Also present is a small primary follicle (*p.f.*). × 220.

Figure 12. Fully grown Graafian follicle about to burst. The discus proligerus has separated from the membrana granulosa to form a corona radiata, and cell division has almost entirely ceased in all the follicle cells. × 220.

Plate 28

Figure 13. Large numbers of mitoses (*m.*) in the germinal epithelium adjacent to a newly forming corpus luteum (*c.l.*) in the late post-ovulation oestrous period. × 400.

Figure 14. Newly formed oogonium (*oo.*) immediately beneath the germinal epithelium (*g.e.*) in the metoestrous period. × 1000.

Figure 15. Stratified lining epithelium (*st.*) of the cervix uteri showing the thin epithelium and the active cell division typical of the third day of dioestrus. × 400.

Figure 16. Stratified lining epithelium (*st.*) of the cervix uteri showing the greatly thickened epithelium and the lack of cell division typical of the pro-oestrous period. × 400.

Plate 29

Figure 17. Endometrium of the corpus uteri to show the lack of mitotic activity on the first day of dioestrus. × 400.

Figure 18. Endometrium of the corpus uteri to show the extreme mitotic activity on the third day of dioestrus. × 400.

Figure 19. Endometrium of the corpus uteri to show the lack of mitotic activity during full oestrus. × 400.

Figure 20. Endometrium of the corpus uteri to show the increased mitotic activity present during metoestrus. × 400.

Plate 30

Figure 21. Mitosis (*m.*) occurring during full oestrus in the epithelium of a uriniferous tubule of the kidney. × 1000.

Figure 22. Mitosis (*m.*) occurring during full oestrus in the transitional epithelium of the urinary bladder. × 1000.

Figure 23. Stratified lining epithelium (*st.*) of the oesophagus showing the lack of mitotic activity on the first day of dioestrus. × 400.

Figure 24. Stratified lining epithelium (*st.*) of the oesophagus showing the active division (*m.*) of the cells of the basal layer during full oestrus. × 400.

PLATE 31

FIGURE 25. Duodenal mucosa showing only slight mitotic activity in the proliferating zone (*p.z.*) on the first day of dioestrus. × 400.

FIGURE 26. Duodenal mucosa showing the large numbers of mitoses (*m.*) in the proliferating zone on the third day of dioestrus. × 400.

FIGURE 27. Rectal mucosa showing the lack of cell division in the proliferating zone (*p.z.*) on the first day of dioestrus. × 400.

FIGURE 28. Rectal mucosa showing the high rate of cell division (*m.*) in the proliferating zone on the third day of dioestrus. × 400.

PLATE 32

FIGURE 29. Mitoses (*m.*) in the cells of the thyroid gland after six injections of oestrone. × 1000.

FIGURE 30. Mitosis (*m.*) in an islet of Langerhans during the full oestrous period. × 1000.

FIGURE 31. Mitosis (*m.*) in the adrenal cortex during the full oestrous period. × 1000.

FIGURE 32. Mitsosis (*m.*) in the adrenal medulla after six injections of oestrone. × 1000.

PLATE 33

FIGURE 33. Division (*m.*) of a pancreatic acinous cell during the full oestrous period. × 1000.

FIGURE 34. Division (*m.*), during early oestrus, of one of the cells of a sebaceous gland (*s.g.*) attached to a hair follicle (*h.f.*). × 1000.

FIGURE 35. Proliferating centre of an intestinal lymph nodule showing some of the large numbers of mitoses (*m.*) which are always present there. Part of the outer zone of small inert lymphocytes (*ly.*) is also visible. × 1000.

FIGURE 36. Numbers of mitoses (*m.*) adjacent to a blood vessel (*b.v.*) in a rapidly growing region of an implanted mammary carcinoma. × 1000.

PLATE 34

FIGURE 37. Section of the skin adjacent to a wound (*w.*) inflicted during the first day of dioestrus and allowed to heal for 2 days. The greatly thickened epidermis shows increased mitotic activity (*m.*) in the swollen cells by the wound edge, but not in the cells (*m.c.*) migrating beneath the wound clot. Unusually active cell division is also apparent in a hair follicle (*h.f.*). × 180.

FIGURE 38. Section of the skin adjacent to a wound (*w.*) inflicted during the first day of dioestrus and allowed to heal for 3 days. The continuity of the epidermis has been re-established (*n.e.*) by the cells which migrated beneath the wound clot (*w.*). Great mitotic activity (*m.*) is now present in the new as well as in the old epidermis, and is also apparent in adjacent sebaceous glands (*s.m.*).. × 180.

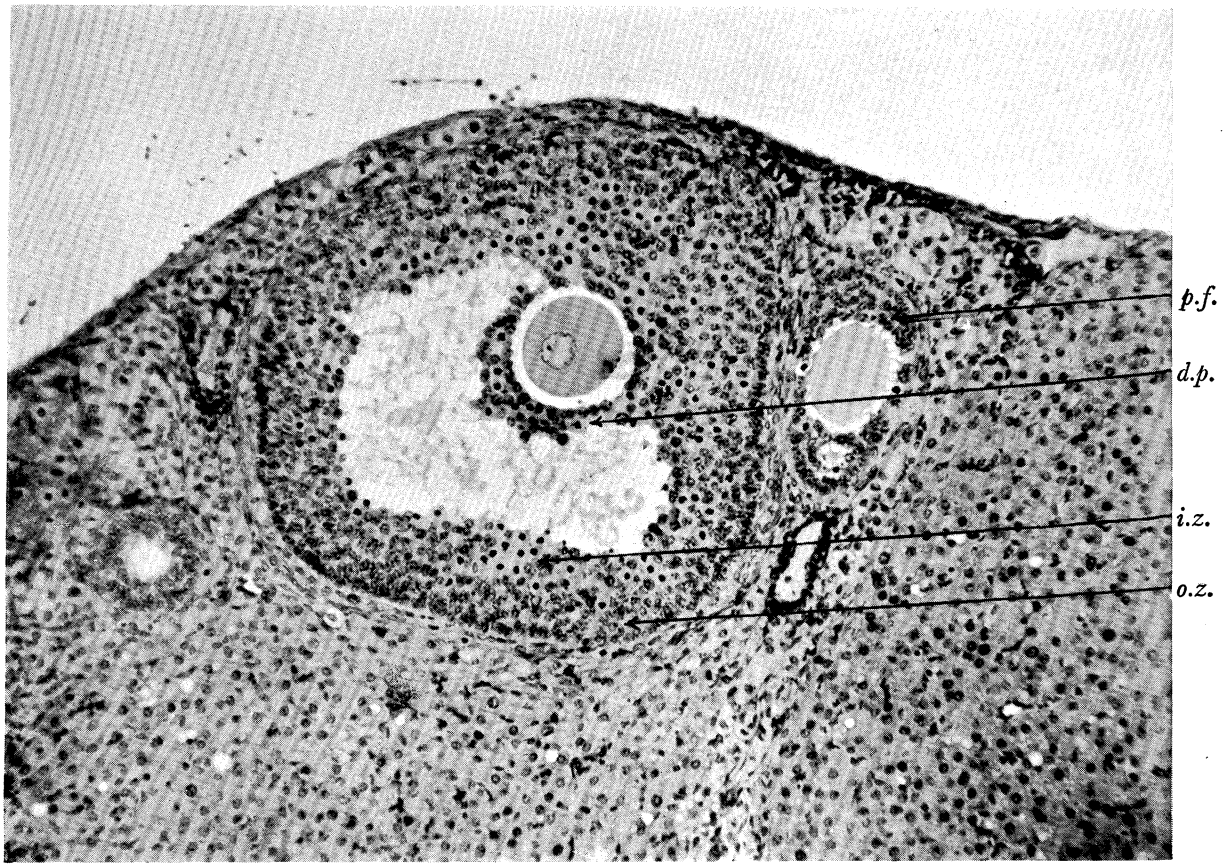


FIGURE 11

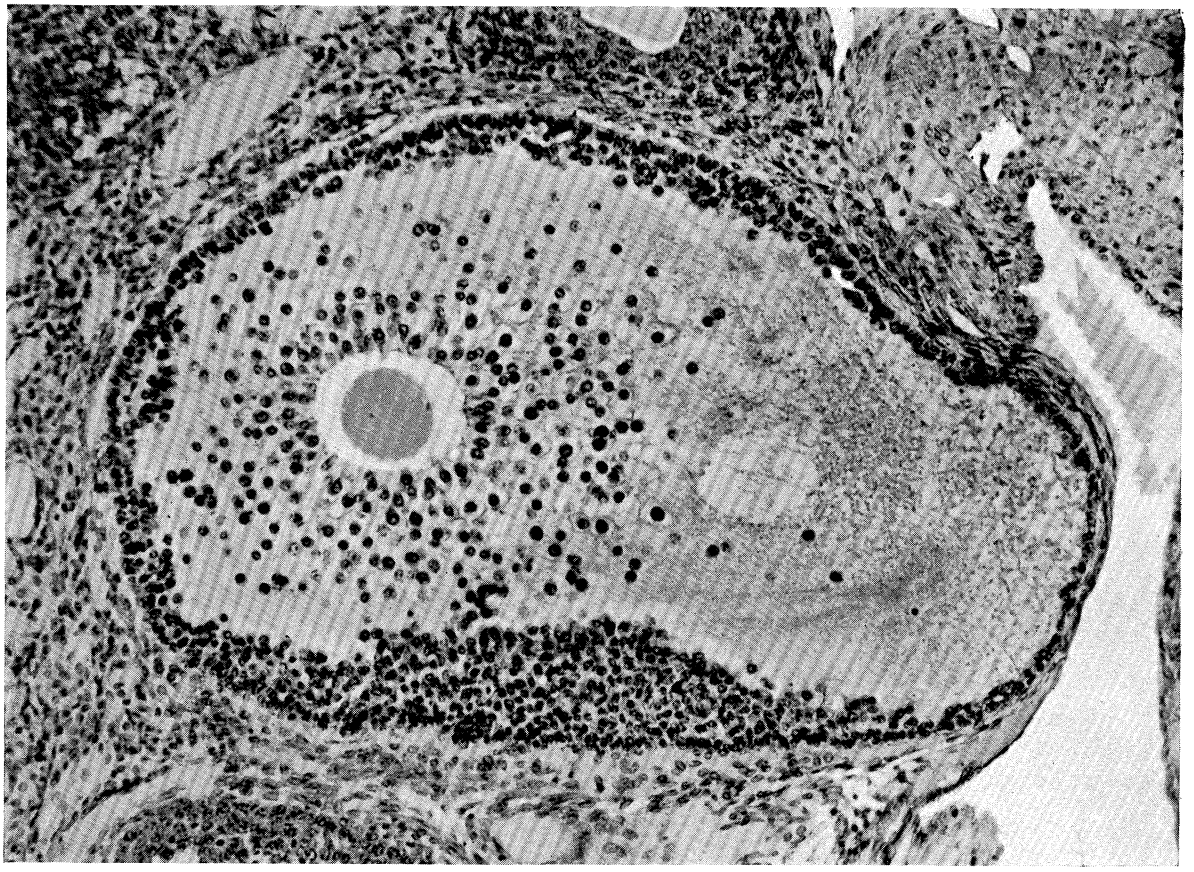
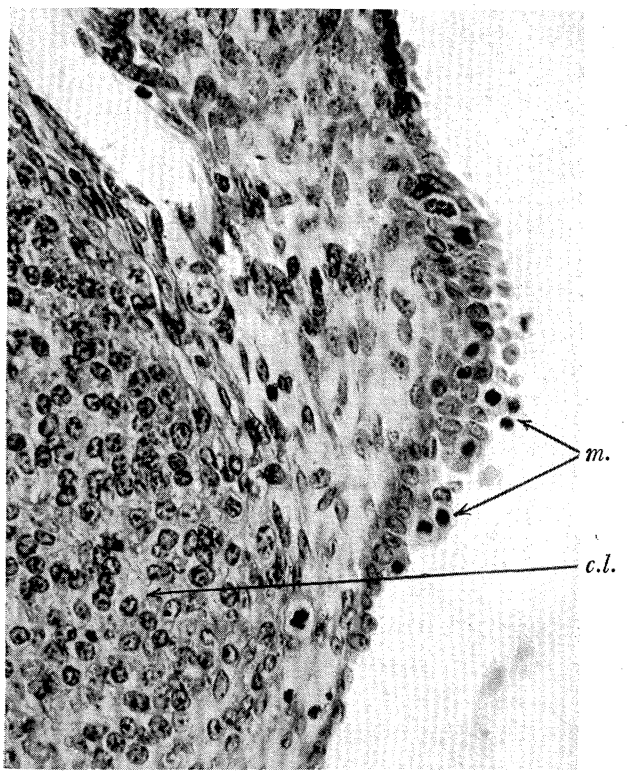


FIGURE 12



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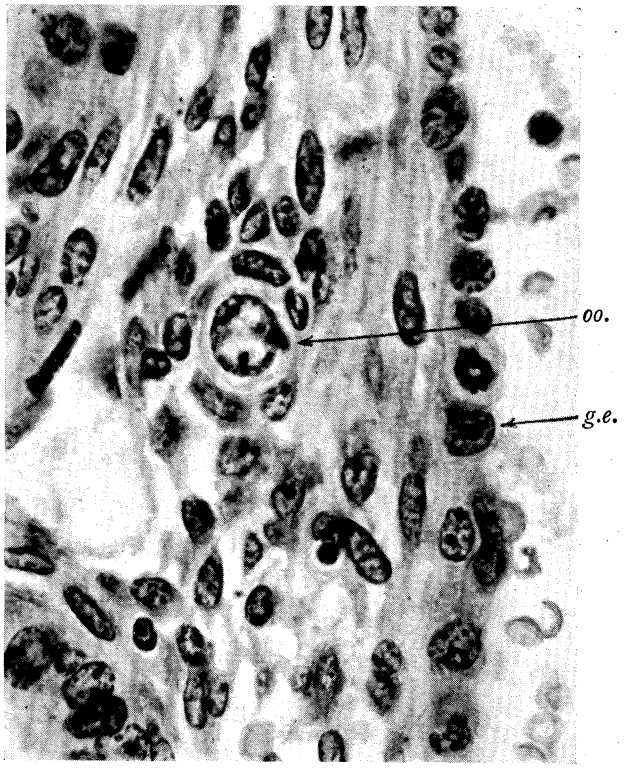

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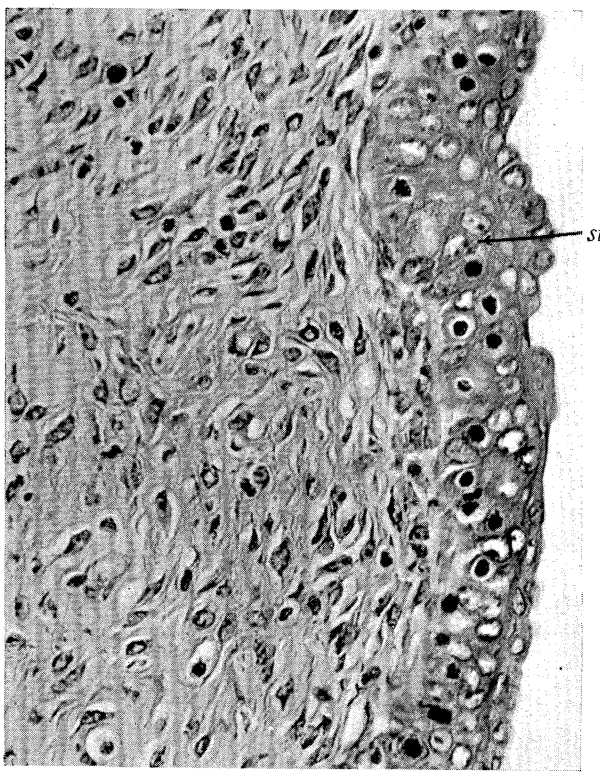

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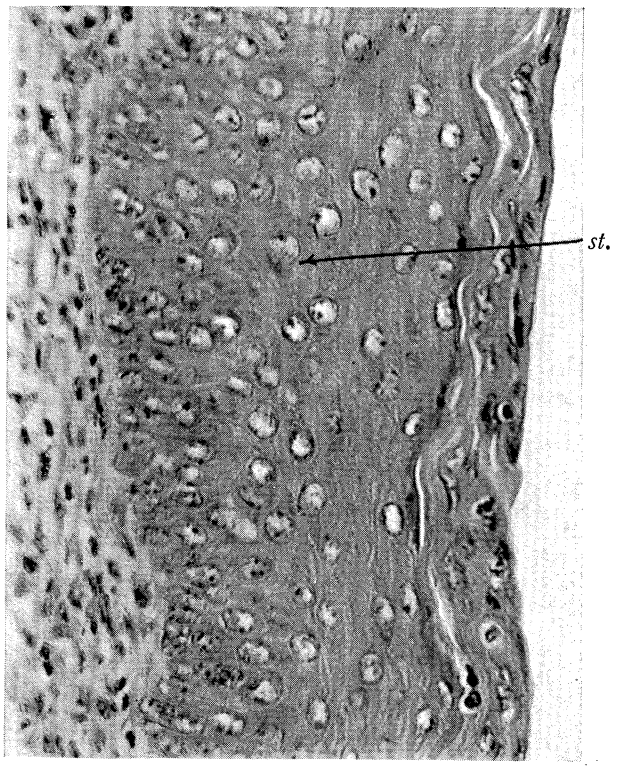

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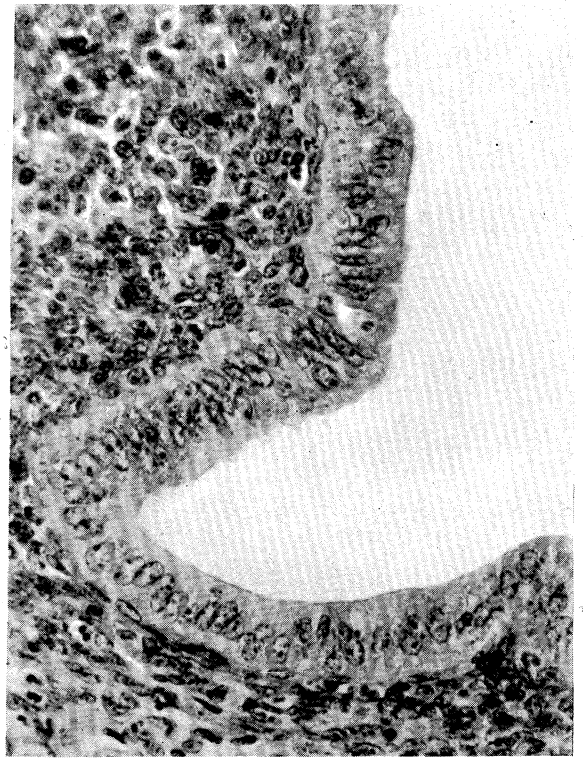

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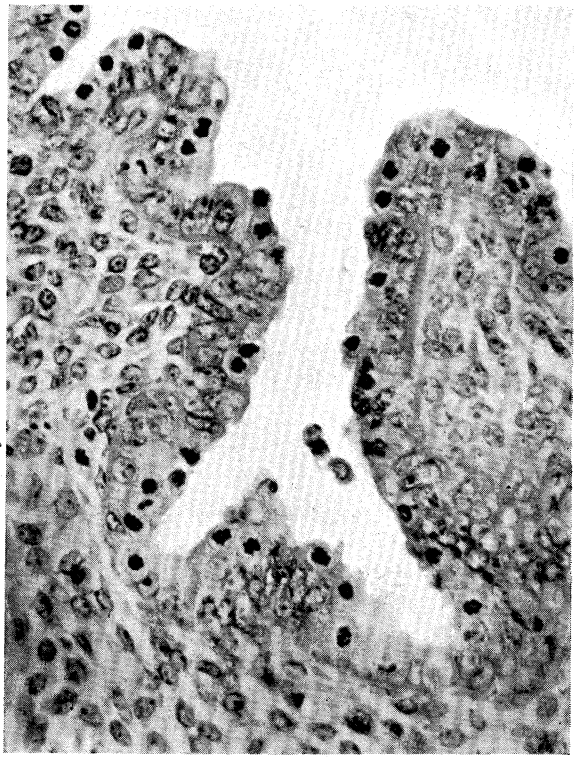

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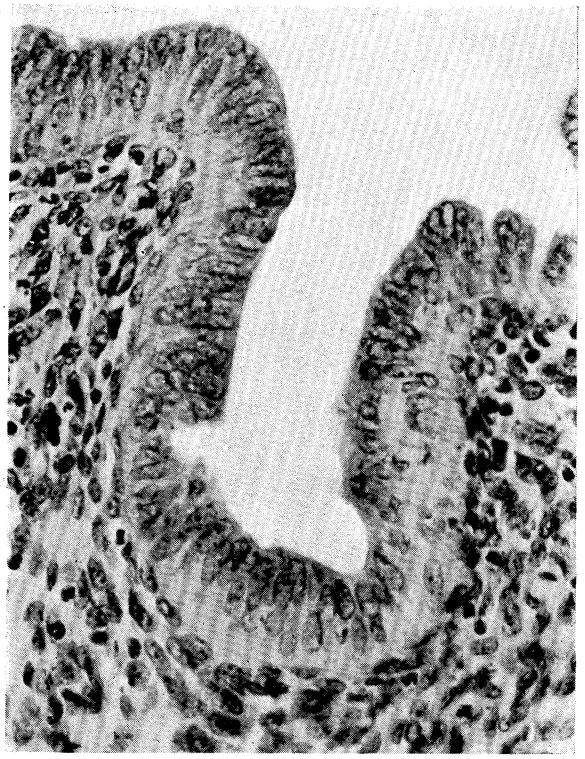

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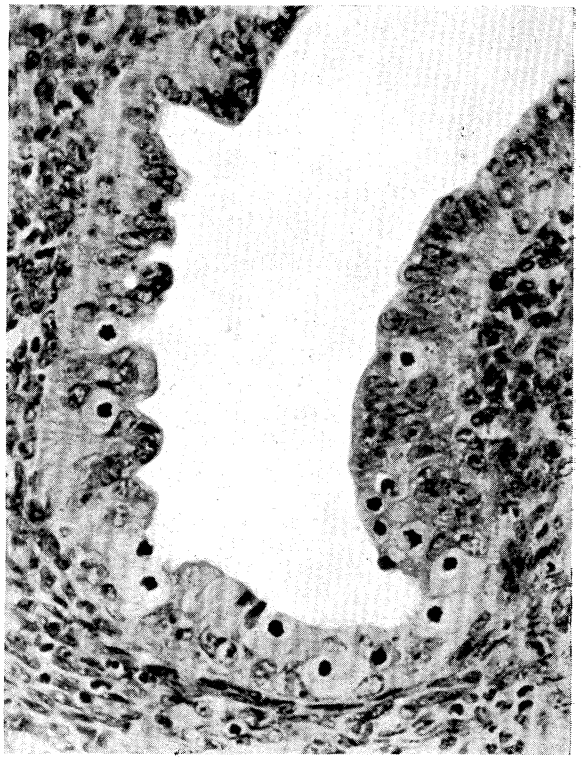

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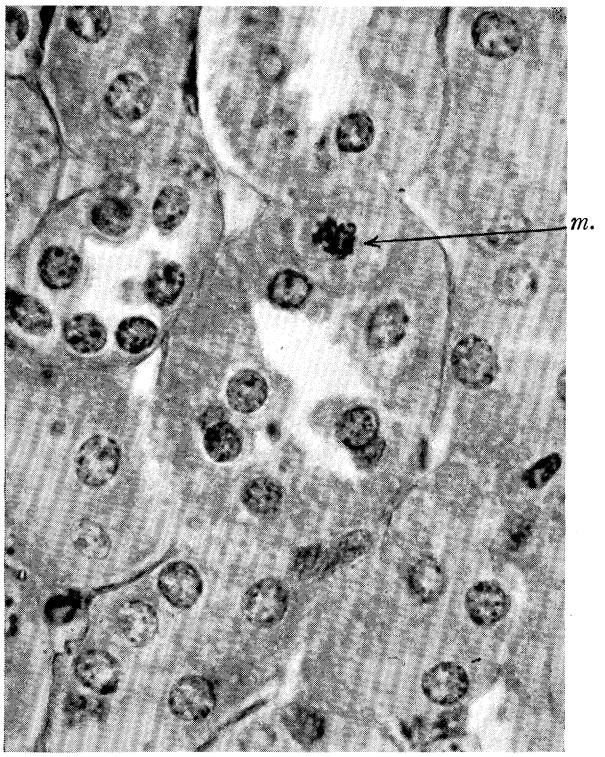

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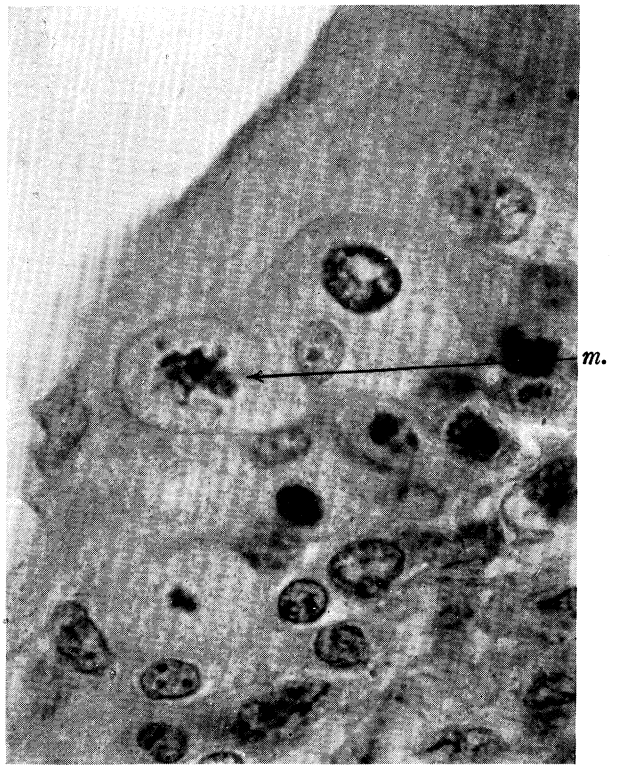

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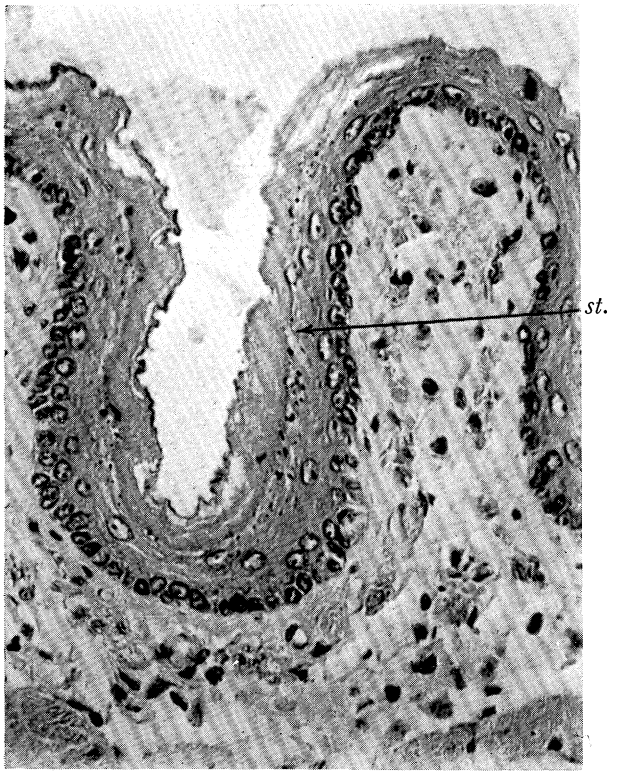

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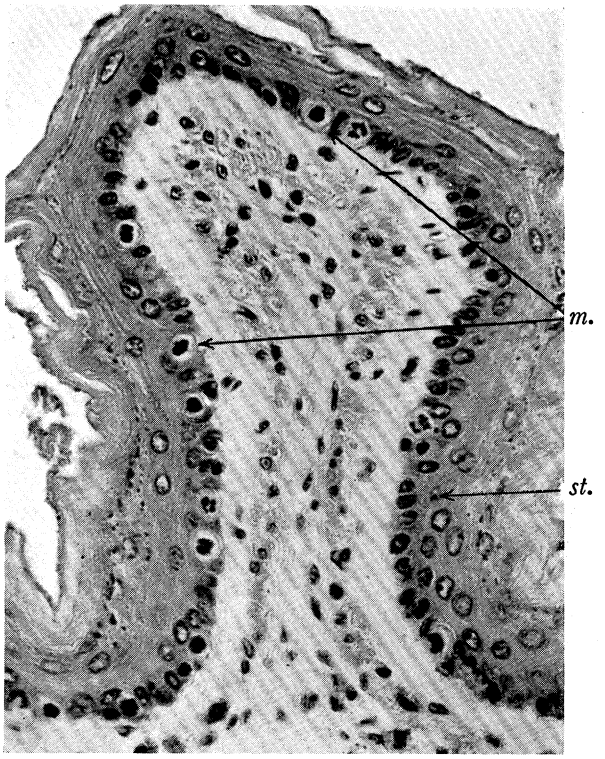

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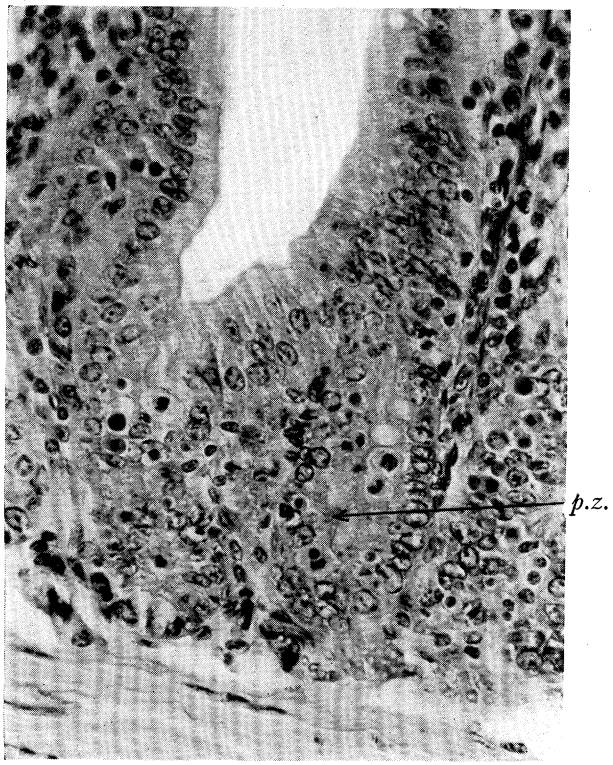

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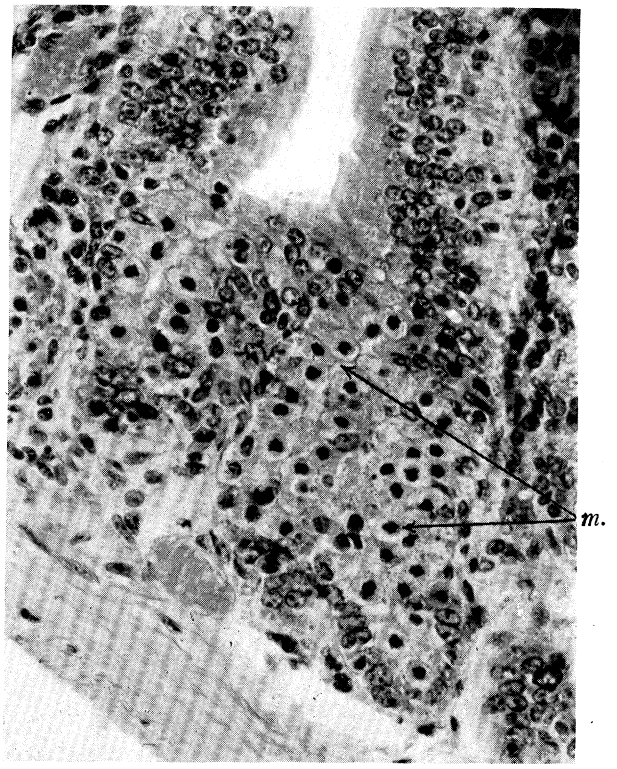

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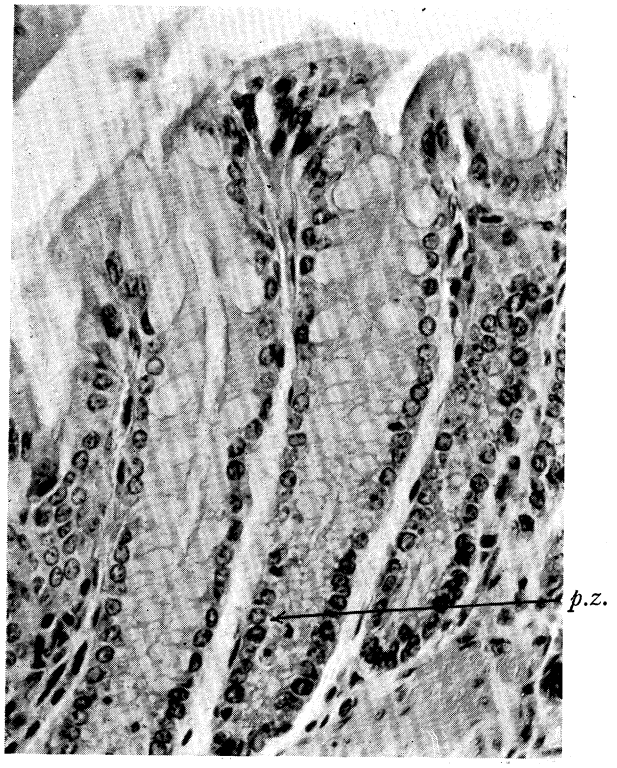

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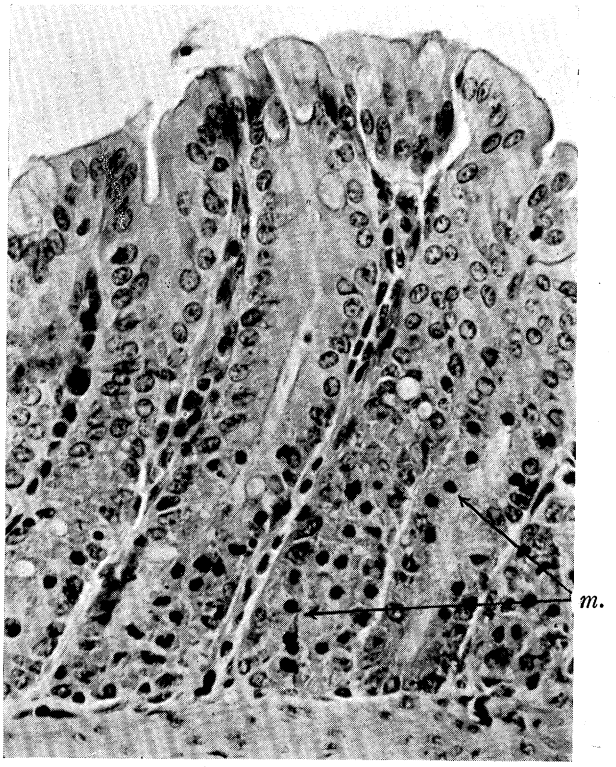

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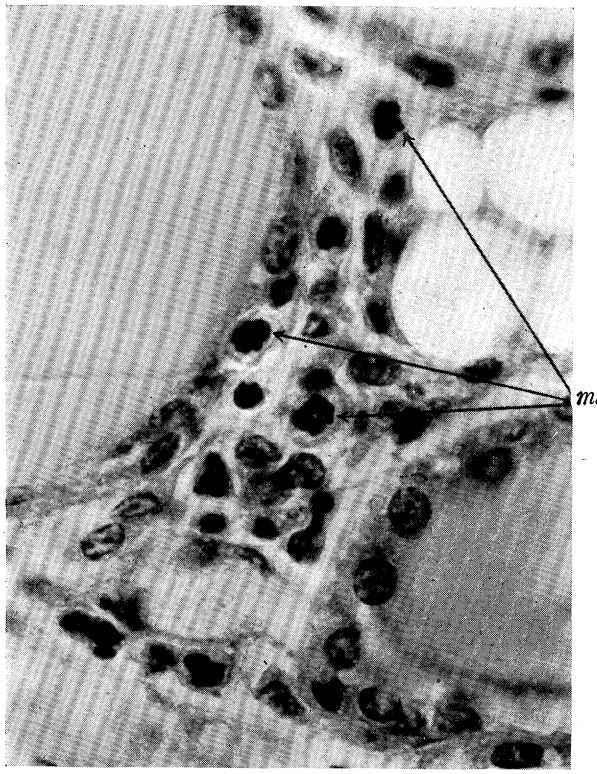

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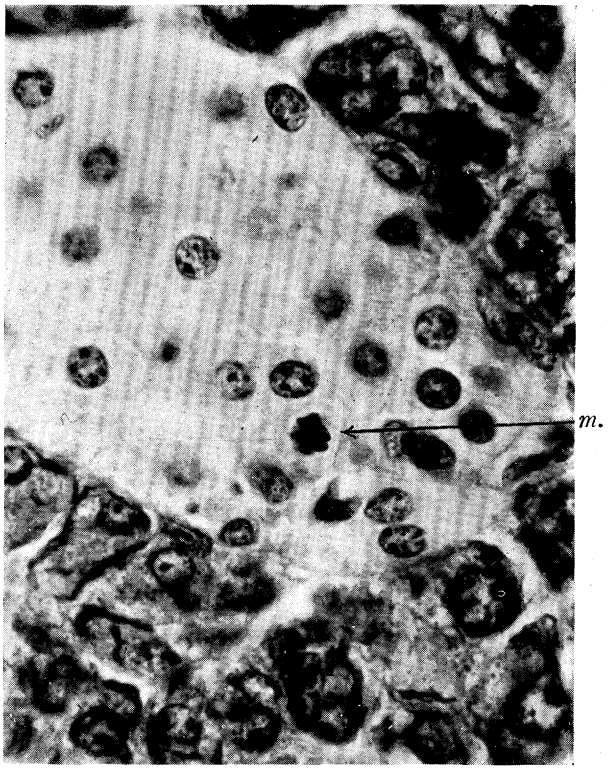
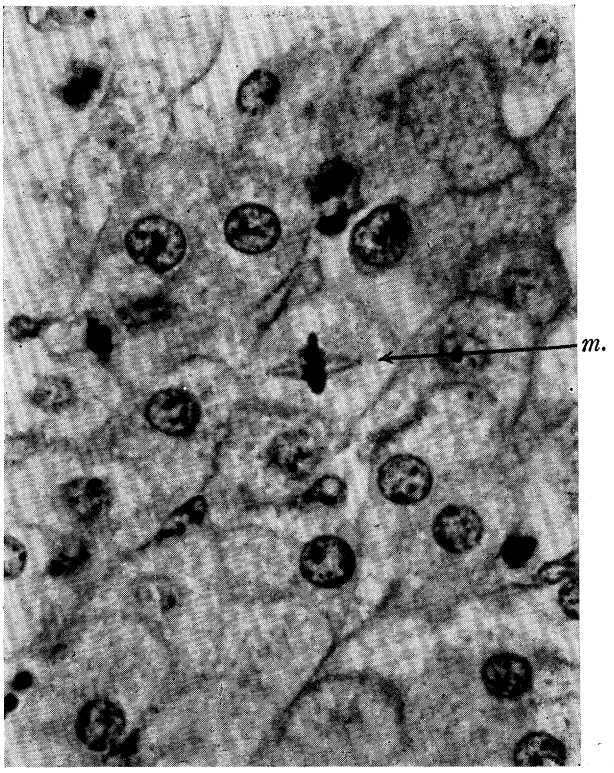


FIGURE 30



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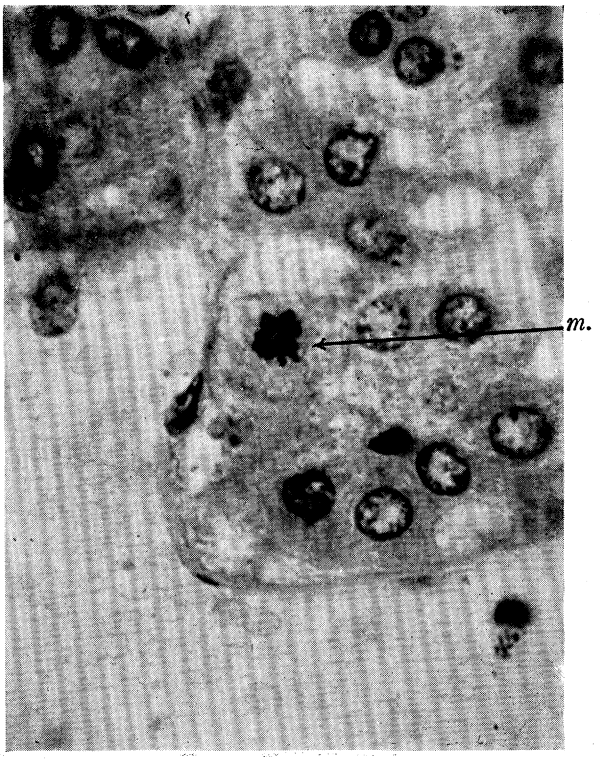

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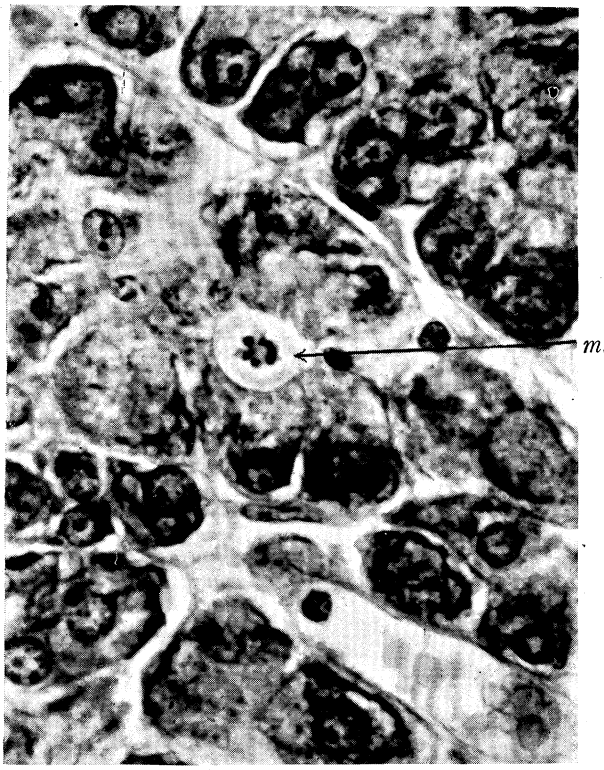

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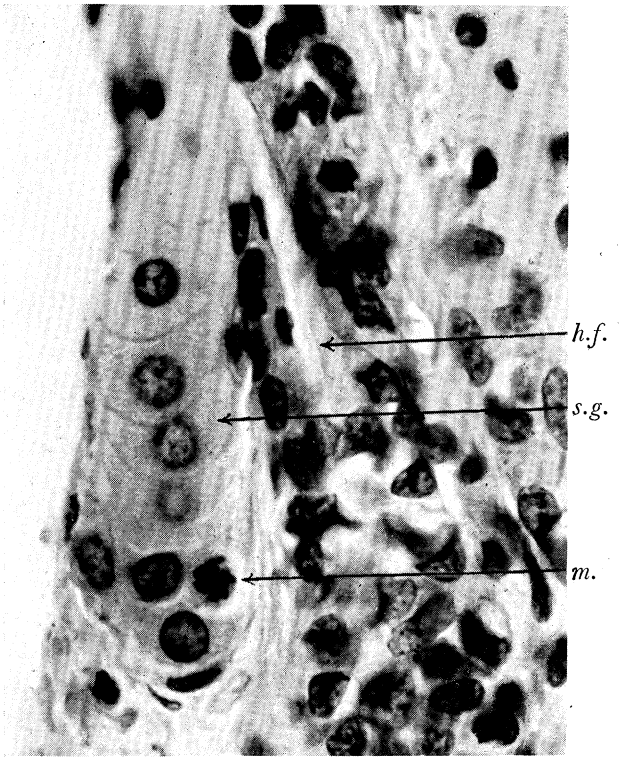

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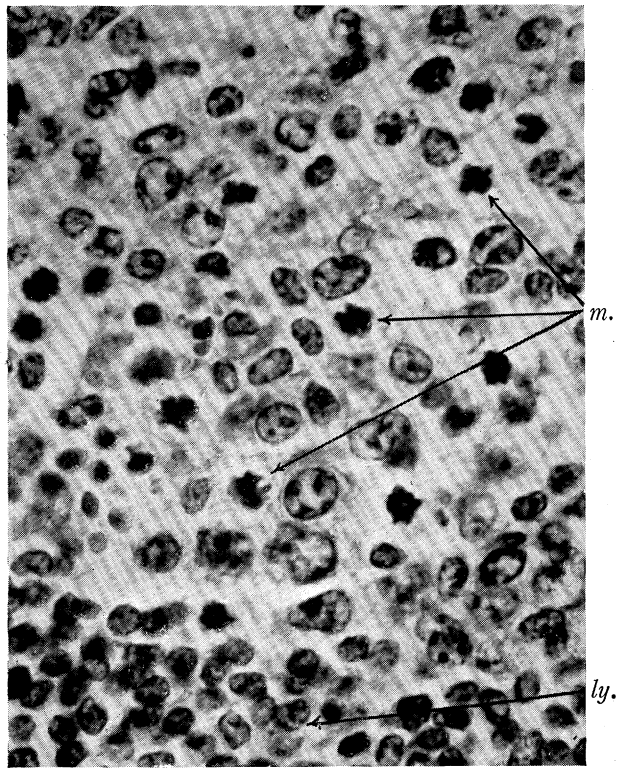

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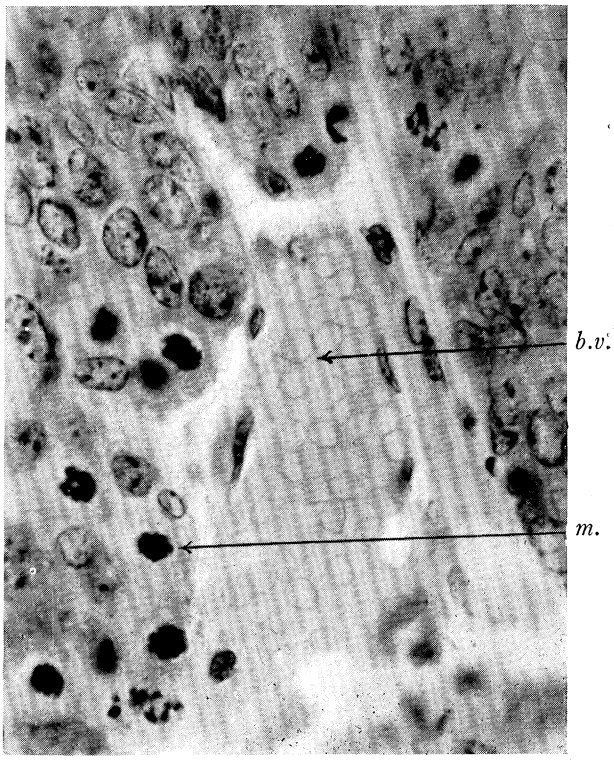
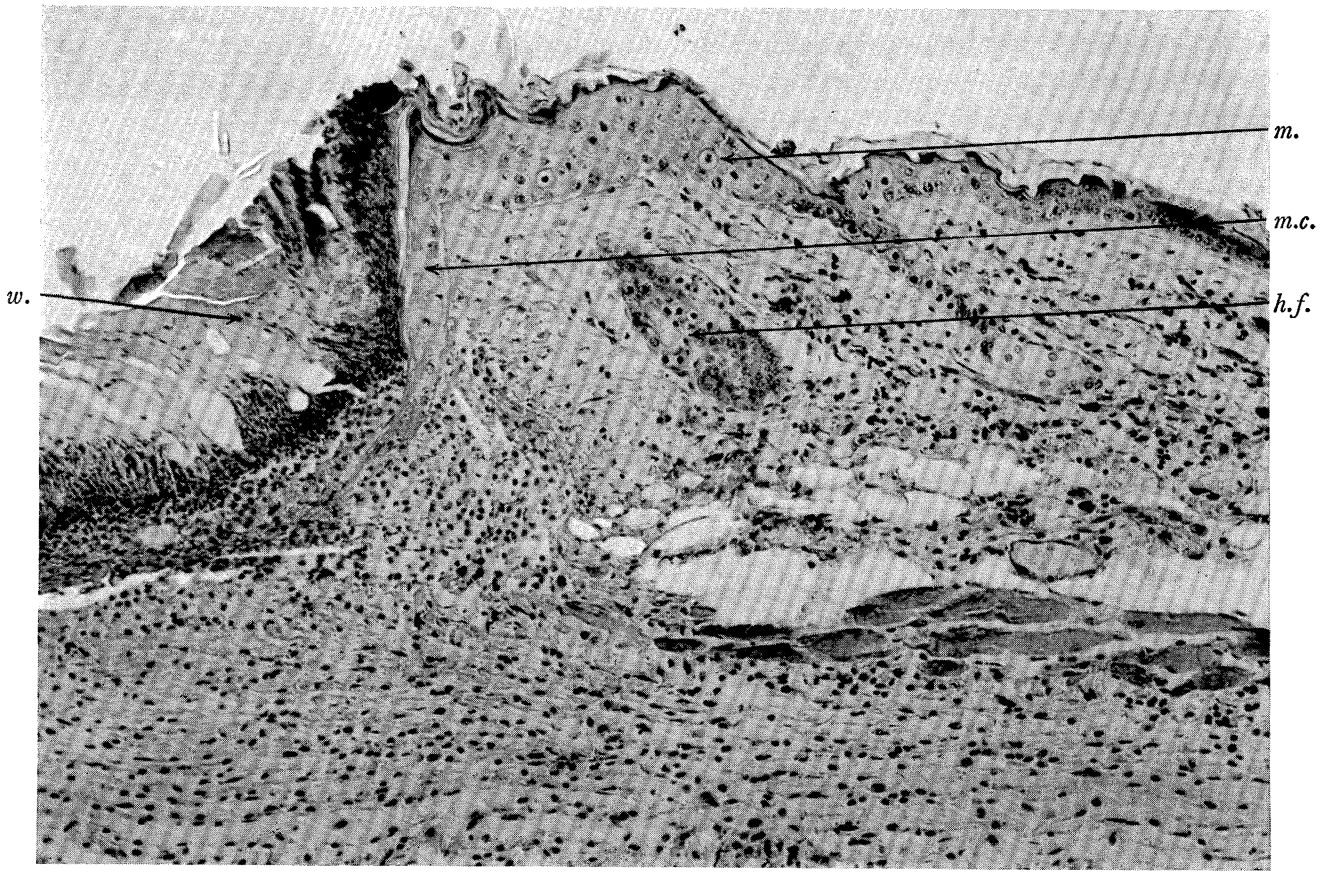


FIGURE 36



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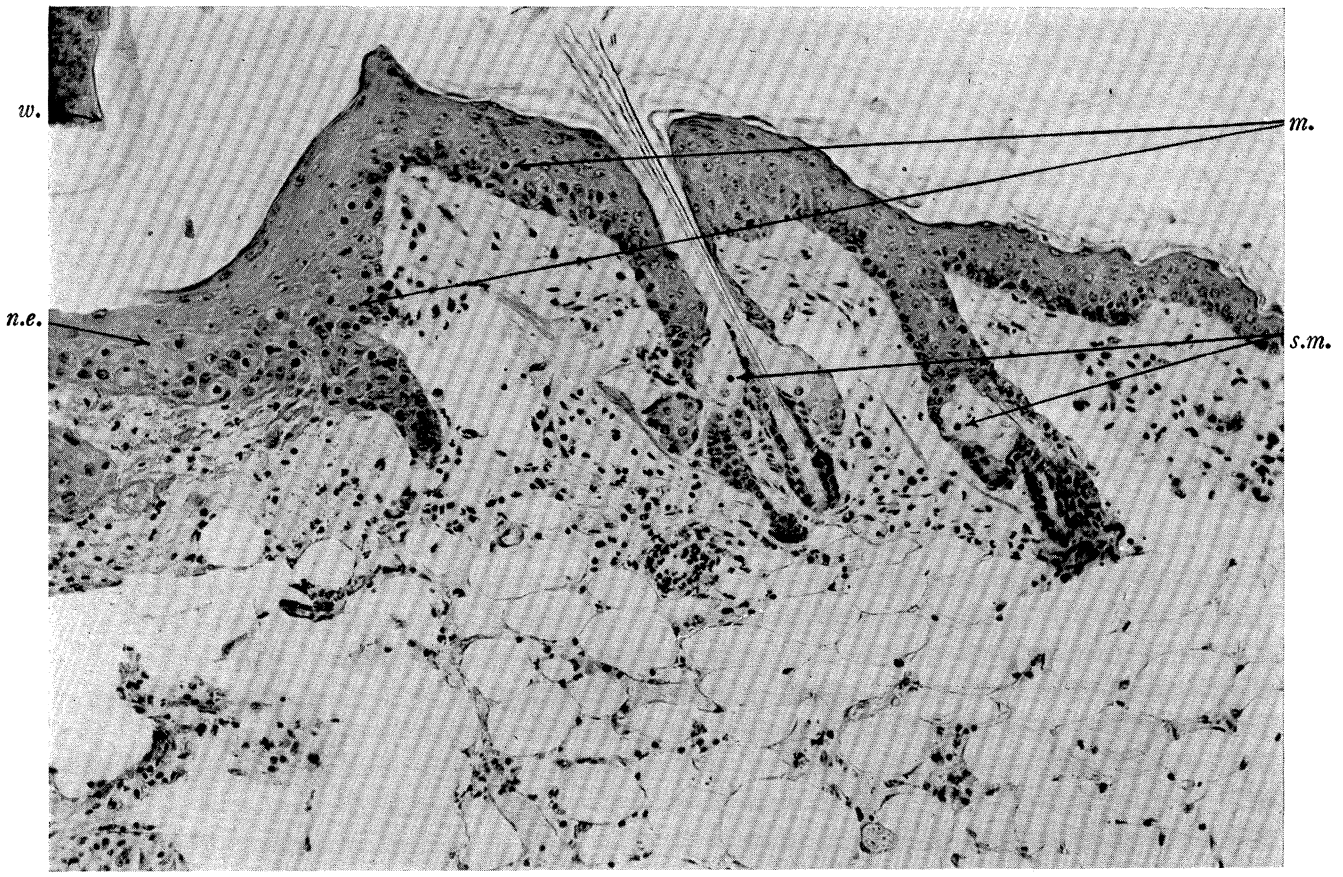


FIGURE 38

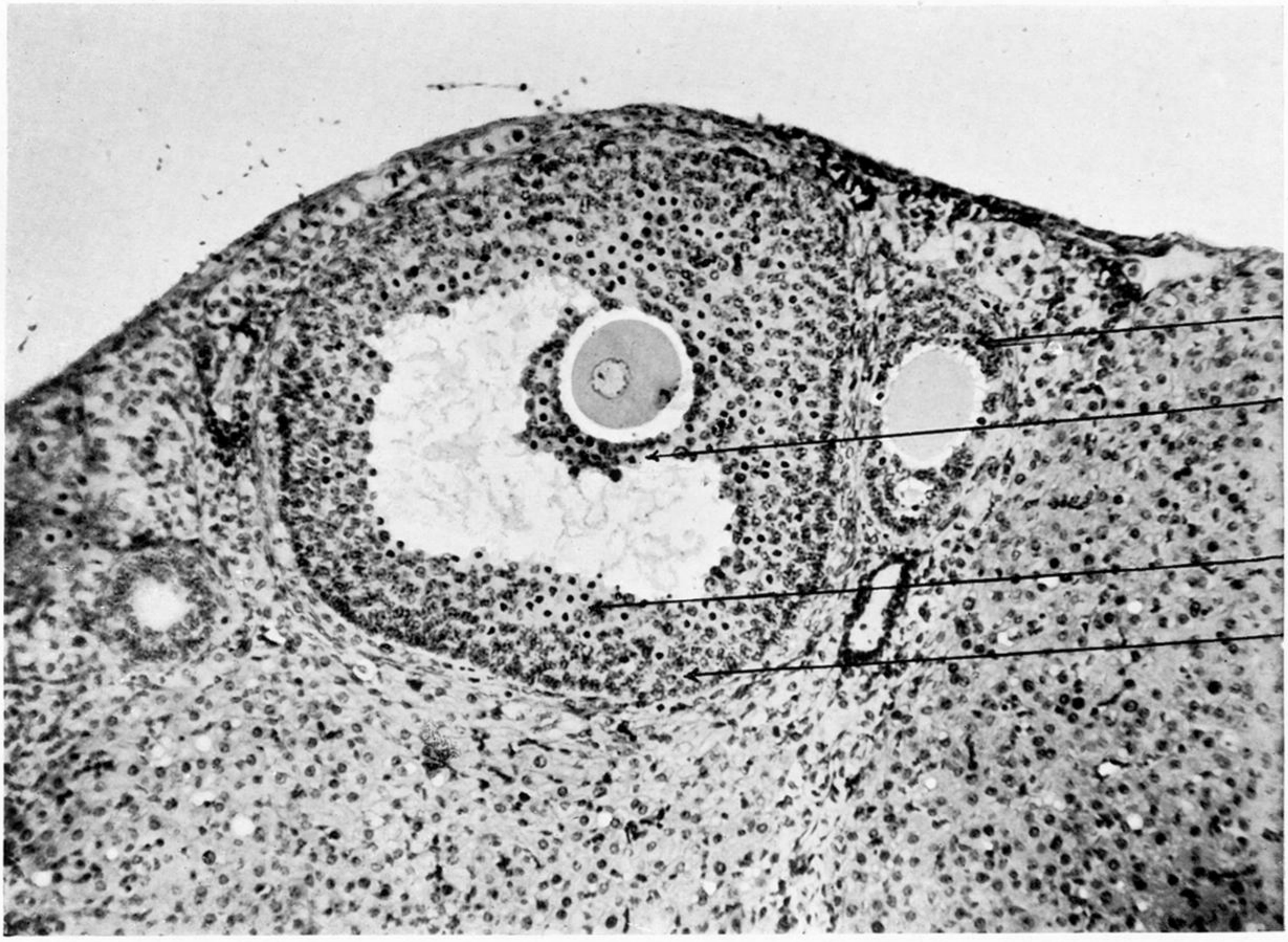


FIGURE 11

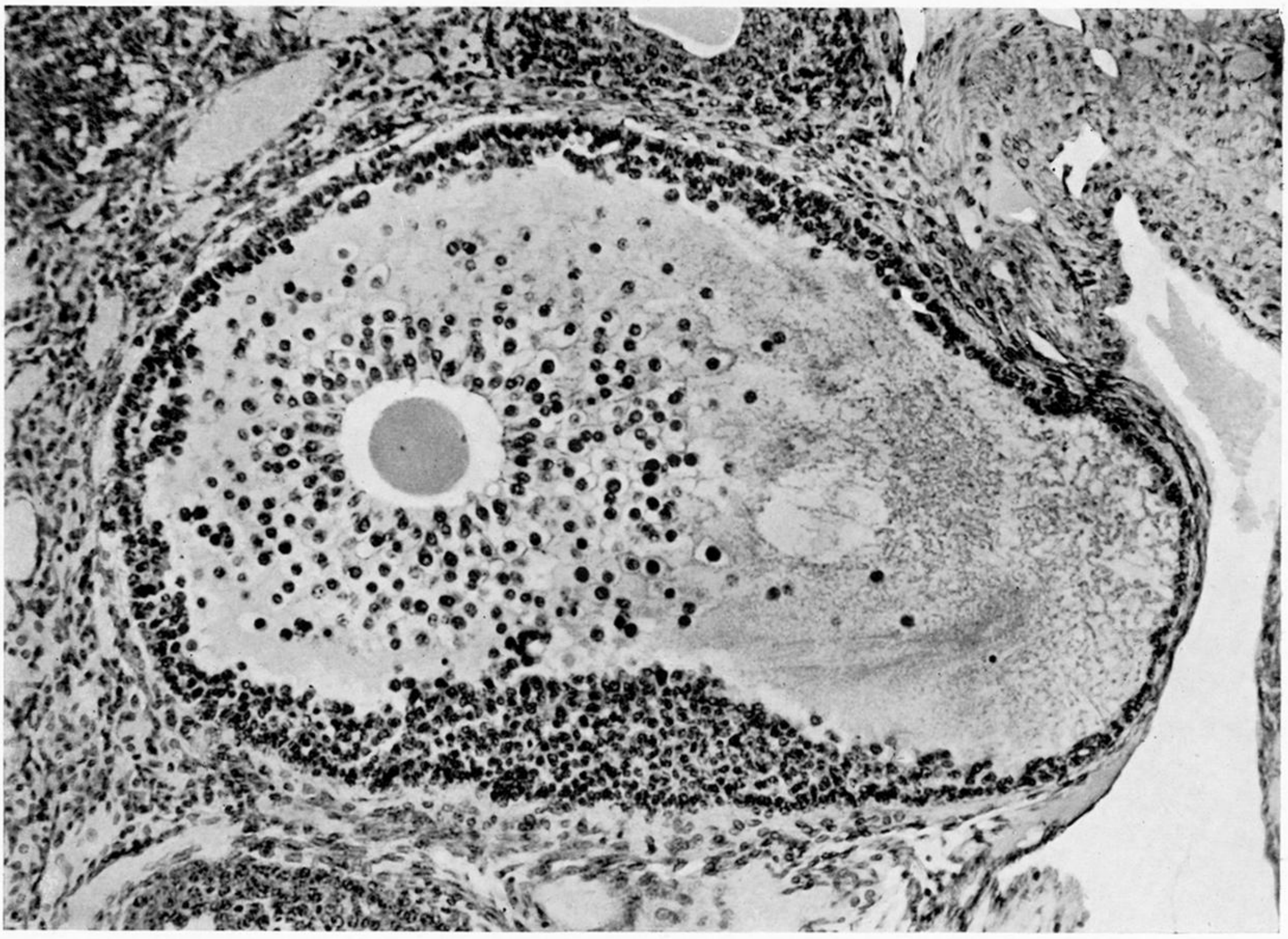


FIGURE 12

PLATE 27

FIGURE 11. Rapidly growing ovarian follicle showing the gradient of mitotic activity in the membrana granulosa. Large numbers of mitoses are present in the discus proligerus (*d.p.*) and the inner zone (*i.z.*) of the membrana granulosa adjacent to the follicular fluid, but elsewhere in the outer zone (*o.z.*) activity is only slight. Also present is a small primary follicle (*p.f.*). × 220.

FIGURE 12. Fully grown Graafian follicle about to burst. The discus proligerus has separated from the membrana granulosa to form a corona radiata, and cell division has almost entirely ceased in all the follicle cells. × 220.

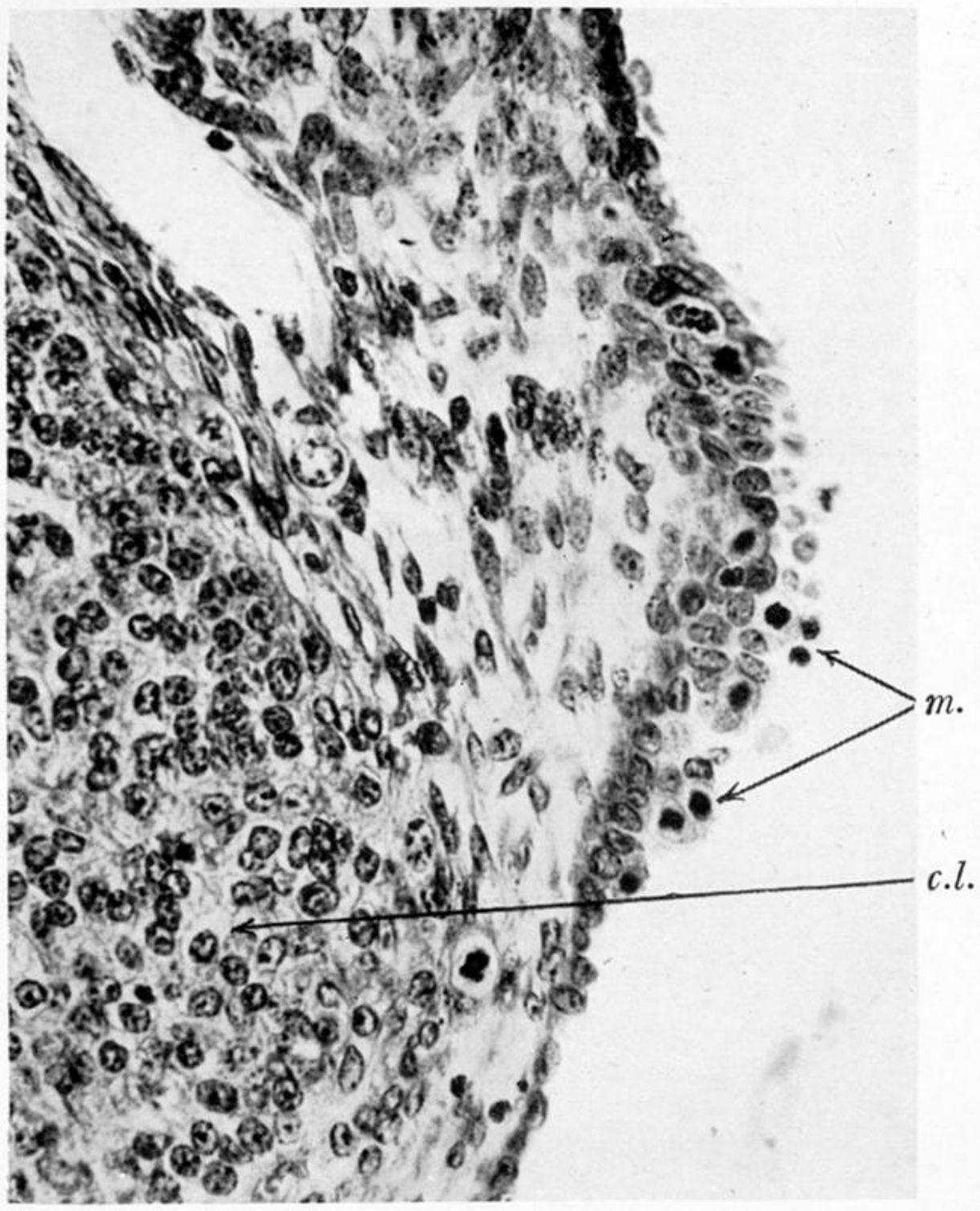


FIGURE 13

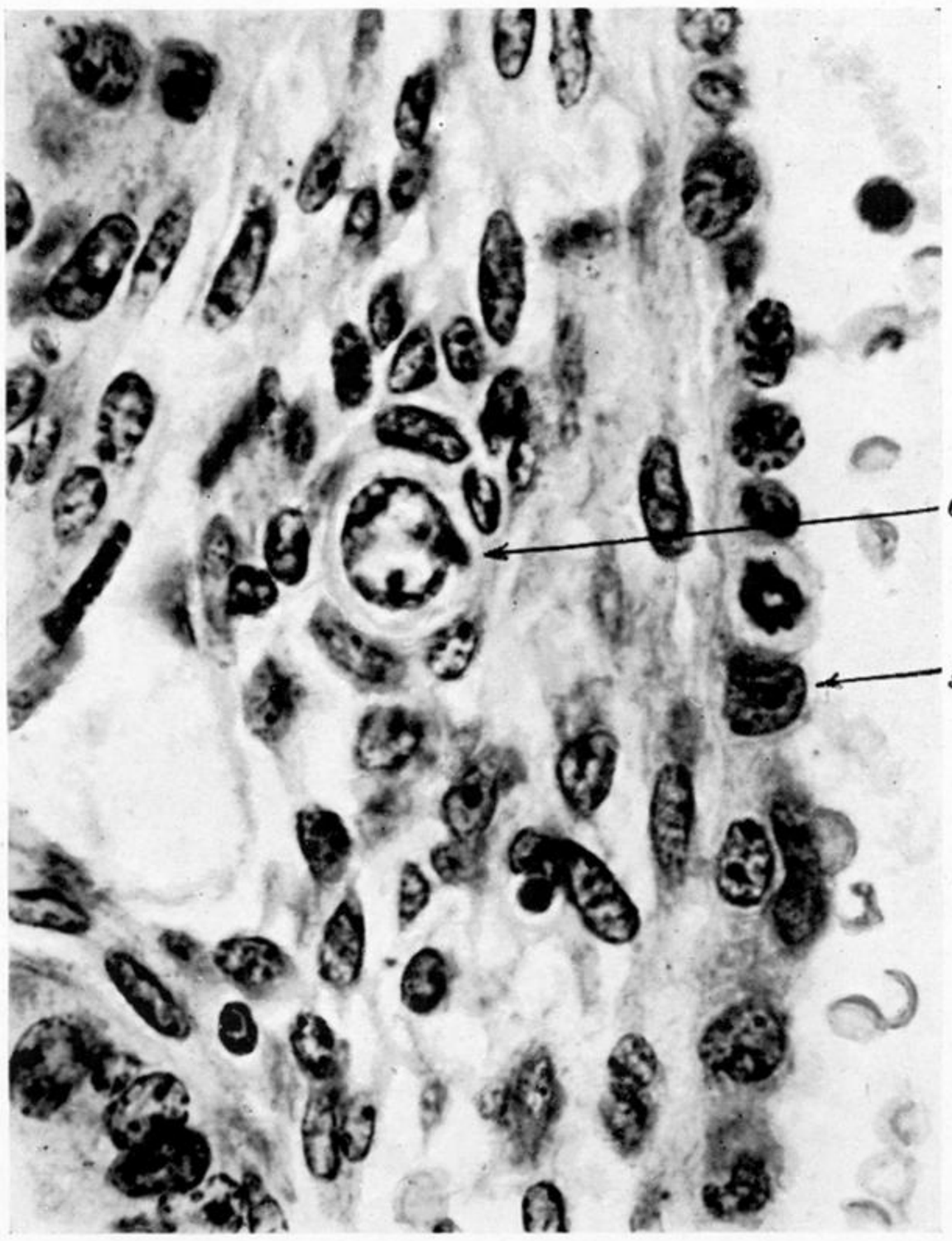


FIGURE 14

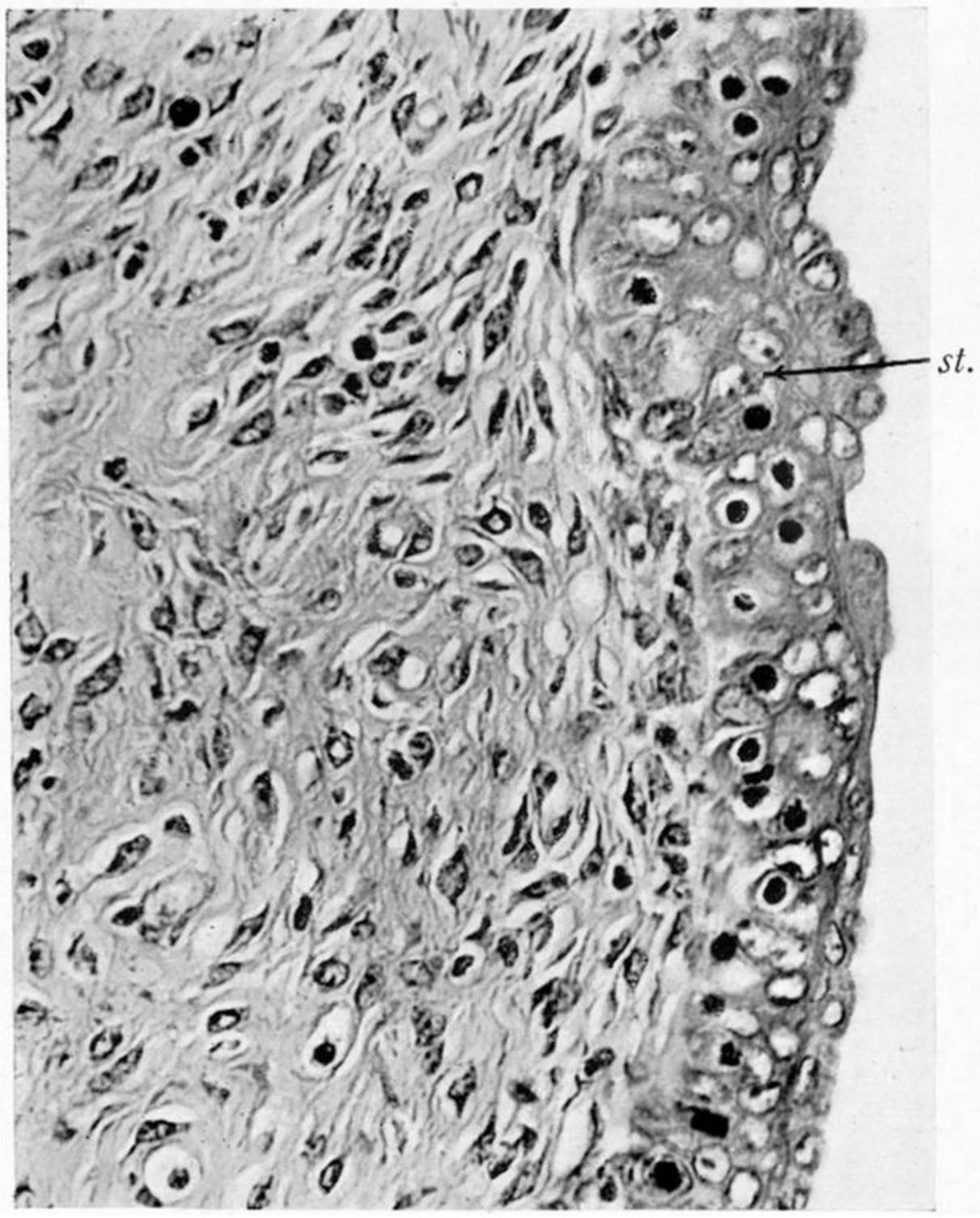


FIGURE 15

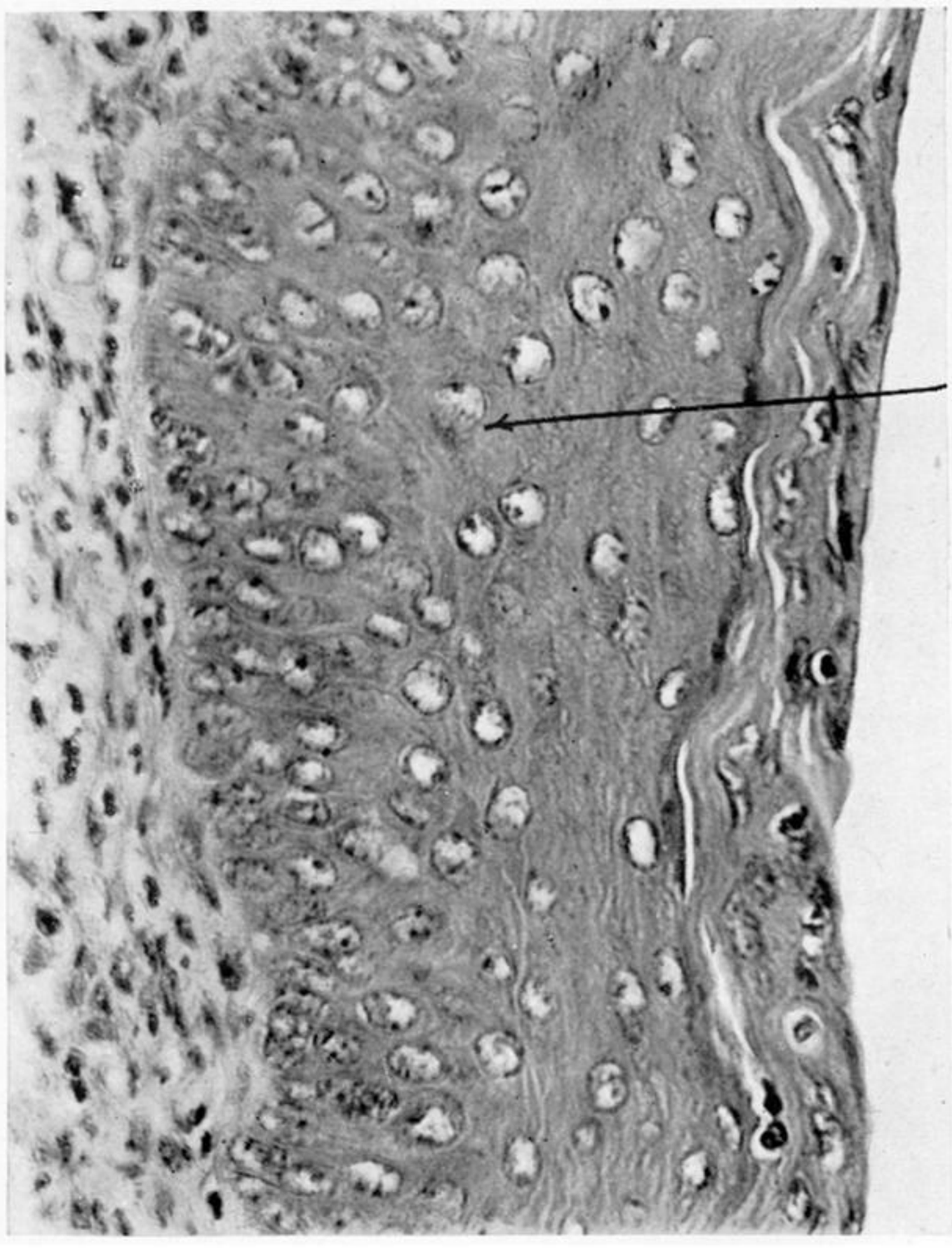


FIGURE 16

PLATE 28

FIGURE 13. Large numbers of mitoses (*m.*) in the germinal epithelium adjacent to a newly forming corpus luteum (*c.l.*) in the late post-ovulation oestrous period. × 400.

FIGURE 14. Newly formed oogonium (*oo.*) immediately beneath the germinal epithelium (*g.e.*) in the metoestrous period. × 1000.

FIGURE 15. Stratified lining epithelium (*st.*) of the cervix uteri showing the thin epithelium and the active cell division typical of the third day of dioestrus. × 400.

FIGURE 16. Stratified lining epithelium (*st.*) of the cervix uteri showing the greatly thickened epithelium and the lack of cell division typical of the pro-oestrous period. × 400.

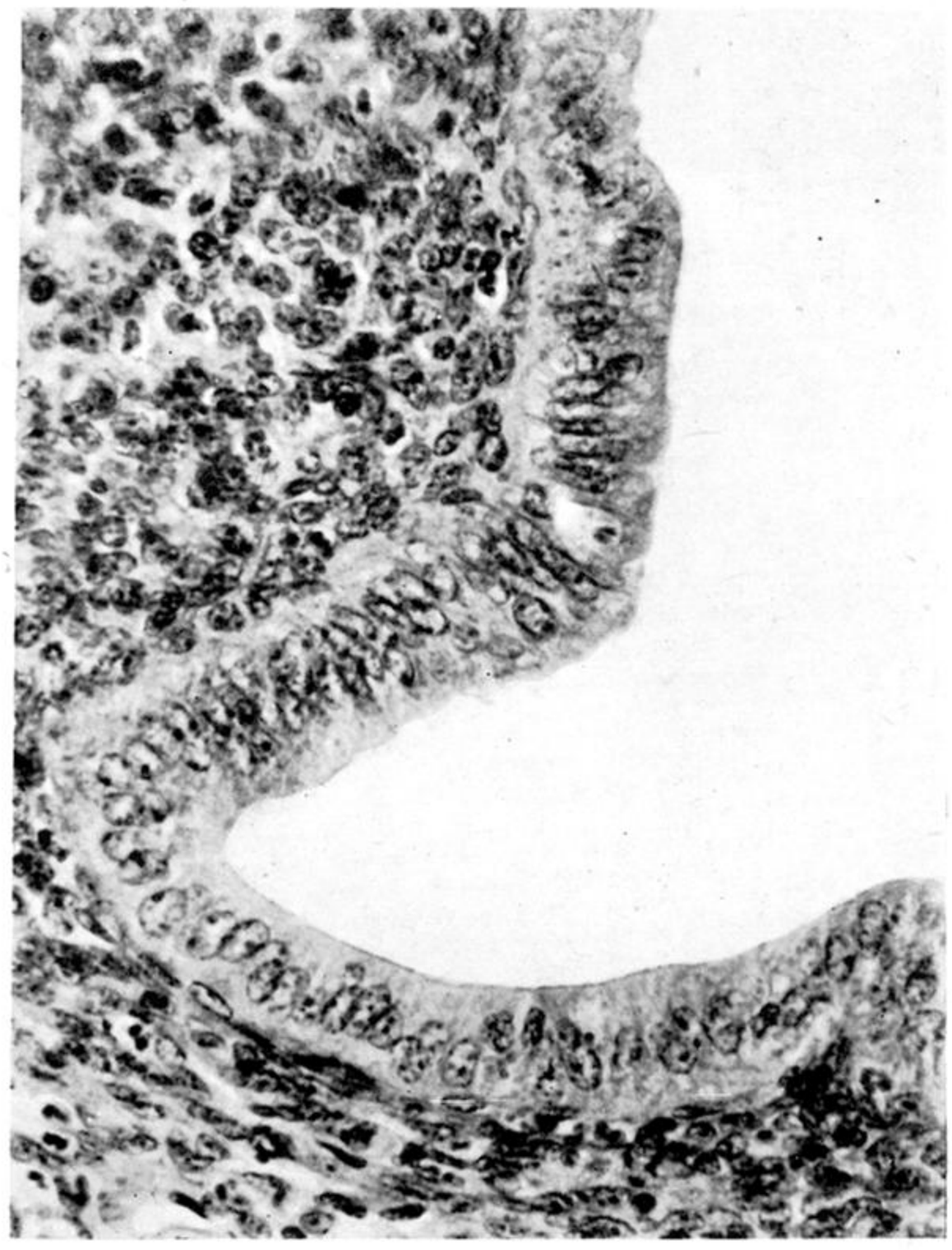


FIGURE 17

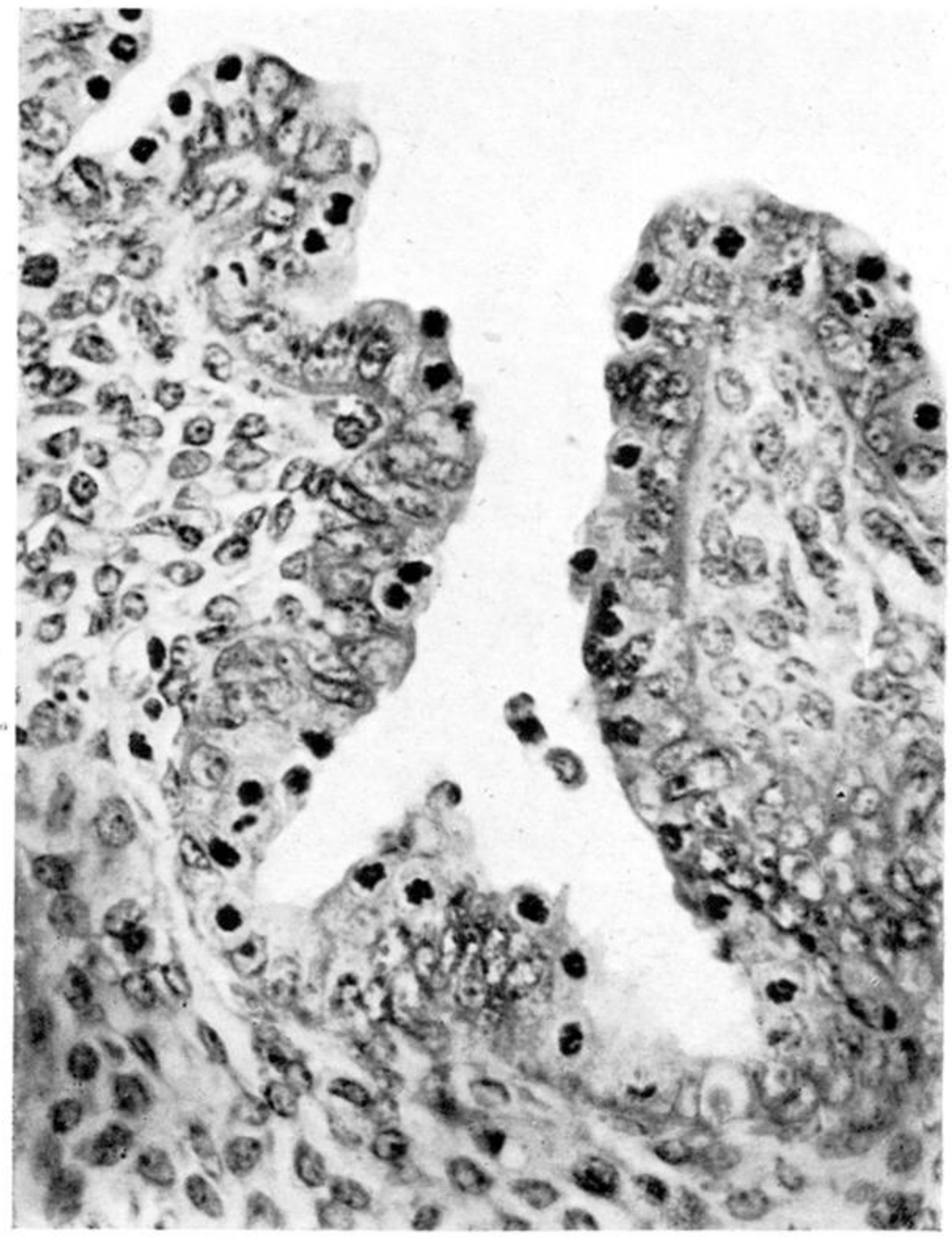
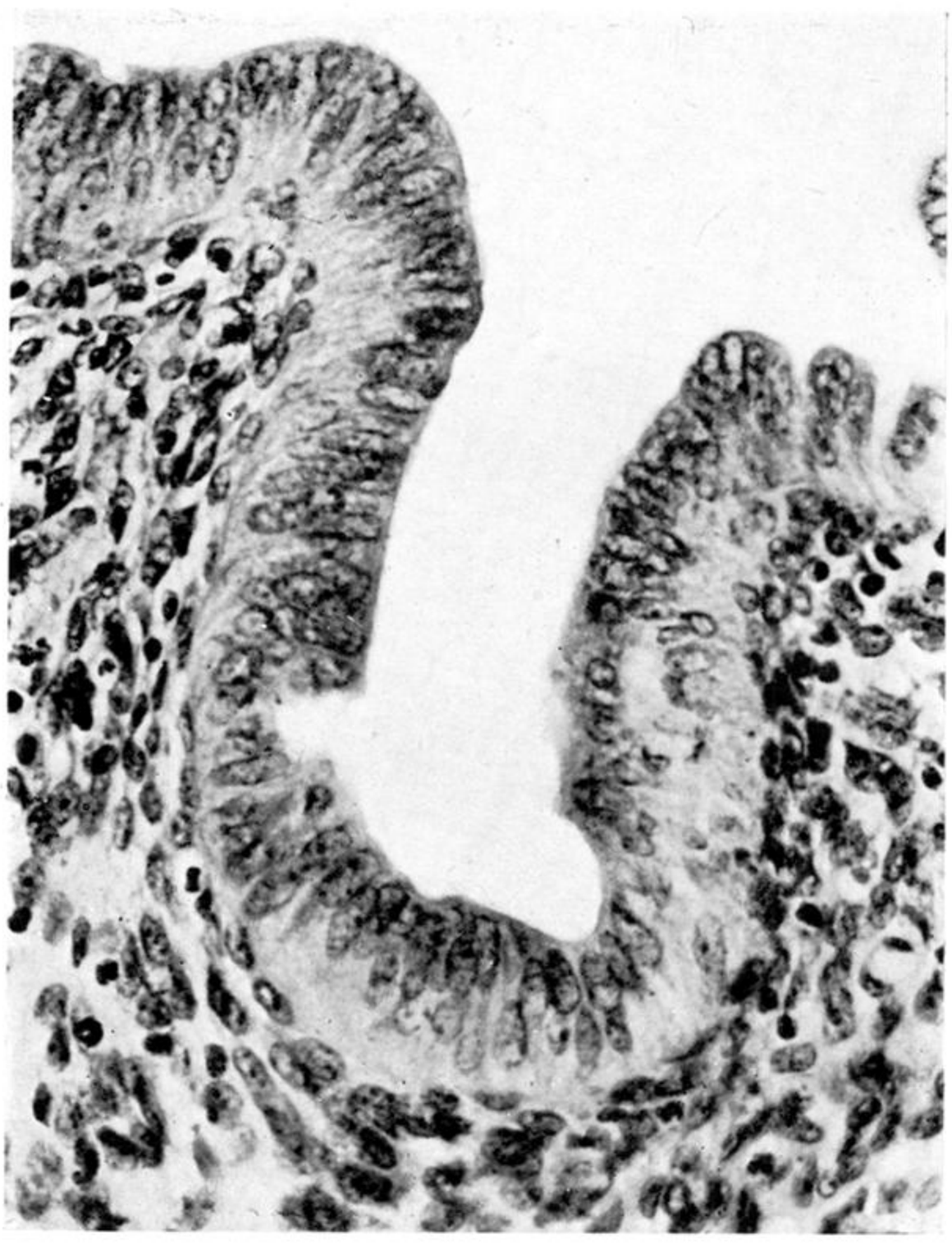


FIGURE 18



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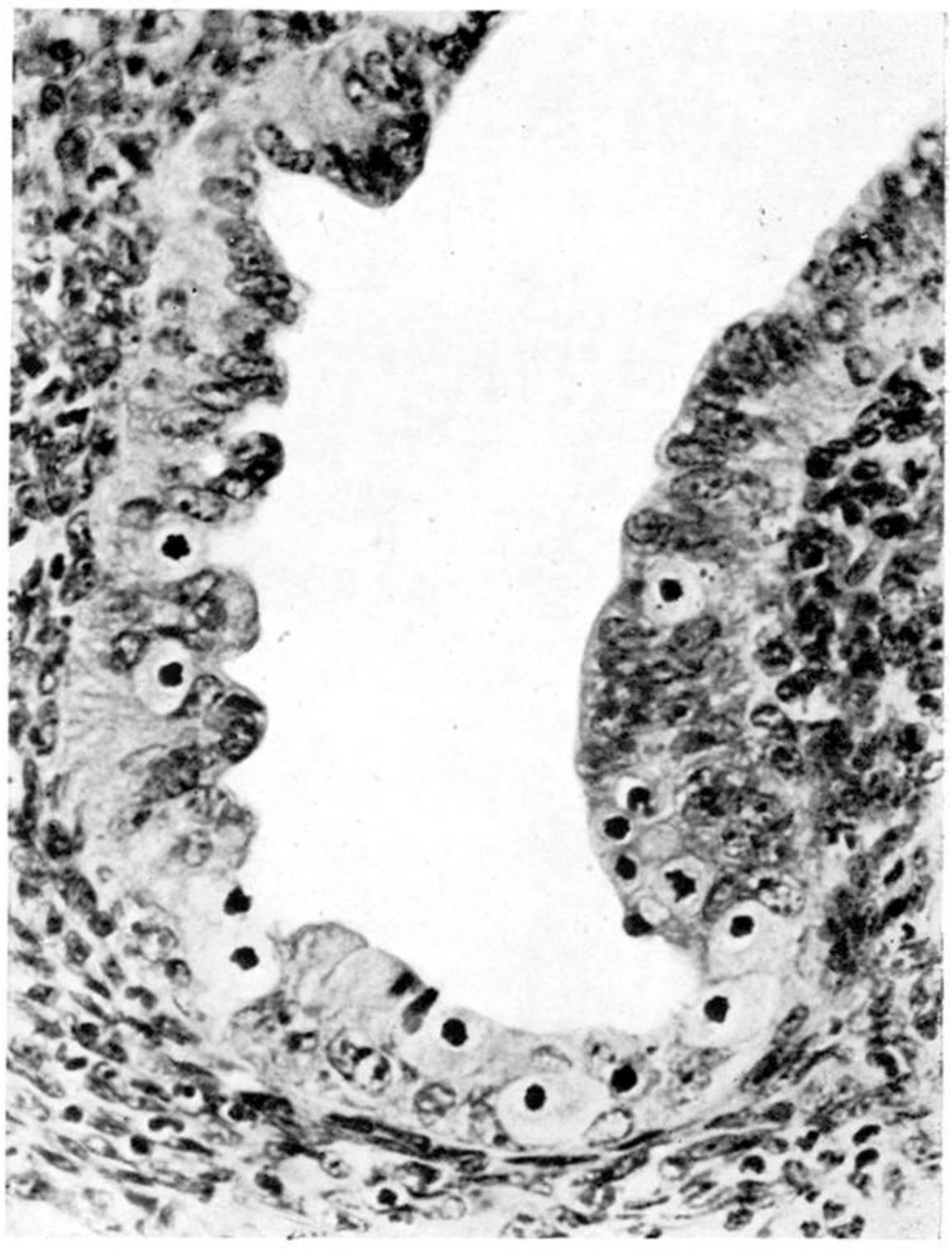

FIGURE 20

PLATE 29

FIGURE 17. Endometrium of the corpus uteri to show the lack of mitotic activity on the first day of dioestrus. × 400.

FIGURE 18. Endometrium of the corpus uteri to show the extreme mitotic activity on the third day of dioestrus. × 400.

FIGURE 19. Endometrium of the corpus uteri to show the lack of mitotic activity during full oestrus. × 400.

FIGURE 20. Endometrium of the corpus uteri to show the increased mitotic activity present during metoestrus. × 400.

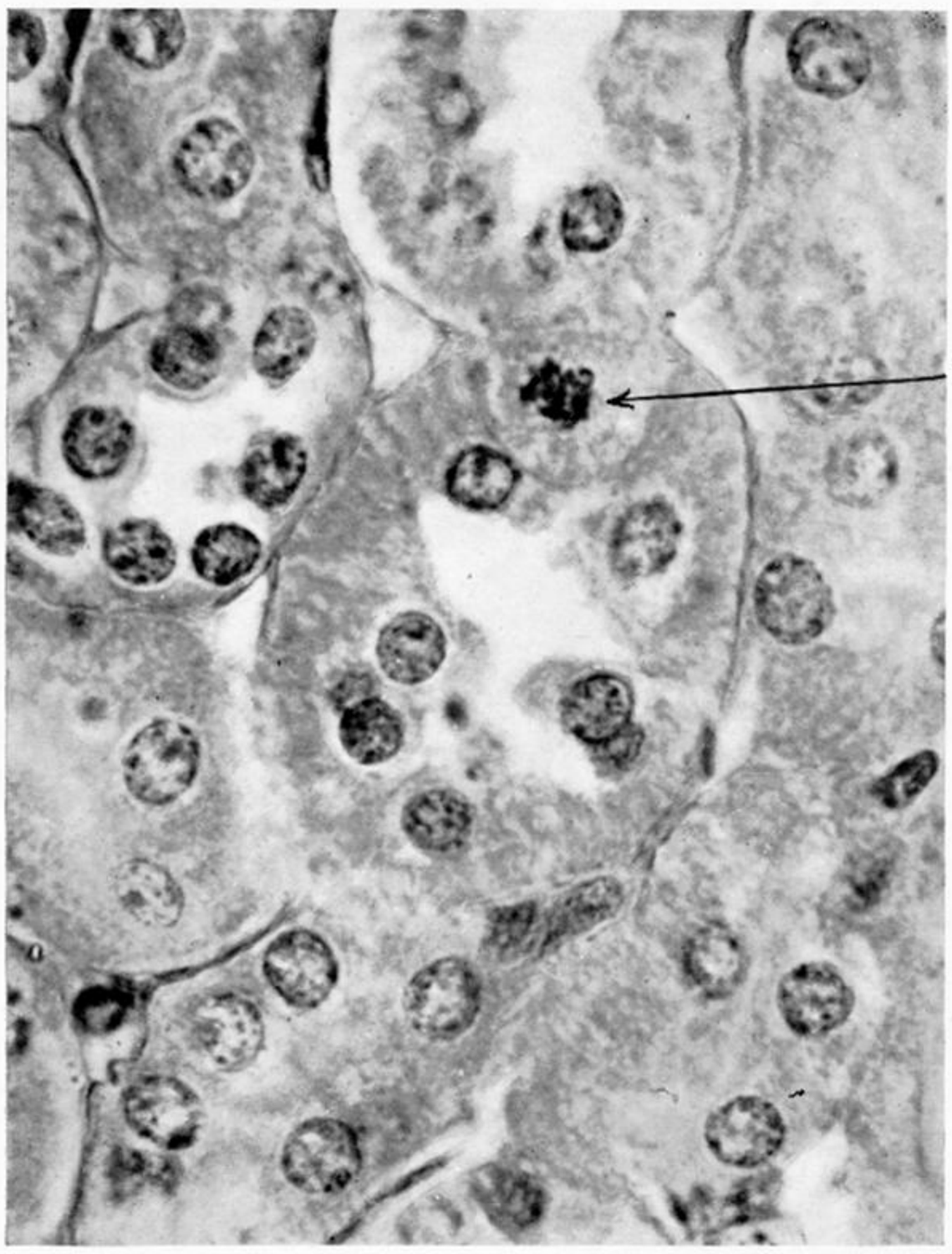

FIGURE 21

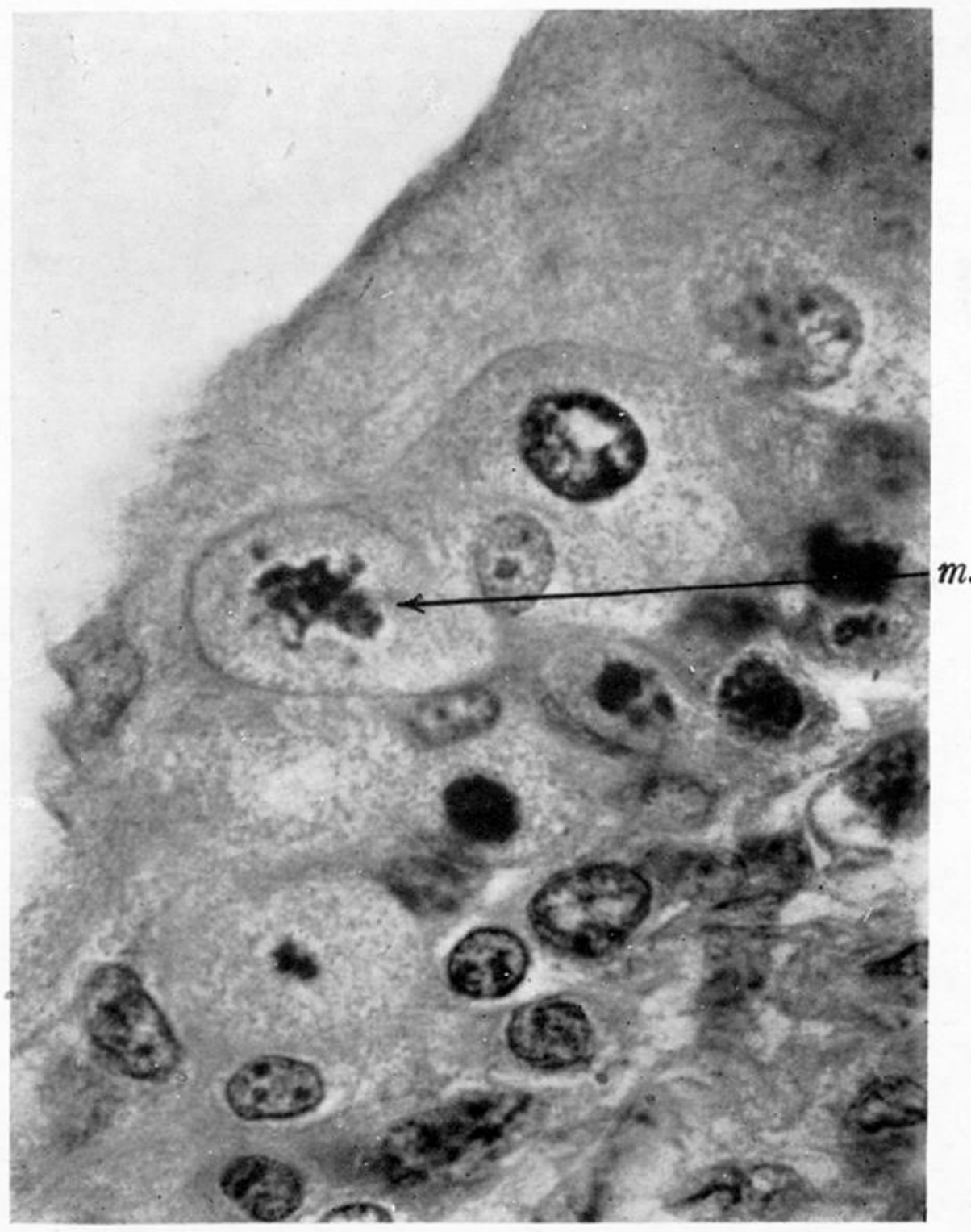

FIGURE 22

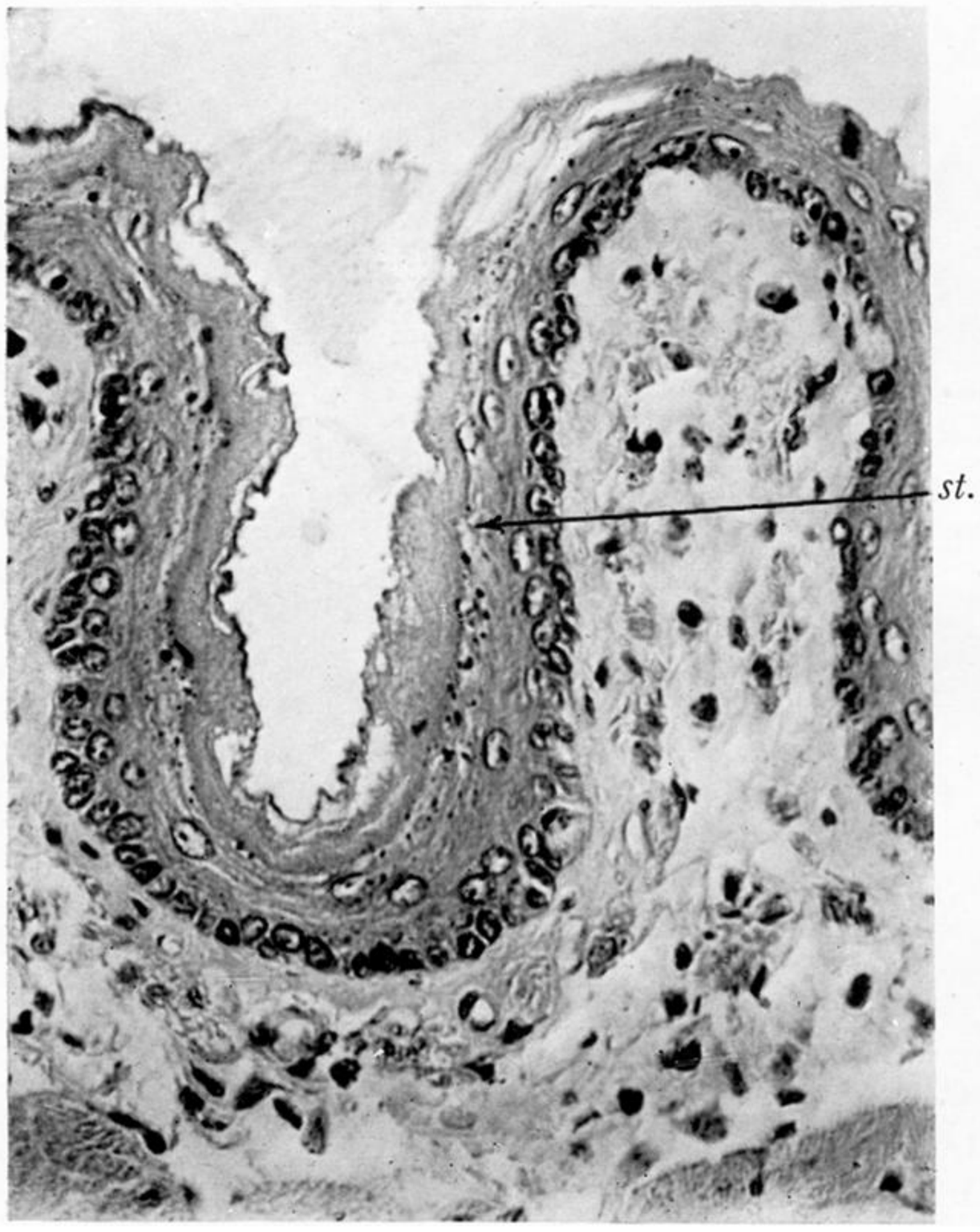

FIGURE 23

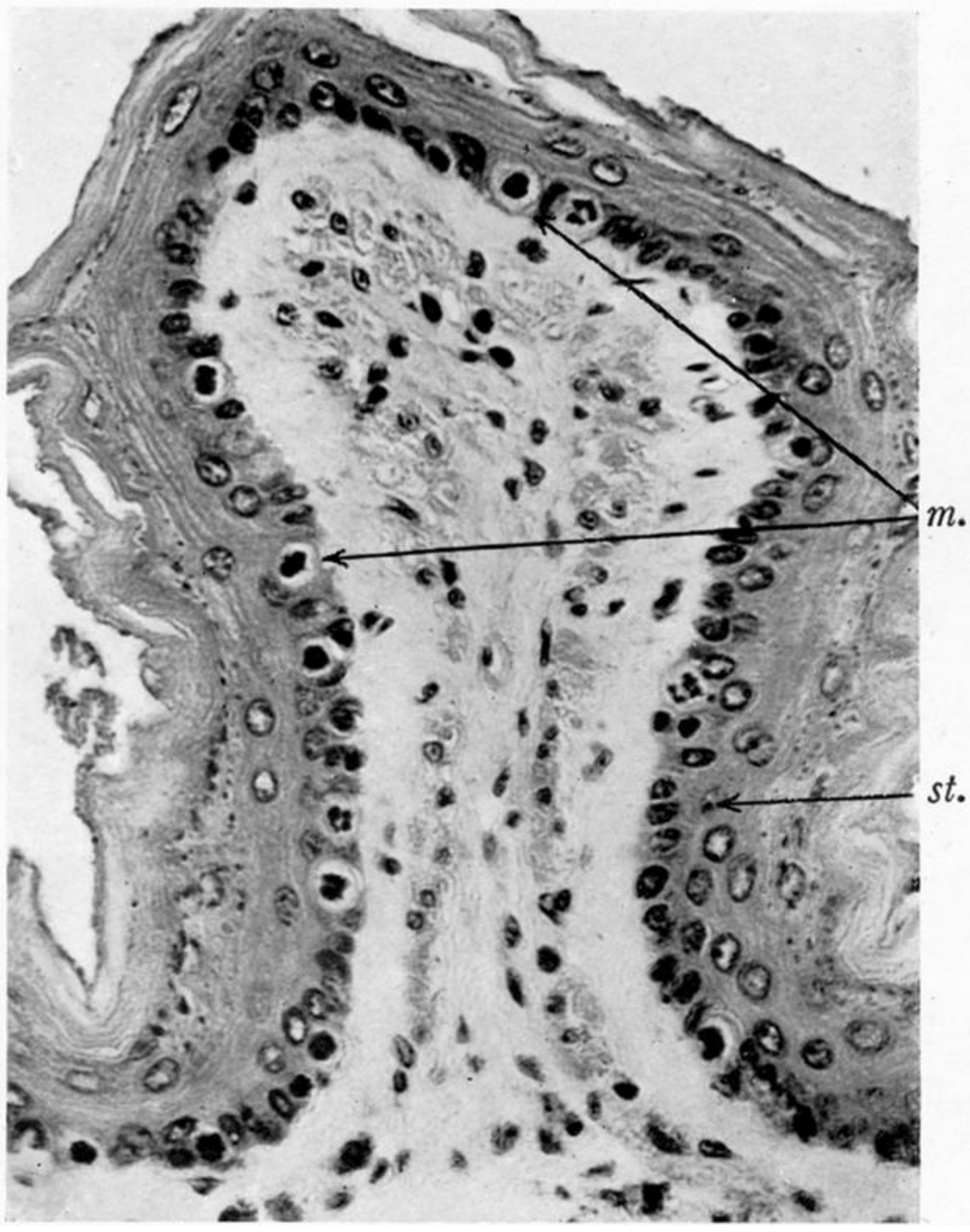

FIGURE 24

PLATE 30

FIGURE 21. Mitosis (*m.*) occurring during full oestrus in the epithelium of a uriniferous tubule of the kidney. × 1000.

FIGURE 22. Mitosis (*m.*) occurring during full oestrus in the transitional epithelium of the urinary bladder. × 1000.

FIGURE 23. Stratified lining epithelium (*st.*) of the oesophagus showing the lack of mitotic activity on the first day of dioestrus. × 400.

FIGURE 24. Stratified lining epithelium (*st.*) of the oesophagus showing the active division (*m.*) of the cells of the basal layer during full oestrus. × 400.

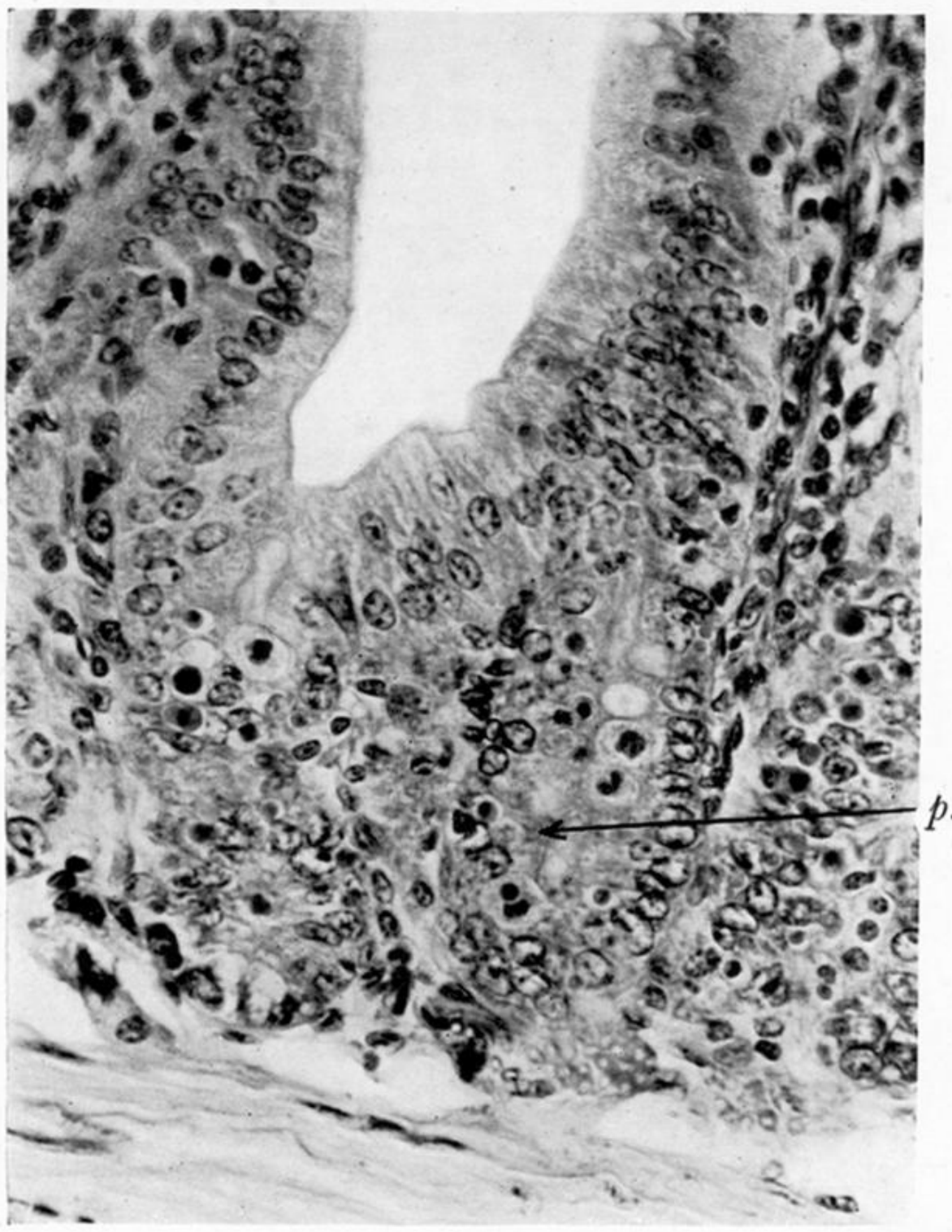

FIGURE 25

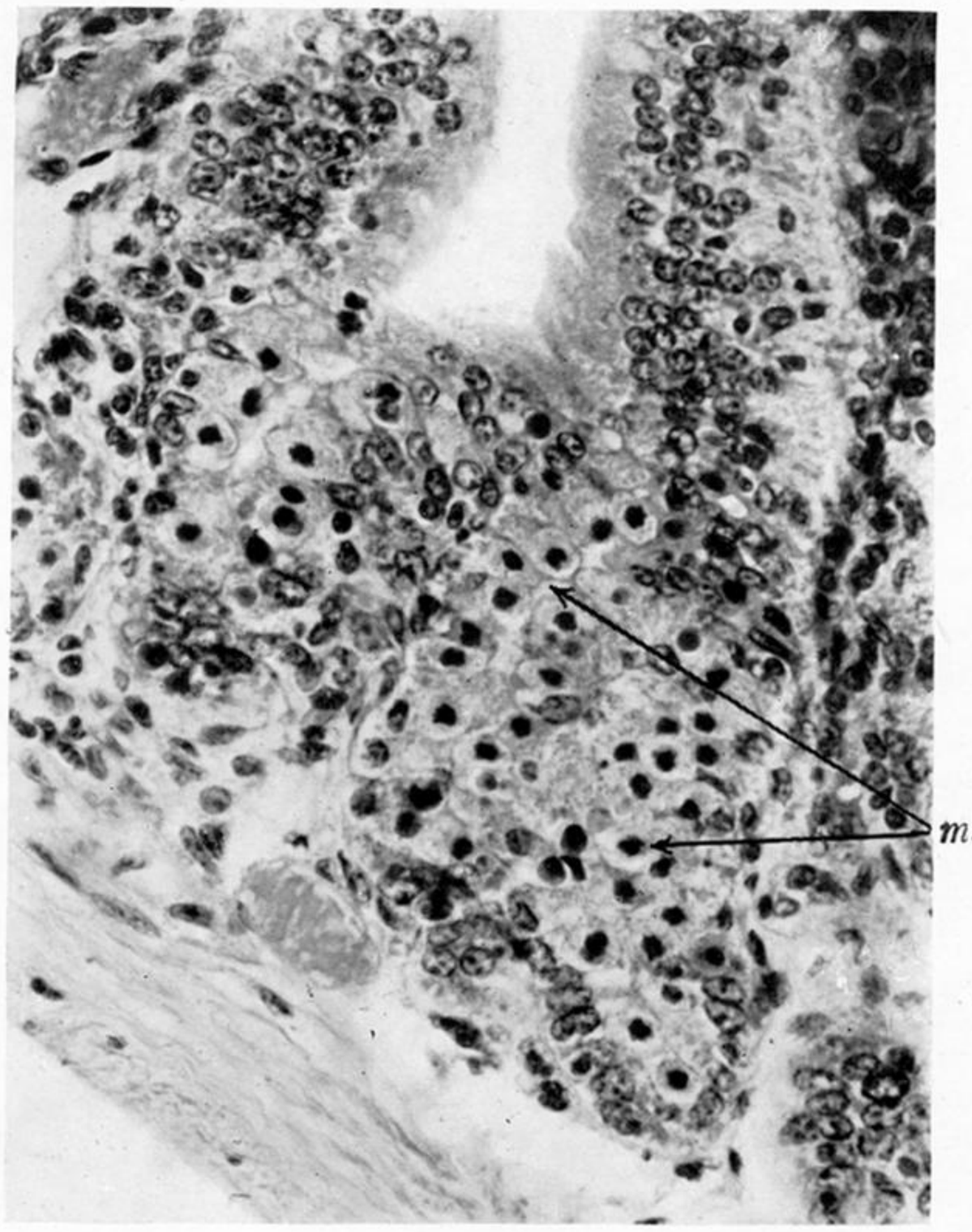

FIGURE 26

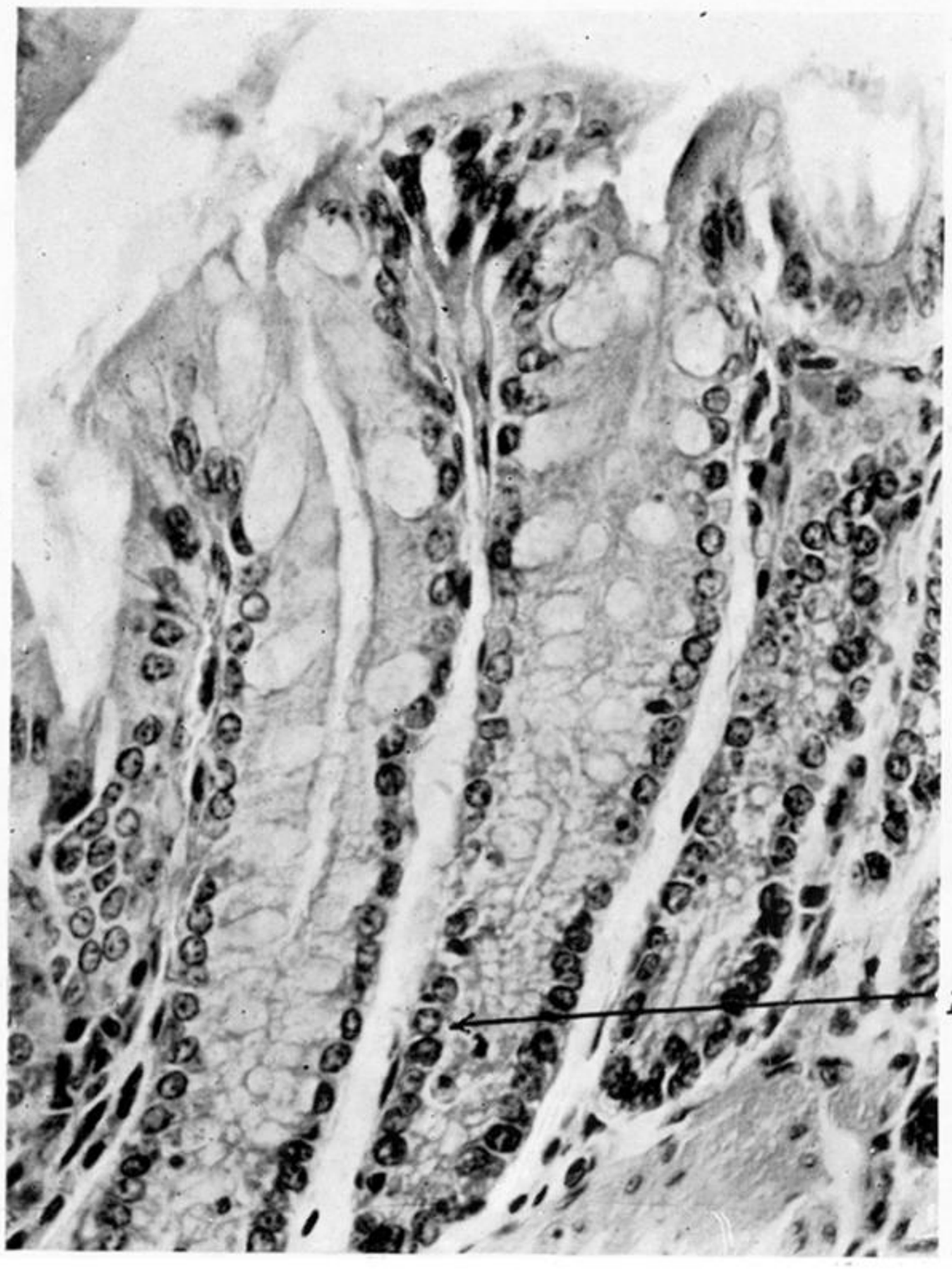

FIGURE 27

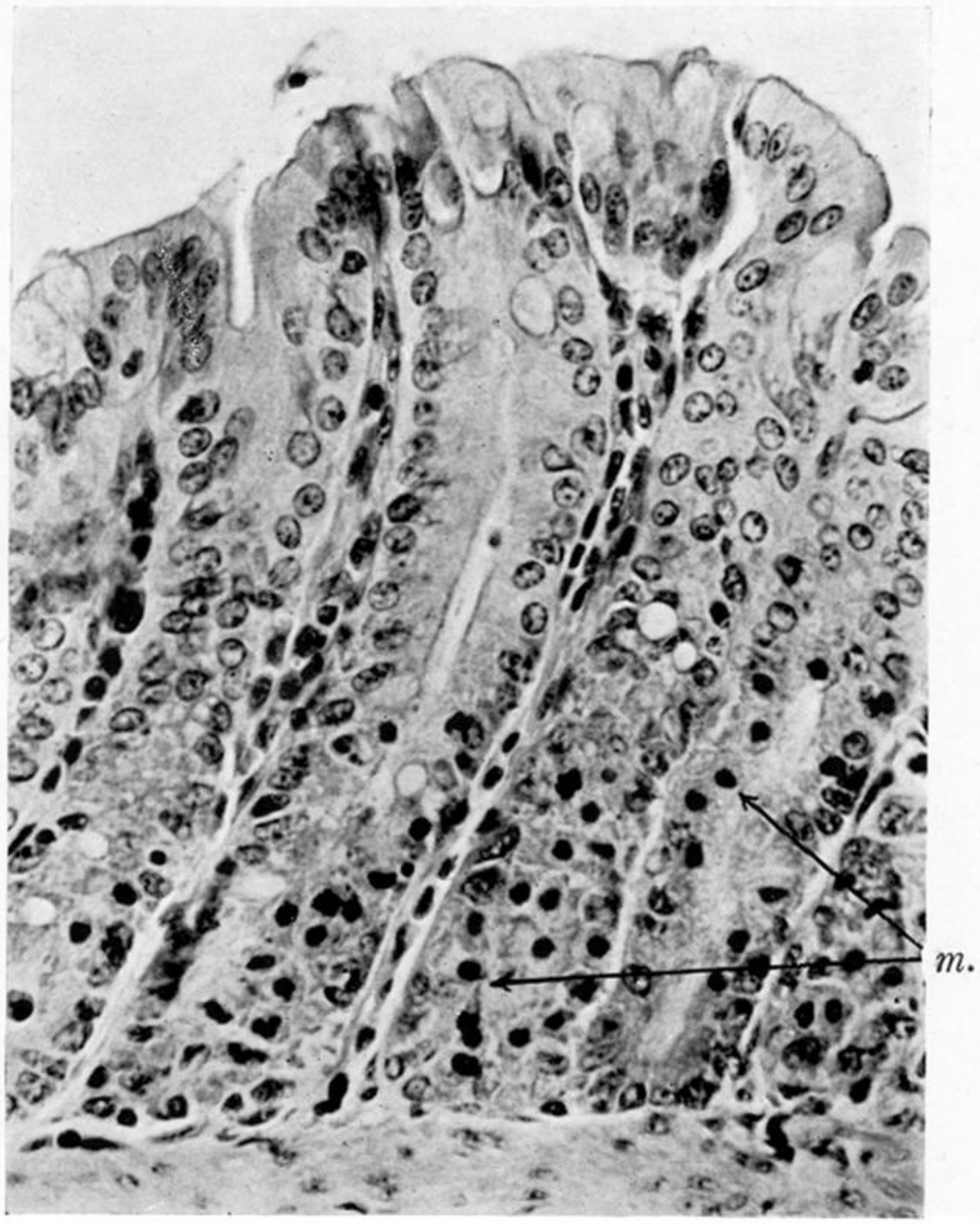

FIGURE 28

PLATE 31

FIGURE 25. Duodenal mucosa showing only slight mitotic activity in the proliferating zone (*p.z.*) on the first day of dioestrus. × 400.

FIGURE 26. Duodenal mucosa showing the large numbers of mitoses (*m.*) in the proliferating zone on the third day of dioestrus. × 400.

FIGURE 27. Rectal mucosa showing the lack of cell division in the proliferating zone (*p.z.*) on the first day of dioestrus. × 400.

FIGURE 28. Rectal mucosa showing the high rate of cell division (*m.*) in the proliferating zone on the third day of dioestrus. × 400.

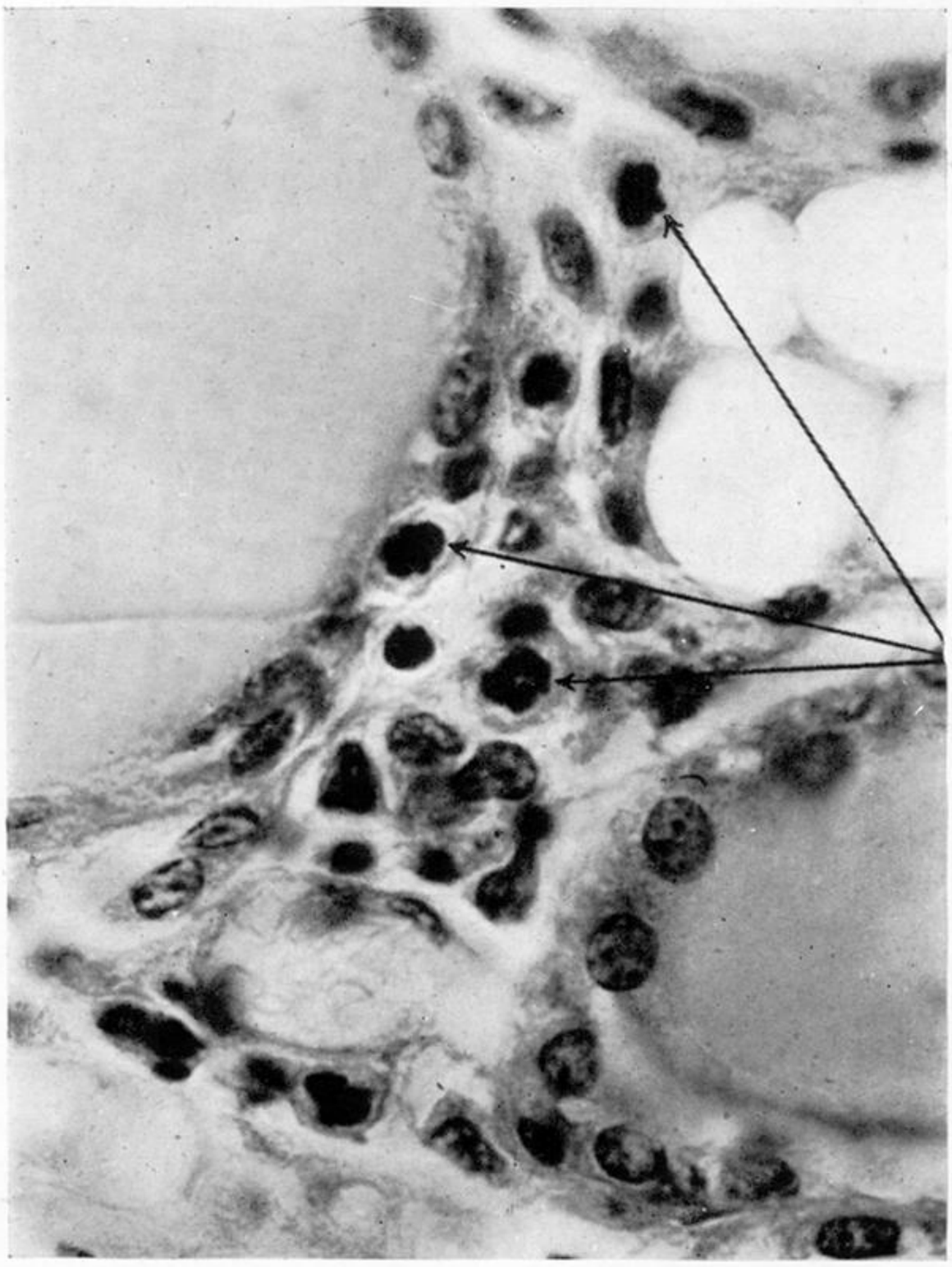

FIGURE 29

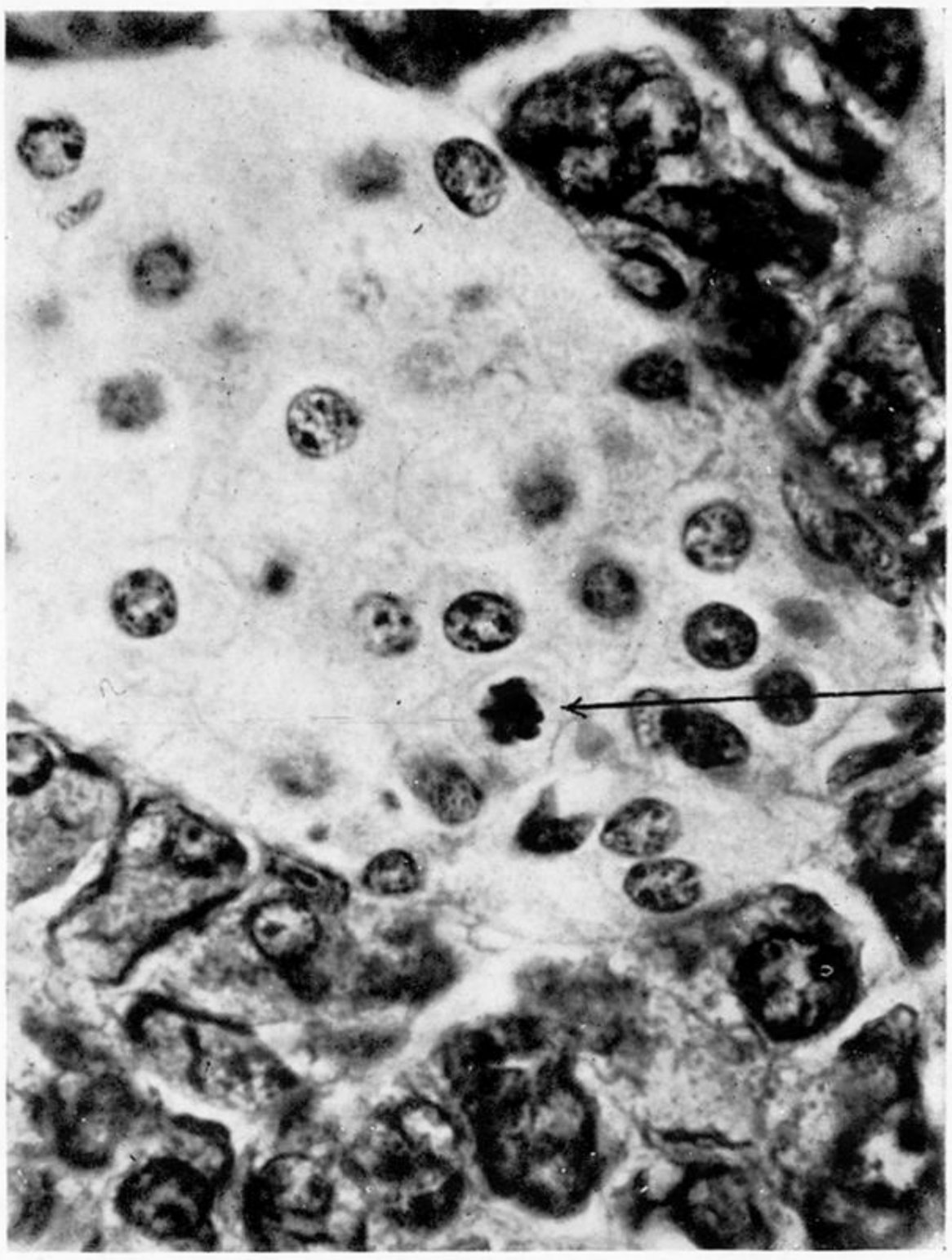

FIGURE 30

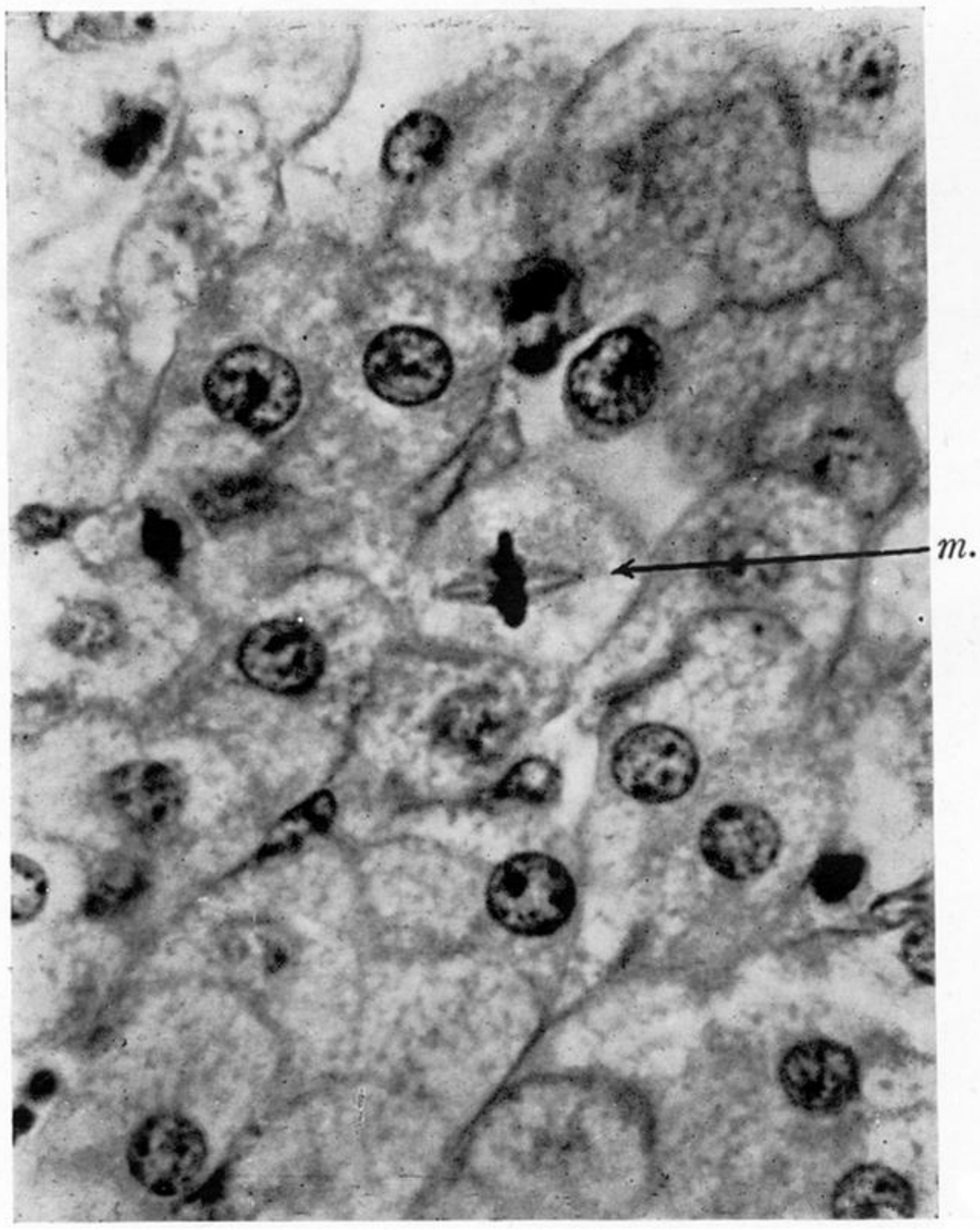

FIGURE 31

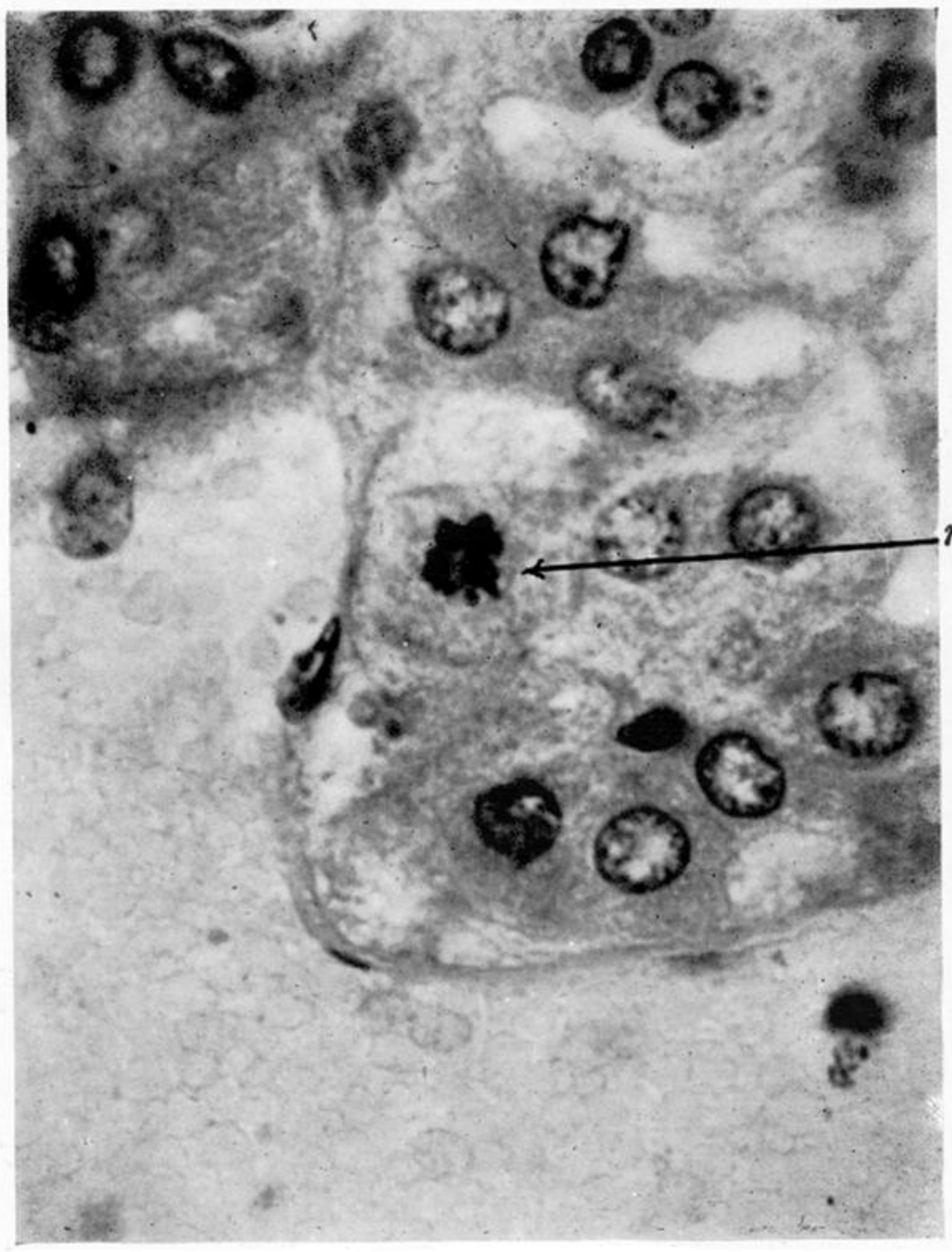

FIGURE 32

PLATE 32

FIGURE 29. Mitoses (*m.*) in the cells of the thyroid gland after six injections of oestrone. × 1000.

FIGURE 30. Mitosis (*m.*) in an islet of Langerhans during the full oestrous period. × 1000.

FIGURE 31. Mitosis (*m.*) in the adrenal cortex during the full oestrous period. × 1000.

FIGURE 32. Mitsosis (*m.*) in the adrenal medulla after six injections of oestrone. × 1000.

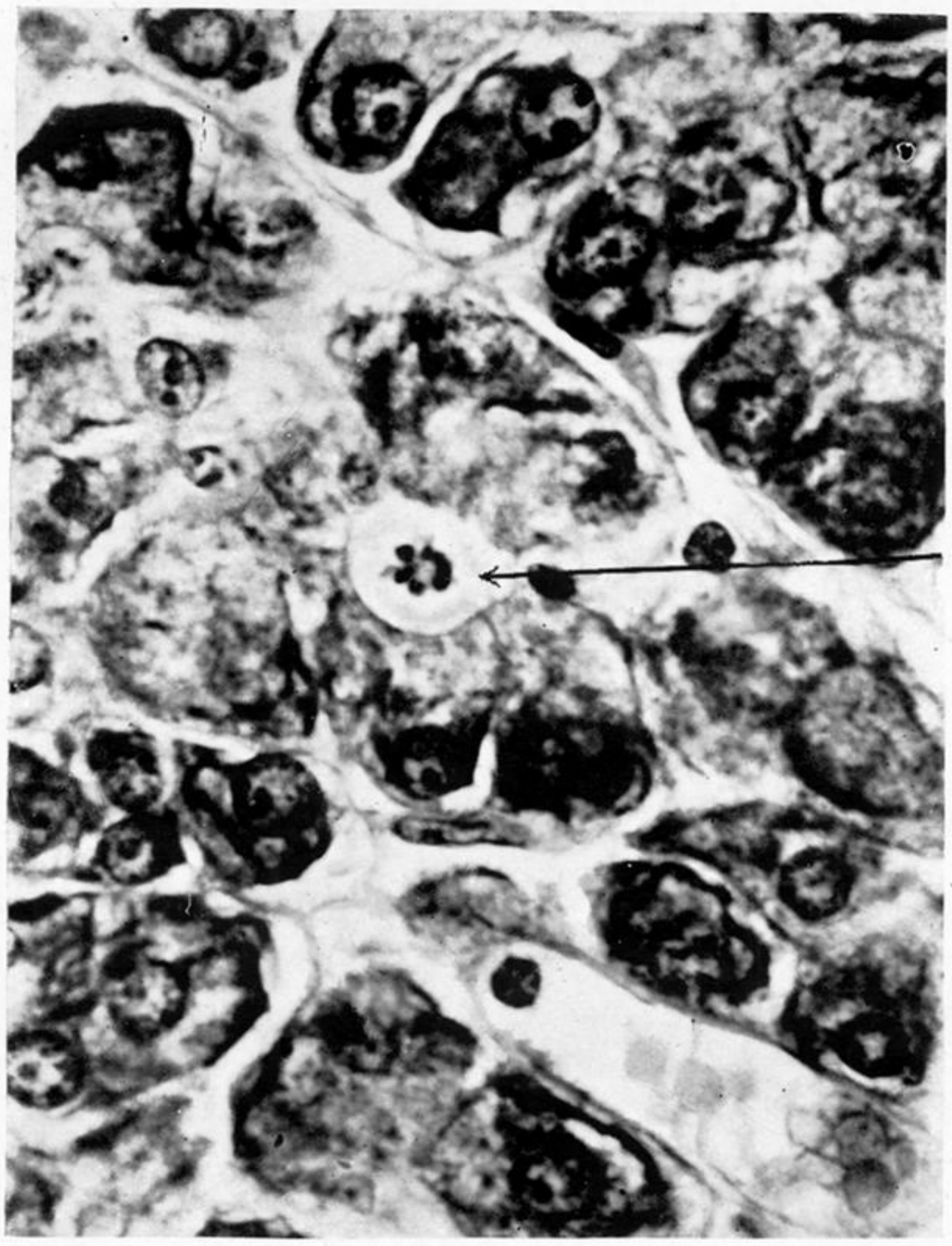

FIGURE 33

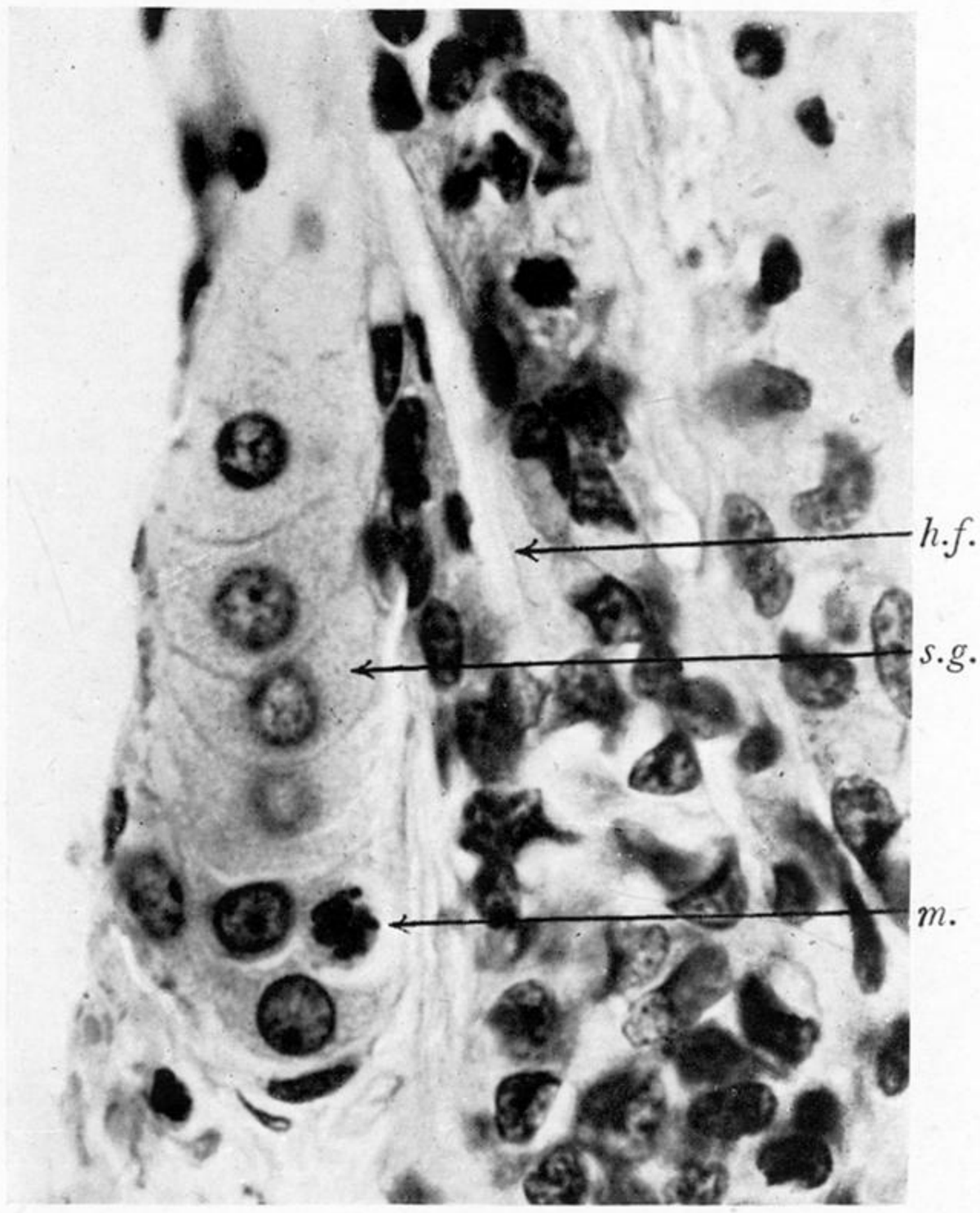

FIGURE 34

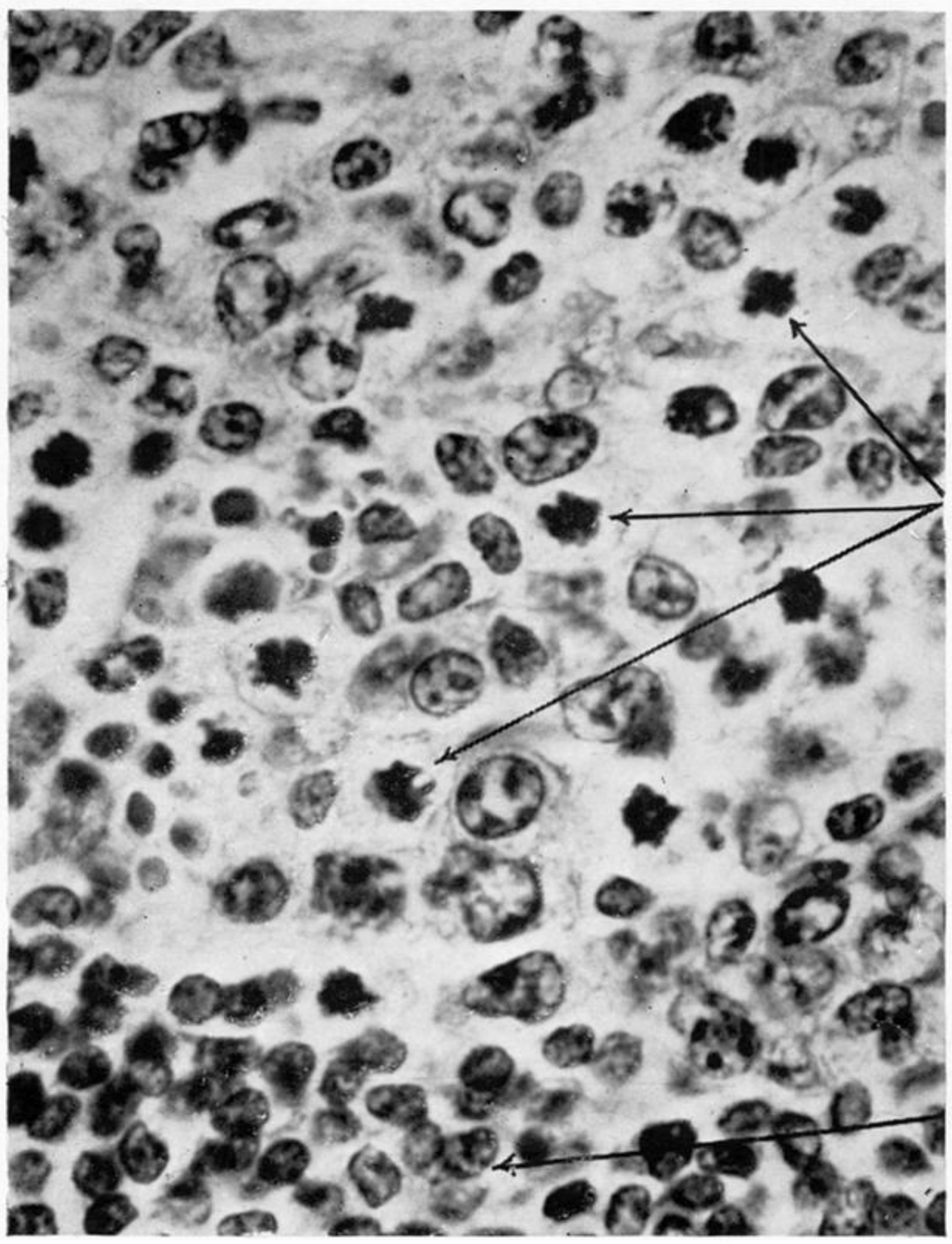

FIGURE 35

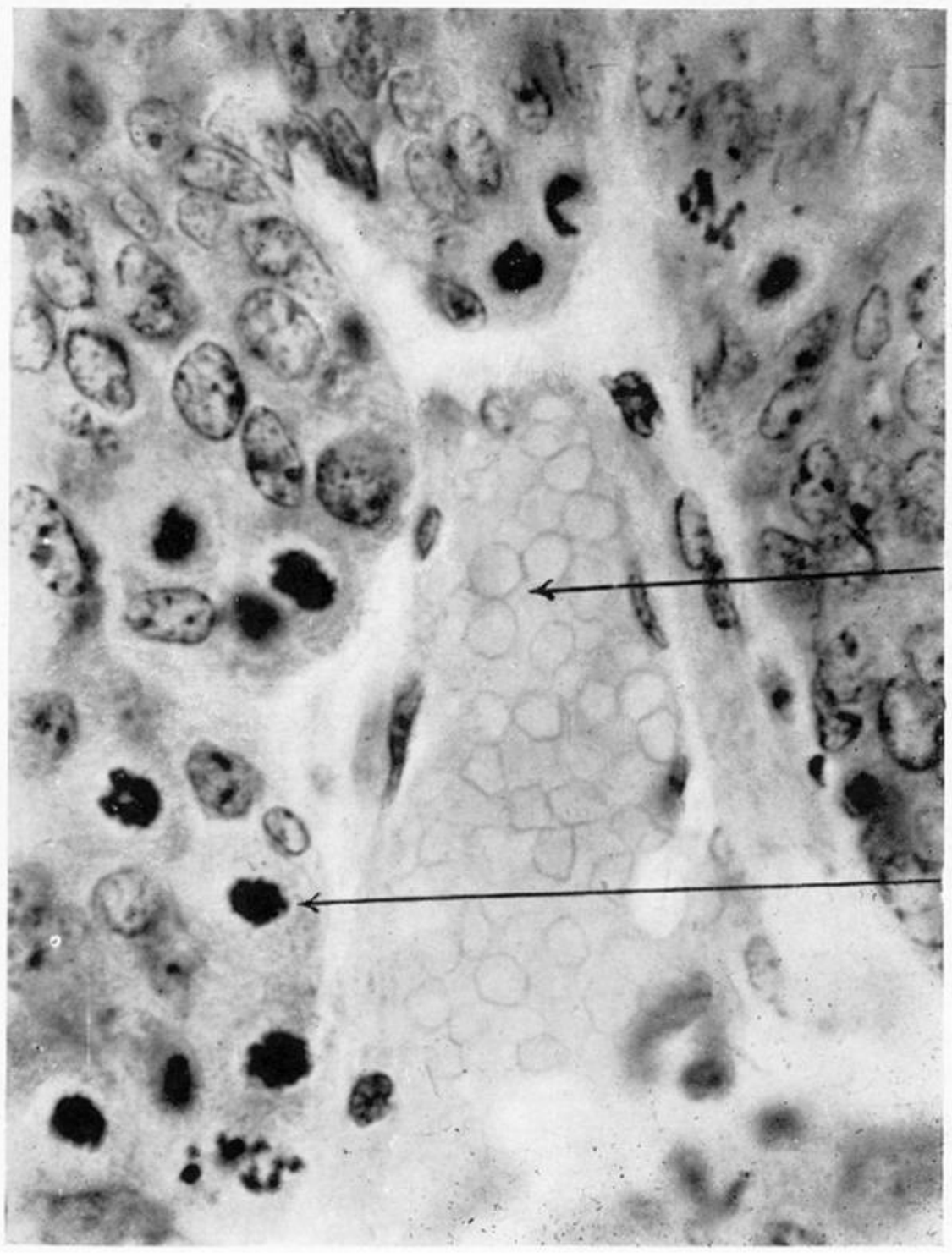

FIGURE 36

PLATE 33

FIGURE 33. Division (*m.*) of a pancreatic acinous cell during the full oestrous period. × 1000.

FIGURE 34. Division (*m.*), during early oestrus, of one of the cells of a sebaceous gland (*s.g.*) attached to a hair follicle (*h.f.*). × 1000.

FIGURE 35. Proliferating centre of an intestinal lymph nodule showing some of the large numbers of mitoses (*m.*) which are always present there. Part of the outer zone of small inert lymphocytes (*ly.*) is also visible. × 1000.

FIGURE 36. Numbers of mitoses (*m.*) adjacent to a blood vessel (*b.v.*) in a rapidly growing region of an implanted mammary carcinoma. × 1000.

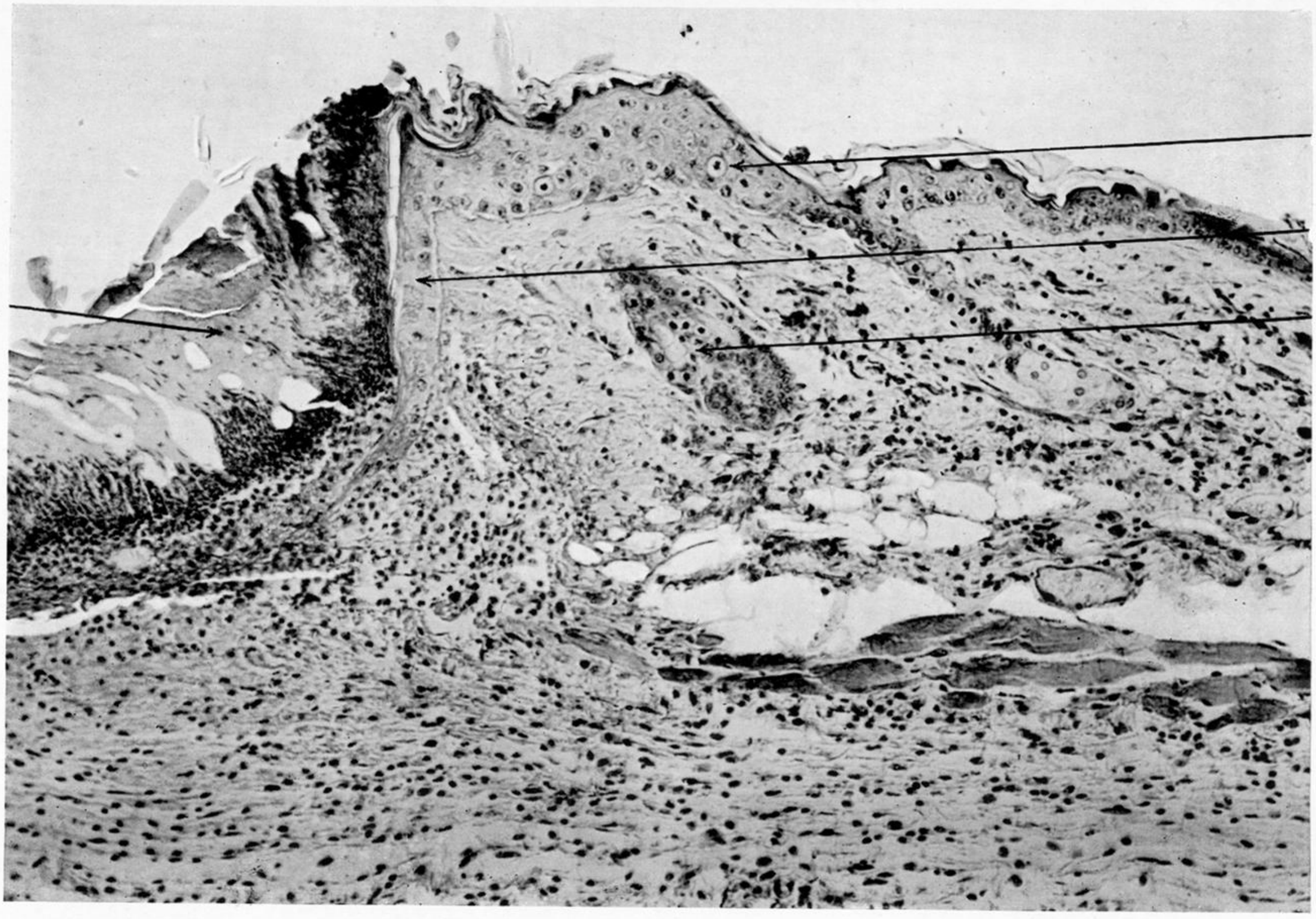

FIGURE 37

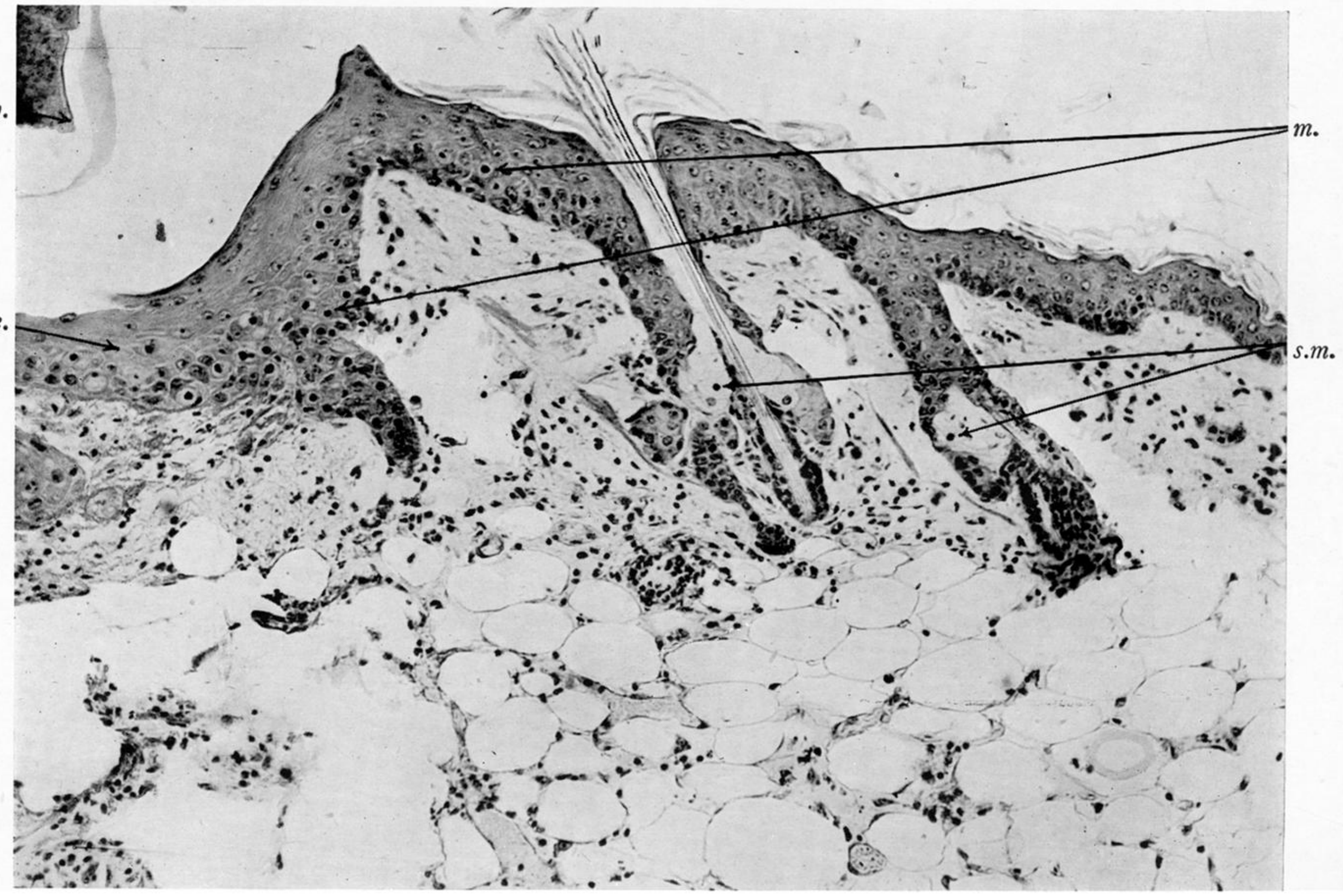

FIGURE 38

PLATE 34

FIGURE 37. Section of the skin adjacent to a wound (*w.*) inflicted during the first day of dioestrus and allowed to heal for 2 days. The greatly thickened epidermis shows increased mitotic activity (*m.*) in the swollen cells by the wound edge, but not in the cells (*m.c.*) migrating beneath the wound clot. Unusually active cell division is also apparent in a hair follicle (*h.f.*). × 180.

FIGURE 38. Section of the skin adjacent to a wound (*w.*) inflicted during the first day of dioestrus and allowed to heal for 3 days. The continuity of the epidermis has been re-established (*n.e.*) by the cells which migrated beneath the wound clot (*w.*). Great mitotic activity (*m.*) is now present in the new as well as in the old epidermis, and is also apparent in adjacent sebaceous glands (*s.m.*).. × 180.