THE ORIGIN OF OVA IN THE ADULT OPOSSUM

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THE ORIGIN OF OVA IN THE ADULT OPOSSUM

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CHAPTER I

INTROCUCTION

The Problem

Many contributions have appeared within recent years dealing with the problems of the origin and history of the definitive germ cells in the mammalian ovary. As a result, many investigators contend that the germ cells which arise previous to the time of birth are the progenitors of the definitive ova in the adult. Others believe that most, and possibly all, of the sex cells which are present at birth degenerate, and the functional ova have their origin from cells of the germinal epithelium. Likewise, conflicting views are held by different authors concerning the time of formation of the functional ova during prepubertal and post-pubertal life. There are workers who have concluded that definitive ova are continually being formed from the germinal epithelium of the adult and have shown a relation between this formation and the oestrous cycle. Therefore, the source and time of origin of the definitive ova still remain as unsettled questions in many mammals. I have made this study on the opossum, Didelphys virginiana, with the aim of determining whether ova are formed from the epithelial covering of the ovary during sexual maturity, and if so to determine how they are formed and to see if there is any relation between the formation and the breeding season. The opossum was used in the investigation for two main reasons. First, the opossum, a primitive mammal, is a native of this vicinity and is found in abundance. Second, it is hoped that an investigation as to the origin of ova in this mammal will add information to the manner in which ova are formed in higher mammals.

Review of Literature

From the literature much information may be found regarding these problems. However, I shall mention only a few opinions concerning the formation of the functional ova after birth and during sexual maturity.

The modern approach to this problem may be said to have begun with the work of Arai in 1920, although he was not the first to suggest that there was acontinuous formation of germ cells through adult life. From a study of the total number of ova in the albino rat during the entire life span, he reported that the definitive oocytes begin to form from the germinal epithelium about ten to fifteen days after birth, occurring most rapidly from fifteen to sixty days, and gradually decreasing with age.[1]

Allen (1923), from his study of the white mouse, concluded that at each normal oestrous period young ova were

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[1]Hoyato Arai, "On the Postnatal Development of the Ovary (albino rat) with Especial Reference to the Number of ova," Am. Jour. Anat., XXVII (1920), 405-462

added to the cortex of the adult ovary, the ova arising from the dividing cells in the germinal epithelium through mitoses. If the long axis of the dividing cell is perpendicular to the surface, or at an angle of more than 20° to 30°, one of the daughter cells is cut off from the epithelium, and is soon surrounded by cells making a young follicle.[2]

Allen and Atcheson (1924) reported that pregnancy severely inhibited postpubertal ovogenesis in the mouse. They observed few mitoses in the germinal epithelium of the pregnant animals in comparison with the number observed in the non-pregnant rats near the time of oestrous.[3]

The results of Allen and Atcheson were confirmed by Papanicolaou (1924) on the guinea pig. He considered ovogenesis a continuous origin of ova from the germinal epithelium from the time of gonadal differentiation to the time of cessation of sexual activity, though the process was modified by seasonal conditions.[4]

An interesting approach to the origin of definitive germ cells was made by Cowperthwaite (1925) in which the occurrence of meiotic phases was considered the criterion of a definitive oocyte. From his study of rat ovaries of

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[2]Edgar Allen, "Ovogenesis During Sexual Maturity," Am. Jour. Anat., XXXI (1923), 439-482.

[3]Edgar Allen and Bellfield Atcheson, "The Effect of Pregnancy upon Postpubertal Ovogenesis," Anat. Rec., XXVII (1924), 179-196.

[4]George N. Papanicolaou, "Ovogenesis During Sexual Maturity as Elucidated by Experimental Methods," Proc. Soc. Exp. and Med., XXI (1924), 393-396.

animals ranging from newly-born to ones having attained sexual maturity, he concluded that young follicles represented a retarded growth condition and they are not derived from a new germ-cell formation. This conclusion was reached by the author chiefly because of a failure to find meiotic stages in the germinal epithelium of any series of ovaries studied.[5]

Contrary to the work of Cowperthwaite, Butcher (1927) found, from his study of the white rat, definite evidence that the germinal potentiality of the peritoneum is not lost at puberty. Butcher believes that the formation of germ cells from the germinal epithelium continues to take place until fecundity is lost at old age. A notable increase in mitotic activity in the epithelium was evident during oestrous, and cells enlarging in the epithelium were usually more common as the oestrous period approached.[6]

The work of Hargitt (1930) confirms the results of Butcher. From his study of adult rat ovaries during different phases of the reproductive cycle, Hargitt found that some new ova are produced from the germinal epithelium during pregnancy, but that the epithelium was most active

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[5]Marion Hazel Cowperthwaite, "Observations of Pre- and Postpubertal Oogenesis in the White Rat, Mus norvegicus albinus," Am. Jour. Anat., XXXVI (1925), 69-90.

[6]Earl O. Butcher, "The Origin of the Definitive Ova in the White Rat," Anat. Rec., XXXVII (1927), 13-30.

between oestrous periods.[7]

Contrary to the work of both Hargitt and Butcher, Heys (1931) concluded from her regeneration studies on the ovaries of rats that there was no evidence to support a belief that ova originate from a peritoneal source.[8]

One of the most recent contributions which has been made pertaining to the origin of ova is that of Allen and Creadick (1937). They observed numerous mitoses in the germinal epithelium of mice ovaries during oestrous, which would probably add new ova to the cortex.[9]

A similar contribution has recently been made by Guthrie and Jeffers (1938) from their work on adult bats. Their work shows that oocytes are formed from the germinal epithelium of the ovary during sexual maturity.[10]

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[7]George T. Hargitt, "The Formation of the Sex Glands and Germ Cells of Mammals. V. Germ Cells in the Ovaries of Adult, Pregnant, and Senile Albino Rats", Jour. Morph. and Physiol., L (1930), 453-469.

[8]Florence Heys, "The Problem of the Origin of Germ Cells," Quart. Rev. Biol., VI (1931), 1-45.

[9]E. Allen and R. N. Creadick, "Ovogenesis During Sexual Maturity," Anat. Rec., LXIX (1937), 191-195.

[10]Mary J. Gutherie and Katherine R. Jeffers, A Cytoogical Study of the Ovaries of the Bats Myotis lucifugus lucifugus and Myotis grisescens," Jour. Morph. and Physiol., LXII (1938), 523-556.

CHAPTER II

MATERIALS, METHODS, AND OBSERVATIONS

Materials and Methods

The materials used in this investigation consisted of ovaries from thirty-two sexually mature opossums which were collected in the Denton area. These varied in weight from 1,221 to 2,020 grams; the average adult weighing about 1,800 grams. Numerous young opossums were collected with these adults which ranged from .08 grams or 8.1 mm. in length (head-rump) to 10.7 or 53.5 mm. in length. These embryos were preserved, but were not used in the investigation.

Collection of the material was begun October 15, and a specimen was collected every two weeks until the latter part of December. After this time numerous ones were collected at short intervals until after the breeding season which was completed in February; specimens were collected thereafter at greater intervals until August.

The specimens were collected alive, brought into the laboratory, killed, weighed and the ovaries were immediately placed in the fixing agent. The young, which accompanied some of the adult females, were weighed, head-rump measurements taken and were preserved entire after slitting the body cavity in order to permit the fixing agent to penetrate the internal organs more readily. In some instances the young were large enough that removal of the ovaries could be accomplished before fixation.

Most of the embryos and ovaries were preserved in Bouin's fluid to which 2% urea crystals were added. The tissue was fixed from 6 to 10 hours. Carnoy's and Flemming's fixing fluids were also used; however, the Bouin's gave the best fixation. The tissue was cut in series from 4 to 10 micra in thickness and was stained with Heidenhain's iron hematoxylin, followed in most cases by eosin as a counterstain.

Observations

Animal collected October 15, weight 1,999 grams.- The animal collected on this date was in the anoestrous period, which is known to be during October, November and December (Hartman 1923).[11] The uteri and oviducts of this specimen were reduced in size, collapsed, and showed no indication of an increased blood supply. Examination of the ovaries revealed that there was no activity from the germinal epithelium and no young ova beneath the epithelium in the tunica albuginea. However, a few older follicles were visible deeper in the cortex of the ovary, but the ovary consisted primarily of scar tissue and of old follicles which had appar-

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[11] C. G. Hartman, "The Breeding Season of the Opossum", Jour. Morph. and Physiol., XL (1923), 142-215.

ently undergone degeneration (Plate 1, figure 1).

Animal collected October 31, weight 1,950 grams.- Numerous young follicles were observed just beneath the germinal epithelium of the ovaries (Plate 1, figure 2). Several empty follicles, which probably had been functional ones in previous seasons, were observed deeper in the cortex of the ovary. No intermediate stages of follicles were visible.

Animal collected November 19, weight 2,130 grams.- The ovaries exhibited several germinal epithelial cells enlarging in situ (Plate 3, figure 6). The epithelium was active in proliferating cells at restricted places, being most active in the region near the hilus of the ovary (Plate 6, figure 12). Deeper in the ovary, in the tunica albuginea, appeared several enlarged cells with follicular cells encircling them. Still deeper in the ovary, were numerous empty follicles and several different stages of developing follicles.

Animal collected December 12, weight 1,518 grams.- Although this specimen was comparatively small, her ovaries showed the presence of a few mature follicles which appeared to be almost ready for ovulation. A few mitotic stages were observed in the germinal epithelium (Plate 5, figure 9). Also, there were several enlarged cells just beneath the epithelial layer, (Plate 4, figure 7), and numerous enlarged cells deeper in the tunica albuginea.

Animal collected January 3, weight 2,096 grams. This was the first specimen collected which showed an enlargement of the genital tract and an increased blood supply leading to these organs. Histological examination of the ovaries revealed that the germinal epithelium was still very active in producing enlarged cells, and as observed in other specimens, the epithelium was most active in the region near the hilus of the ovary.

Animals collected January 11, weight 1,980 and 2,078 grams respectively.- The germinal epithelium was still active in these specimens and there was no particular change observed in the ovaries of these over that of the specimen collected January 3. However, the genital tracts of these were much more enlarged and showed a greater congestion of blood.

Animal collected January 18, weight 1,985 grams.- The walls of the uteri were even more congested with blood than found in the previous ones, and the entire reproductive tract was greatly enlarged. Externally, the ovaries appeared to be enlarged and exhibited prominent projections which resembled blisters protruding from the surface. Histological examination revealed that the projections were mature follicles near the surface of the ovary, apparently ready for ovulation. A few enlarged cells were observed in the germinal epithelium.

Animal collected January 28, weight 1,987 grams.- This was the first specimen collected which was pregnant. Twenty gastrula stages of development were found in the folds of the two uteri. The ovaries contained several empty follicles and numerous corpora lutei, which was to be expected since the animal was pregnant. In this and in other animals in which ovulation had occurred, the basement membrane was easily observed in the empty follicles and sharply differentiated from the theca interna. The germinal epithelium of the ovaries was not active in giving rise to new cells, but a few very young follicles were observed just beneath the epithelium in the tunica albuginea.

Animal collected February 8, weight 1,790 grams.- Three embryos were found in the pouch of this adult which were 21.1 mm. in length (head-rump). A few enlarged cells were visible in the germinal epithelium which seemed to occur at restricted places, still appearing in greater abundance near the hilus of the ovary. A few empty follicles and corpora lutei were visible.

Animal collected March 31, weight 1,302 grams.- This small female had eight embryos in her pouch which were 53.5 mm. in length. This adult likewise had numerous empty follicles in the ovaries and what appeared to be corpora lutei. Only a few enlarged cells were observed in the germinal epithelium, but numerous young follicles were found in the tunica albuginea.

Animal collected June 6, weight 1,840 grams.- Six embryos 43 mm. in length were found in the pouch of this adult. The young were probably without question born during a second breeding period, which begins about three months after the onset of the first breeding period (Hartman 1923).[12] The ovaries were practically filled with empty follicles and several corpora lutei were observed (Plate 2, figure 3). The germinal epithelium of these ovaries was not active in forming enlarged cells and no mitotic figures were present, but a few young follicles still remained deep in the tunica albuginea.

Animal collected July 15, weight 2,001 grams- There were no young found in the pouch of this specimen, but her mammary glands were still enlarged and contained milk, which was evidence that she was probably still suckling her young. The ovaries were somewhat reduced in size, and exhibited numerous empty follicles and a few corpora lutei (Plate 2, figure 4). No enlarged cells were observed in the germinal epithelium or tunica albuginea, and only a very limited number of young follicles were observed, these appearing deep in the tunica albuginea.

Animal collected August 3, weight 1,620 grams.- The ovaries of this specimen contained more young follicles than the previous one, but numerous empty follicles and a few corpora lutei were observed in the deeper parts of the gonads. No activity was visible in the germinal epithelium, and no young ova were present just beneath the epithelium in the tunica albuginea. The young follicles which were present appeared in the deeper parts of the tunica albuginea (Plate 3, figure 5).

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[12] Ibid.

Formation of Ova

In the adult opossum there seems to be two methods by which new ova are produced in the ovaries. First, single cells of the germinal epithelium enlarge in position and become separated from the epithelial layer of cells; these move into the tunica albuginea. Here each enlarged cell becomes surrounded by smaller neighboring cells and forms a young follicle (Plates 3 and 4, figures 6, 7, and 8). Second, an epithelial cell may divide by mitosis which will give rise to a cell just beneath the germinal epithelium (Plate 5, figure 9). This cell may likewise form a follicle by becoming surrounded by smaller neighboring cells; however, this method is much less common than the former.

The processes involved may be followed in more detail. Plate 3, figure 6, shows a typical germinal epithelial cell enlarging in situ. In this and in other cases it can be shown that the slightly enlarged cells really represent typical cells of the surface layer which have increased in size and assumed a more spherical form; transitions may be found between the flattened or cubic epithelial cells and the large spherical cells. After attaining some size, the enlarged cells move from the epithelial layer and sink into the tunica albuginea where they are encircled by flattened and elongated cells already present in the tunica albuginea (Plate 4, figures 7 and 8). Often several cells appear to enlarge at one region in the germinal epithelium and move in groups into the tunica albuginea. This is particularly true of the region near the hilus of the ovary (Plate 6, figure 12).

On plate 5, figure 9, a germinal epithelial cell is represented in a typical telophase stage of mitosis. The spindle is located at an angle almost perpendicular to the surface of the ovary; thus, a daughter cell will be contributed to the underlying tissue. A cell contributed in this manner may be destined to become an ovum since it is enlarged and is similar to a cell which moves from the epithelium without division, or it may be one which will encircle an ovum in the formation of the follicle. It appears that only a few ova are contributed in this manner.

Frequency of New Ova Production

It is difficult to determine accurately the frequency with which new ova are produced in different phases of adult life. Actual counting is practically out of the question, so estimates must be made. However, relative numbers can be determined and comparisons can be made using relative numbers as a basis.

Considering first the number of oocytes and young follicles, it is found that very few are present at the beginning of the anoestral period. Within a few weeks the number begins to increase and a maximum number is reached before the onset of the breeding season. After pregnancy, the number of oocytes decreases rather rapidly until only a very few are present in the ovaries at the end of the breeding season.

This decrease occurs because there is a cessation in the formation of new ova, and the ones already developed are given off at ovulation. However, a few ova continue to form during pregnancy and throughout the breeding season. The source of the new oocytes is known, since a correlation exists between the activity of the germinal epithelium and the increase in oocyte number. When the number of oocytes is smallest (soon after the close of the breeding period) the germinal epithelium is least active; when the number of oocytes is greatest (in the latter part of the anoestral period), there are found more cases of enlarged germinal epithelial cells and ingrowing groups of the same.

Three things seem to be shown by these comparative studies. First, some new ova are produced during the period of pregnancy from the germinal epithelial cells. Second, the greatest activity of the germinal epithelium takes place in the ovaries of non-pregnant animals during the latter part of the anoestrous period. Third, the correlation between the number of oocytes present in adult ovaries and the activity of the germinal epithelium is close enough to show that new ova are constantly being formed in the ovaries of adult animals.

CHAPTER III

DISCUSSION, SUMMARY, AND CONCLUSIONS

Discussion

The results of this investigation on the origin of ova in the adult opossum seem to substantiate the changing concept that the germinal potentiality of the mammalian ovary is continued throughout the reproductive period of adult life. Arai (1920), Allen (1923), Allen and Atcheson (1924), Papanicolaou (1924), Butcher (1927), Hargitt (1930), Allen and Creadick (1937), Gutherie and Jeffers (1938), and others have found this condition in the forms they studied. There is no doubt that the germinal epithelium of the opossum ovary continues to be active during adult life, but I cannot estimate how many of these cells will become functional ova. Papanicolaou (1924) observed in the guinea pig that ingrowing groups of the epithelium might form ova, interstitial cells, follicle cells, or luteal cells.[13] I made no attempt to determine the destiny of all the cells which had their origin from the germinal epithelium, but it is only reasonable to believe that many of the ingrowing cells were not destined to form ova. This probably explains the belief of some investigators that only interstitial or follicle cells may be derived from

[13]Papanicolaou, *op. cit.*, pp. 393-396.

the germinal epithelium. League and Hartman (1925) explain that the presence of anovular follicles in the opossum ovary is due to an ingrowth of epithelial cells which fail to become associated with old ova already present.[14] I cannot justify the claim that ova cannot be produced anew in the adult and that the germinal epithelium can only form follicles or interstitial cells.

Hargitt (1930) gave two methods by which new ova are produced in the adult rat. First, by enlargement of cells in the germinal epithelium which become surrounded by smaller neighboring cells, and the group then moving into the tunica albuginea, forming a young follicle. Second, a number of germinal epithelial cells round into a mass, and without enlargement the entire group moves into the albuginea, where one of the cells may enlarge to form an ovum, whereas, the others remain small and form follicle cells.[15] The first method is similar to that which I have described in the opossum, but I find no evidence of ova formation by the second method which Hargitt has described. This observation conforms to the results obtained by Gutherie and Jeffers (1938) from their work on adult bat ovaries. Their studies indicate that oocytes are formed during sexual maturity by the movement of enlarged cells from the germinal epithelium into the

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[14]Bessie League and Carl G. Hartman, "Anovular Graafian Follicles in Mammalian Ovaries," Anat. Rec., XXX (1925), 1-14.

[15]Hargitt, op. cit., pp. 453-469.

underlying tissue.[16] Several investigators mention only one method by which ova are formed in the adult and that is by mitotic division of cells in the germinal epithelium. Allen (1923), Allen and Atcheson (1924), Butcher (1927), and Allen and Creadick (1937), have concluded from their studies that new ova arise in the adult by mitosis of cells in the epithelium. I find that a few ova arise in this manner in the opossum, but the number is very small in comparison with the number having their origin by the enlargement of the germinal epithelial cells in situ and their subsequent movement into the deeper cortex of the ovary.

The fact that there is a correlation between the number of oocytes present in the adult ovaries and the activity of the germinal epithelium is further proof that the enlarged and dividing cells of the epithelium form new ova. Hargitt (1930) found a correlation between the number of enlarged cells in the germinal epithelium and the number of ova in the ovaries of adult rats. He calls attention to the fact that there are probably many investigators who have failed to observe this correlation because the enlarged cells in the epithelium are often difficult to distinguish.[17]

The observation that the activity of the germinal epithelium was not uniform over the entire ovary was easily made

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[16]Gutherie and Jeffers, op. cit., pp. 523-556.

[17]Hargitt, op. cit., 453-469.

in most of the specimens used in this investigation. Papanicolaou (1924) observed this in his study of guinea pig ovaries.[18] Other than his report, I find no author making mention of this observation. Possibly the lack of uniform activity is due to a better blood supply at certain regions of the ovary. The greatest activity of the epithelial layer was near the hilus of the gonad, and since the hilus is the region where blood vessels enter the ovary it is only reasonable to believe that the supply of blood would be greater here. Thus, this seems to be a determining factor in causing the increased activity.

In regard to the frequency of new ova production, I find that my results are comparable to those obtained by Allen (1923), Allen and Atcheson (1924), Papanicolaou (1924), Butcher (1927), Hargitt (1930), and Allen and Creadick (1937). Hartman (1923) found that the breeding season of the opossum in this region began in January following a three months anoestrous period. He reported that a first brood was weaned in about three months and immediately following, most of the females became pregnant again. Thus, a second litter would be at the weaning stage about July or August.[19] Using these results as a basis, I began collecting material in October. The first specimen, which was collected October 15, showed no indication of epithelial activity, but the one collected

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[18]Papanicolaou, <u>op. cit.</u>, pp. 393-396.

[19]Hartman, <u>op. cit.</u>, pp. 142-215.

sixteen days later exhibited numerous young ova just beneath the germinal epithelium; however, only a few enlarged cells were observed in the epithelium. Unfortunately, no specimens were collected between October 15 and 31, but I am of the opinion that I would have found an abundant proliferation of cells from the epithelial layer of the ovary from animals which should have been collected during this time. Specimens collected after October 31 showed a great activity of the germinal epithelium until the time of pregnancy was approached. Pregnancy seemed to have a marked inhibiting effect upon the production of ova, but the process did not cease altogether until after the second breeding period which was about the first of June. From data herein presented, I am of the opinion that the ovary of the opossum undergoes a period of rest during the months of July, August and September, which will account for the lack of numerous young ova and follicles during this time.

A summary has been given of the investigations which show that ova continue to be formed anew through adult life in such higher mammals as the rat, mouse, bat, and guinea pig. Since this adult formation applies to the opossum, one of the lowest mammals, it is suggestive that we are dealing with a typical mammalian process.

Summary and Conclusions

1. New ova are formed during adult life of the opossum which is shown by a correlation between the activity of the germinal epithelium and the number of oocytes and young follicles present in the ovaries.

2. A few new ova are produced from the germinal epithelium of ovaries during pregnancy and oestrous, but it is in the ovaries of non-pregnant animals near the close of the anoestrous period that the germinal epithelium is most active.

3. The majority of new ova have their origin from the germinal epithelial cells which enlarge in situ and subsequently move into the tunica albuginea.

4. A few ova are contributed by mitosis of cells in the germinal epithelium.

5. The germinal epithelium is most active in producing new ova near the hilus of the ovary.

PLATE I

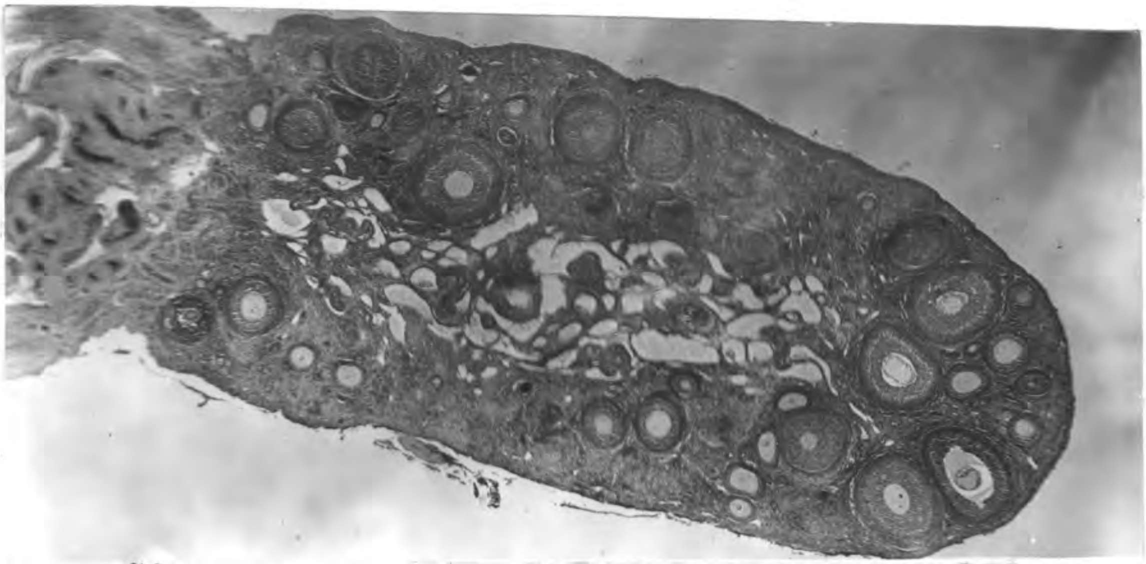

Fig.1. Microphotograph of a longitudinal section of the ovary from a specimen collected October 15. Note the numerous old follicles and scar tissue, and the lack of young ova.

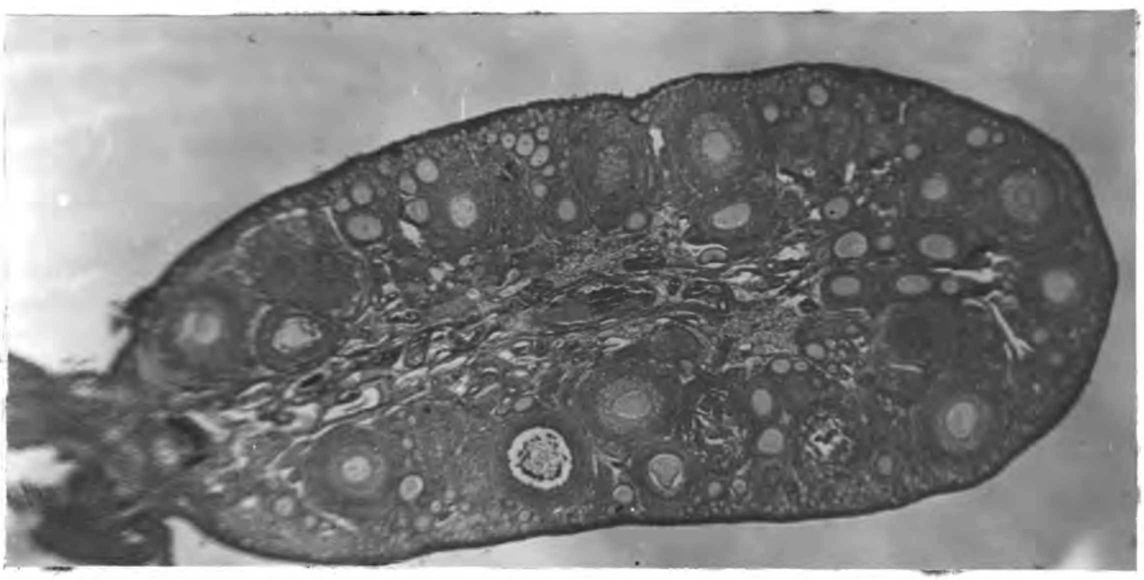

Fig.2. Microphotograph of the ovary from a specimen collected October 31. Note the numerous small cells just beneath the epithelium of the ovary.

PLATE II

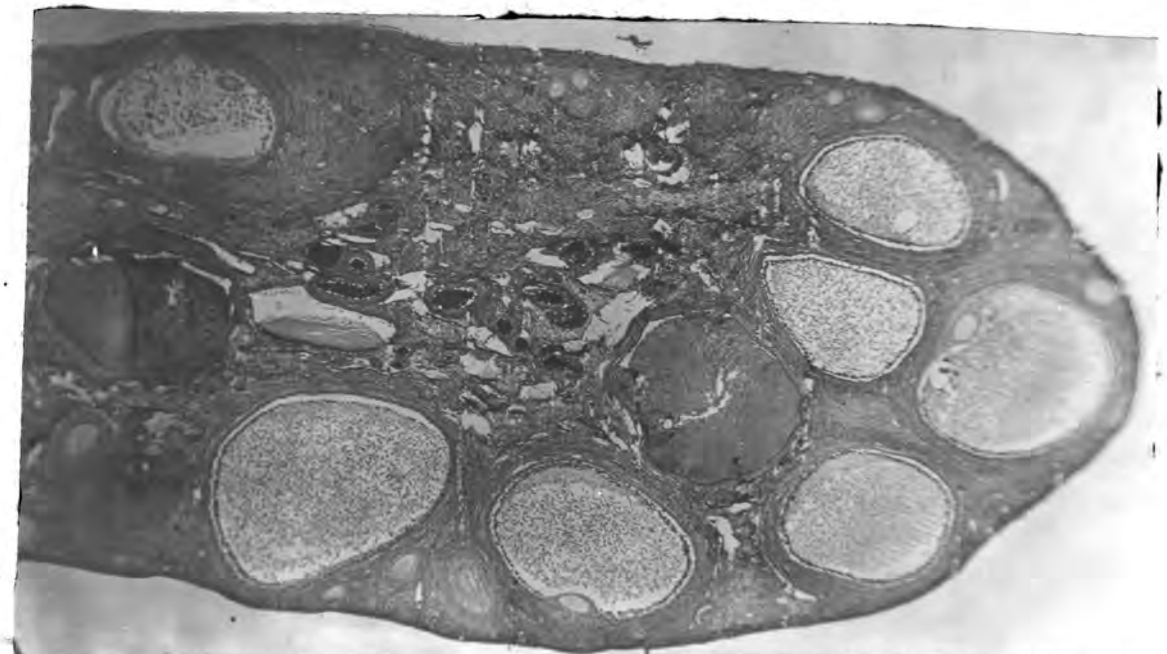

Fig.3. Microphotograph of the ovary from a specimen collected June 6. Several empty follicles are observed at the surface of the ovary and two corpora lutei are visible in the center of the section.

Fig. 4. Microphotograph of the ovary from a specimen collected July 15. Note the decrease in size of the ovary, the numerous empty follicles, and lack of ova.

PLATE III

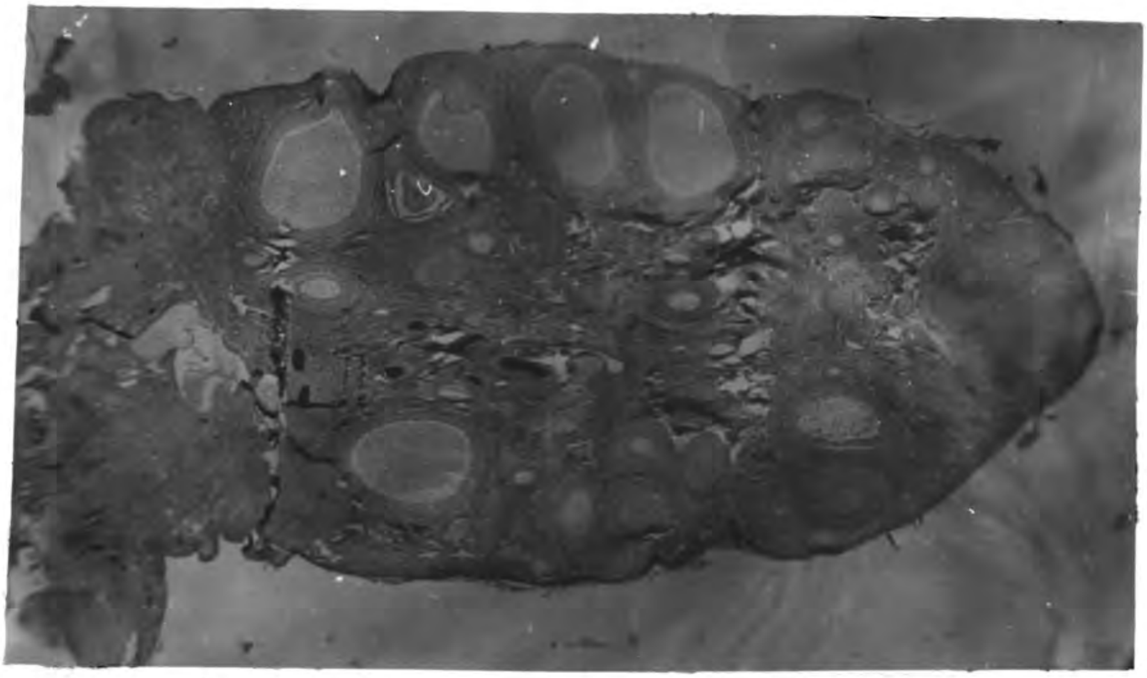

Fig. 5. Microphotograph of the ovary from the specimen collected August 3. Numerous empty follicles are visible and a few young follicles are observed.

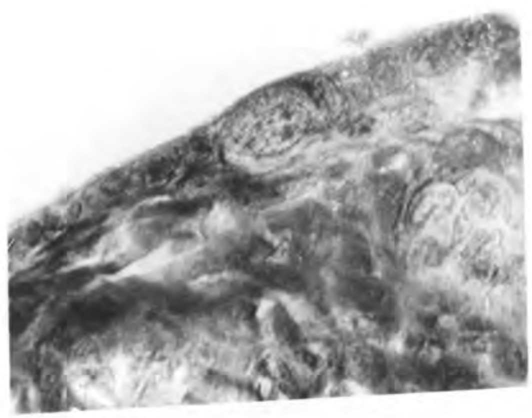

Fig. 6. Microphotograph showing an enlarged cell in the germinal epithelium. This was taken from the ovary of the specimen collected November 19.

PLATE IV

Fig. 7. A microphotograph taken under high magnification of a portion of the ovary from the specimen collected December 12. Several enlarged cells are visible just beneath the germinal epithelium.

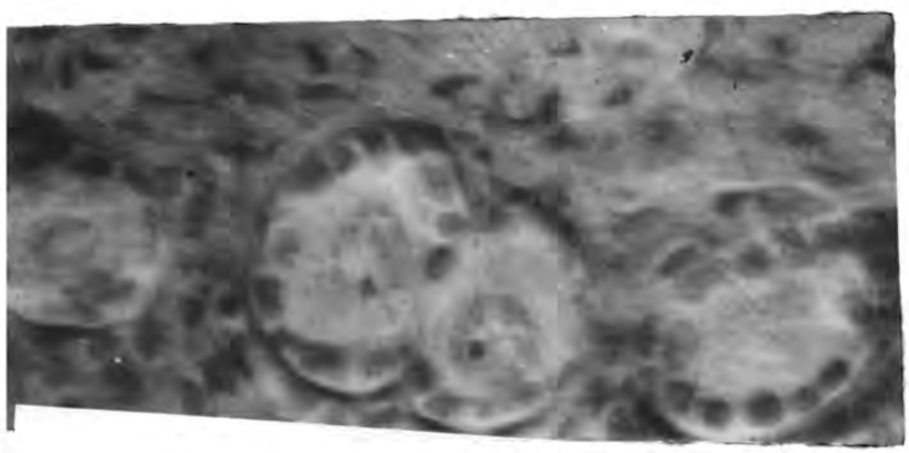

Fig. 8. A microphotograph which shows young follicles in the deeper part of the tunica albuginea. This was taken from the ovary of the specimen collected December 12.

PLATE V

Fig. 9. Camera lucida drawing showing a germinal epithelial cell in the telophase stage of mitosis. This was made from the ovary of the specimen collected December 12.

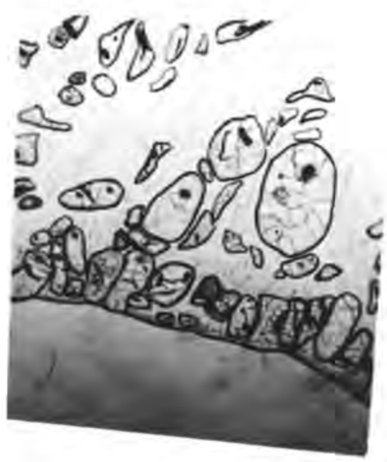

Fig. 10. Camera lucida drawing showing enlarged cells moving from the germinal epithelium into the deeper cortex of the ovary. This was made from the ovary of a specimen collected January 3.

PLATE VI

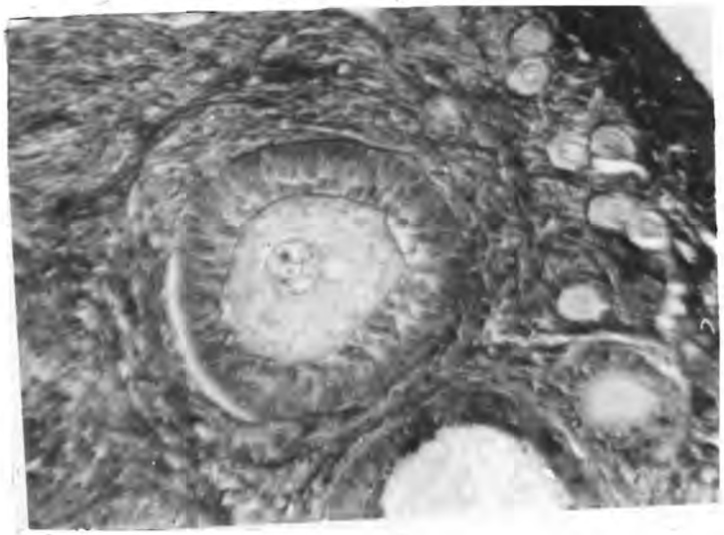

Fig. 11. Microphotograph showing a typical mature follicle. This was taken from the ovary of a specimen collected January 24.

Fig. 12. Camera lucida drawing which shows the increased activity of the germinal epithelium near the region of the hilus. This was made from the ovary of the specimen collected November 19.

BIBLIOGRAPHY

Allen, Edgar, "Ovogenesis During Sexual Maturity," Am. Jour. Anat., XXXI (1923), 439-482.

Allen, Edgar, and Atcheson, Bellfield, "The Effect of Pregnancy upon Postpubertal Ovogenesis," Anat. Rec., XXVII (1924), 178-196.

Allen, E., and Creadick, R. N. "Ovogenesis During Sexual Maturity," Anat. Rec., LX (1937), 191-195.

Arai, Hoyato, "On the Postnatal Development of the Ovary (albino rat) with Especial Reference to the Number of Ova," Am. Jour. Anat., XXVII (1920), 405-462.

Butcher, Earl O., "The Origin of the Definitive Ova in the White Rat. (Mus norvegicus albinus)." Anat. Rec., XXXVII (1927), 13-30.

Cowperthwaite, Marion Hazel, "Observations of Pre- and Postpubertal Oogenesis in the White Rat, Mus norvegicus albinus," Am. Jour. Anat., XXXVI (1925), 69-90.

Gutherie, Mary J. and Jeffers, Katharine R., "A Cytological Study of the Ovaries of the Bats Myotis lucifugus lucifugus and Myotis grisescens," Joun Morph. and Physiol., LXII (1938), 523-556.

Hargitt, George T., "The Formation of the Sex Glands and Germ Cells of Mammals. V. Germ Cells in the Ovaries of Adult, Pregnant, and Senile Albino Rats," Jour. Morph. and Physiol., L (1930), 453-469.

Hartman, C. G., "The Breeding Season of the Opessum," Jour. Morph. and Physiol., XL (1923), 142-215.

Heys, Florence, "The Problem of the Origin of Germ Cells," Quart. Rev. Biol., VI (1931), 1-45.

League, Bessie and Hartman, C. G., "Anovular Graafian Follicles in Ma mmalian Ovaries," Anat. Rec., XXX (1925), 1-14.

Papanicolaou, George N., "Ovogenesis During Sexual Maturity as Elucidated by Experimental Methods, "Proc. Soc. Exp. and Med., XXI L1924), 393-396.