Cytological and Histological Studies on Abnormal Follicles of Mature Mice

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(I) INTRODUCTION

During these thirty years, the remarkable growth of knowledge of the physiology of mammalian ova in several parts of the world has serried to a great extent the analysis of very complicated 'mechanisms of ovulation following advances of cytological methods, on the basis of fundamental physiological and morphological studies of ovulation.

The cyclic changes of the sex organ are more pronounced and complicated in

mammals than in other animals; they are known as an oestrous cycle, and occur at different intervals of days or weeks according to the species. It is only during a period of the oestrus that the mature ova are produced in the ovary, and the female becomes sexually receptive, so that fertilization can occur. The oestrous cycle has so far been most closely studied in rodents, particularly in the Muridae, since they breed in every season through the year in caged conditions. Mature female mouse ovulates repeatedly about once every about 5 days from the age of 60 days. The typical oestrous cycle begins with the active secretion of the gonadotrophic hormone from the anterior pituitary. This secretion commences when the animal has grown to maturity. It is thought that at this early stage it is the follicle stimulating hormones which is produced in great quantity. The follicles stimulating hormones of the anterior pituitary induce rapid growth of Graafian follicles, and a part of this growth is achieved by mitosis and another part by the secretion into the antrum of the follicular fluid and of oestrogen. At a final point, the rate of oestrogen secretion has a depressing effect on the rate of pituitary secretion and increases the rate of secretion of interstitial cell stimulating hormone. Thus, about the time of ovulation the supply of follicle stimulating hormone and of oestrogen is diminishing, and the interstitial cell stimulating hormone is then mainly responsible for the luteinization of cells of the ruptured Graafian follicles to form the corpus luteum. KNAUER (1896) seems to be the first who studied the mechanism of ovulation, and STOCARD & PAPANICOLAOU (1917) dealt with the maturation process in the Graafian follicles using the smear method with vaginal fluid in the guinea pig. ALLEN (1923) and HISAW (1947) put foreward a theory on the oestrous cycle in the Muridae. Also, MAKINO (1941) reported the cytological features of normal follicles in mature ovaries of mice (*Mus musculus* L.). On the other hand, ASAMI (1920), HARTMAN (1926), ENGLE (1927), TAKEWAKI (1937), LANE (1938), PLISKE (1940), MAKINO & SIGEMORO (1946) and SIGEMORO (1947) reported on atretic follicles which contained abnormal ova with polyovular follicles in mice and rats; they showed that a great number of ova were produced in mammalian ovaries during the oestrous, but the majority of them underwent degeneration, only a few attaining maturation, and developing into functional ova. To be regret, however, most of these investigators have dealt with merely morphological observations of the atretic ova, and failed to inquiry into their behavior and changes involving the maturation phases leading to their disintegration. Further detailed observations have been left for the analysis of mechanism by which atretic ova would be produced. Here, further investigation is invited to study effects of some hormonal substances upon the formation and fate of atretic ova.

The present author's former study has been dealt with cytological observations on abnormal mature ova which followed the maturation course in the normal sexual cycle of mature mice. The results indicated that the degeneration of abnormal ova was caused by the disintegration taking place in the egg cell, in accordance to the view emphasied by ENGLE (1927). On the other hand, there are another veiw that the disintegration occurs in graulosa cells reported by ASAMI (1920), BRANCA (1925),

EREUD & VEDDER (1938) and PLISKE (1949). This view is of suggestive that the disintegration may be due to the influence of sex stimulating hormones. Probably, a follicle stimulating hormone from the anterior pituitary would induce rapid growth of the Graafian follicle; the growth may be partly achieved by multiplication of follicle cells and partly by the hormonal secretion into the follicular fluid. It has been known that the follicular fluid contains a large amount of oestrogen; the latter may increase the mitotic activity of follicle cells. Since the pituitary induces the active secretion of the follicular fluid as well as oestrogen, it may be the oestrogen that practically causes the real growth of the follicle. Based on the results obtained the suggestion may be possible that the ovum which undergoes degeneration without being growing into the functional follicle would be produced under the influence of certain sex hormones. But, a question remains as to whether any kind of hormones affects, or controls, the growth of Oögonium, or primary and secondary follicles.

Further, the present author has studied cytologically with particular concern the influences of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormone from the placenta upon atretic mature ova in mice. Also, the influence of the pregnant mare serum of horses on the abnormal mature ova of mice has been investigated by the author in order to see the fate of abnormal ova.

Furthermore, the investgation has been extended by the author in order to learn the difference in number of normal, abnormal mature ova and polyovular follicles in mature ovaries of mice derived from different strains obtainable in different laboratories of Japan, since no such investigation has been carried out by any author.

Some histochemical studies of normal and abnormal mature ova in mouse ovaries have been a further subject of investigation undertaken by the author. There have been published recently several reports on histochemical studies of atretic ova in the mammalian ovary by HARTER (1948), VINCENT & DORNFELD (1948), DEANE (1952), ISHIDA (1953, 1954), and some others. The present study has been carried out in some detailed for the analysis of histochemical nature in normal and abnormal ova, use being made of two different mouse strains.

The data obtained in the present investigations was described being separated into four parts in this paper. Part I is devoted to the cytological studies on normal and abnormal mature ova, and polyovular follicles in mature ovaries of *Mus musculus* (Swiss albinos): they were observed at different phases of the oestrous cycle with the use of the vaginal smear method.

In Part II is described an cytological effect of sex stimulating hormones on large abnormal follicles and ova in *Mus musculus* (Swiss albinos). The study was subdivided into two parts. The first dealt with the effect of the mixture of anterior pituitary sex gland stimulating hormone and sex gland stimulating hormones from placenta upon the fate of abnormal follicles. The second was the effect of the mixed hormones as above and the pregnant mare serum of horses upon abnormal follicles. It was hoped in these studies to examine whether or not the administration of sex stimulating hormones derived from the anterior pituitary gland, placenta gland

and pregnant mare serum of horses would reduce the number of abnormal mature ova and polyovular follicles.

Part III describes the results of the cytological investigation of some comparative features of normal, abnormal mature ova and polyovular follicles in mature ovaries in the following four mouse strains; Swiss albinos, D-240 albinos, B-72 albinos and dd/Ma albinos.

Part IV gives accounts of the histochemical observations of normal and abnormal mature ovaries of mice studied at four stages of the oestrous cycle. The occurrence and localization of glycogen and RNA were the subjects mainly dealt with in ovaries of *Mus musculus* (Swiss albinos and D-240 albinos).

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(II) MATERIAL AND METHODS

Unmated mature female mice (*Mus musculus*) at 70 to 120 days of age furnished the material for the present study; they are Swiss-albinos derived from the Agriculture Department of Tokyo University and D-240 albinos, B-72 albinos and dd/Ma albinos derived from the Makino Laboratory, Zoological Institute, Hokkaido University. They were all purely bred in the author's laboratory.

At the start of experiments, vaginal smears were daily made from each mouse and inspected under the microscope. Individuals which showed a normal sexual cycle were used as material for study. The sexual cycle of mice generally shows a considerable variation. Especially, a few mice from S-strain and D-240 strain were observed to shown an abnormal sexual cycle. They showed an abnormally long dioestrus cycle. They were not used as material for the present study. In every specimen, weight was recorded every 5 days during the course of experiments.

The methods for study are summarized as follows:

Part I. The ovaries taken from 30 individuals of S-strain mice were used as material. They were removed and fixed with Bouin's solution. The sections were made according to the usual paraffin method, and stained with Heidenhain's iron-haematoxylin.

Part II. Twenty-five individuals of S-strain mice were received injections of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormones from placenta ("Synaphorin", Teikoku Hormone MFG., Co.) 2 R.U. every day for 3 successive days in a period of the oestrus stage. Starting on the 5 days before the oestrus stage, 25 individuals were injected with "Synaphorin" 2R.U. per day for 3 successive days and again with pregnant mare serum of horses, ("Serotropin", Teikoku Hormone MFG., Co.) 50 I.U. Twenty-four hours after injection, the ovaries were removed and fixed with Bouin's solution. The sections were made

according to the usual paraffin methods and stained with Heidenhain's iron-haematoxylin. Seventeen individuals were reserved as controls.

Part III. A comparative cytological investigation was made with ovaries derived from 30 individuals of Swiss albinos, from 30 individuals of D-240 strain mice, from 20 individuals of dd/Ma strain mice, and from 20 individuals of B-72 strain mice. They were fixed with Bouin's solution, and treated with the method as described above.

Part IV. For histochemical investigations, the ovaries taken from 8 individuals of Swiss albinos and 2 individuals of D-240 strain mice provided the material. Fixing fluids for the demonstration of glycogen and RNA were 95 % alcohol, Zenker's fluid and 10 % formalin. The sections were made according to the usual paraffin method, and stained with Schiff's periodic acid modified by Lilie for the observation of glycogen. For RNA, thionin staining method was adopted.

The work was carried out during a period ranging from early spring to late autum every year, since the animals breed rather well in those seasons.

(III) RESULTS OF OBSERVATIONS

Part 1. On abnormal follicles in mature ovaries of mice at different phases of the oestrous cycle

(1) Normal mature follicles of mice

The first maturation division of the oocyte is completed prior to ovulation in the mouse ovary as in other mammals. The normal maturation division of the oocyte was observed only in the Graafian follicle which follows advancing regularly the course of the maturation. Functional mature ovum showing a normal sexual cycle was found in the Graafian follicle before its rupture. Such a mature follicle was observed occupying a position at the periphery of the ovary. The ovum lying in the Graafian follicle is surrounded by a gelatine membrane, called zona pellucida, and the latter again by corona radiata. Surrounding the corona radiata there are granulosa cells which enclose a huge cavity filled up with the fluid, liquor folliculi. In the mature follicle being 217 to 290μ in diameter, the mature ovum has a diameter of 51 to 82μ under a fixed condition. MAKINO (1941) reported that the egg of mouse (*Mus musculus* L.) has a diameter ranging from 65 to 70μ. SOBOTTA and BURCKHARD (1910) reported that the diameter of rat egg are 60 to 65μ. YAMANE (1937) are measured 110 to 120μ in diameter of the rabbit egg. The size of the mouse egg nearly approximate that of the rat. The observations indicated that the egg diameter seems to differ by different strains of mice under study: dd/Ma mice has a diameter ranging 75 to 80μ, the follicle being 286 to 350μ, the B-72 mice 78 to 80μ, the follicle being 286 to 340μ, D-240 mice 73 to 80μ, the follicle being 243 to 357μ. It is evident from the above results that there is no striking difference in egg-diameter by difference of mouse strain. Following the penetration of the spermatozoon the ovum generally reduces in diameter.

After the disappearance of the nuclear membrane, the chromosome arrange

on the equatorial plate to form the first spindle in the ovum. After the division of chromosomes, the outer pole of the spindle which upheaves on the egg surface gets extruded as the first polar body. The ovum thus undergoes the first maturation division prior to ovulation and still further advances in its maturation course up to the metaphase stage of the second division. The maturation process in the ovum stops at this stage, untill insemination occurs following ovulation. The detailed descriptions involving the maturation phenomena in the normal mature ovum in mice were given in the paper of MAKINO (1941). It has been generally accepted that ovulation occurs prior to the completion of the second maturation division, and that during ovulation the process leading to the second division is arrested, lying quiescent, as it were in the metaphase condition. Insemination is therefore a necessary anticipation to the second maturation division. It is therefore evident that the results of this study is of considerable significance in giving a cytological proof to the results experimentally attained by YAMANE (1930, 1935a, b, 1937) in the rabbit egg *in vitro*. He reported that spermatozoa showed a twofold action consisting in dispersing sticky follicular cell-mass surrounding the ovum and activating the second maturation division in artificial insemination *in vitro* with rabbit ova. The formation of the second polar body following the penetration of spermatozoon into the ovum was deduced to be a proteolytic action of enzyme presumably of the trypsin group. In an ovary, the author observed an ovum which shows the first and second polar bodies in the unmated mouse. This may be an example showing abnormal maturation divisions in an atretic follicle (Fig. 42).

The author has chanced to observe some Graafian follicles of a normal appearance which show a great antrum.

The changes of the nucleus of the ovum advancing towards the first maturation division seems to proceed successively during a short duration. After the disappearance of the nuclear membrane has successively taken place, the chromsomes make their arrangement on the equatorial plate and thus the first polar spindle is formed (Figs. 1-14). After the separation of chromsomes the outer pole of the spindle which upheaves on the egg surface, gets extruded as the first polar body (Figs. 15-16). The ovum enters into the second maturation division carrying the first polar body, and lies practically free from the granulosa cells as well from the corona radiata cells, being suspended in a naked state in the liquor folliculi (Figs. 17-18). It advances in its maturation course up to the metaphase stage of the second division. The maturation course in the ovum stops at this stage, until insemination occurs following ovulation.

For the sake of convenience, the stages of the normal follicle are divided into three general types as follows:

(1) Primary follicles; any follicle having one or more cell layers with no antrum or a few antrum.

(2) Secondary follicles; any follicle with a prominent antrum and the ovum starting in the maturation division.

(3) Corpora lutea; a crude categorgy including any follicular structure after rup-

ture.

Primary follicles refer to small primary follicles with six to eight rows of granulosa cells, but in which the antrum folliculi has not yet been fully formed. This type are abundant in ovaries of young mice (Fig. 67).

Secondary follicles show all follicles which have an antrum in each (Fig. 11). In the present study, the secondary follicles advancing towards the maturation course were observed in 157 examples from 30 individuals under study. They showed diameter of 200 to 350μ. One hundred-nine follicles from 28 ovaries of 14 individuals were at the phase of proestrus; 20 follicles from 10 ovaries of 5 individuals were at the stage of oestrus; 28 follicles from 14 ovaries of 7 individuals showed the dioestrus stage (Table 1).

The number of normal mature ova in average at four stages of the oestrous cycles was 7.8 at the proestrus, 4.0 at the oestrus, 4.0 at the metoestrus and 0 at the dioestrus. The ovulation seems to be taken place during a period from about 12 to 20 hours in the oestrous stage. The proestrus stage is just before the rupture of ova. The number of normal mature ova at this stage is 7.8 on an average with a variation range from 1 to 9 (Table 10). SATO (1959) dealing with some observations on natural ovulation in mature ova, reported the average number of mature ova as 8.2 in D-240 strain *in vitro*.

(2) Abnormal mature follicles

The atretic follicles are generally divided into two types. Type 1 refers to the small primary follicle with several rows of granulosa cells: some of them shows the antrum folliculi consisting of several rows of granulosa cells, while some others contain no antrum folliculi. Type 2 deals with all follicles which advance to maturation phase; they mostly have no antrum, though a few show an antrum. The follicles belonging to type 1 are found in the ovum before the maturation course, whereas type 2 in the ovum undergoing the maturation division. The object of this work is to study the morphology of the ovary of type 2.

The number of ovary of type 2 as observed in 60 ovaries was 85, while normal mature follicles were 157 in number, as given in Table 1. In the phase of the oestrous cycle, fifty-eight follicles from 28 ovaries of 14 individuals were at the proestrus stage; 15 follicles from 10 ovaries were the oestrus stage; 8 follicles from 14 ovaries were the metoestrus stage; 4 follicles from 8 ovaries were the dioestrus stage.

The number in average of abnormal ova in four stages of the oestrous cycle were 4.1 at the proestrus, 3.0 at the oestrus, 1.1 at the metoestrus, and 1.0 at the dioestrus. Total number of abnormal ova was 2.8 in an average, while normal mature ova were 5.2 (Table 10). It was observed based on the vaginal smear method that the atretic follicles at the proestrus stage are very frequent through the oestrous cycle. The data presented here suggest a numerical relation between the abnormal and normal mature ova in an ovary.

Some of the abnormal mature ova showed the chromosomes which arrange on the equatorial plate with the first polar spindle, having no nuclear membrane and

antrum (Figs. 19-36). Some others showed the second polar spindle at metaphase accompanied by the first polar body in the follicle with a few antra (Figs. 37-41): this type of abnormality is rather common in mature ovaries. Some others showed the completion of the second division offering the second polar body, though the antrum folliculi has not been formed (Fig. 42). There were also found abnormalties of chromosomes (Figs. 43-44), and the first division showing a tetra-polar spindle (Figs. 60-61). The irregularity in shape of the ovum was also common; in the extreme case, the ovum contained many vacuoles in the ooplasm (Figs. 58-59).

ENGLE (1927) in the mouse and SIGEMORO (1947) in the rat have emphasized that in the course of degeneration the disintegration takes place in the egg body. On the other hand, ASAMI (1920), BRANKA (1925), EREUD & VEDDER (1938) and PLISKE (1940), all in rats, have pointed out that the disintegration occurs in the granulosa cells. Based on the data so far obtained, it seems probable that in the course of degeneration the disintegration takes place in both in the egg body and in the granulosa cells and as a result it exerts some influence on the hormone secretion in the mature ovary. Especially, the hormone preparations admininstered probably affect only the follicles of larger size as shown type 2.

From the results of observation with ovaries of 30 specimens of mice, it is evident that those showing abnormality in egg body were found in 73 examples, while those showing abnormality in granulosa cells were observed in 12 examples in total. In the eggs at the proestrus stage from 14 individuals, the ratio of the egg body abnormality to granulosa cell abnormality was 49 : 9, in those at the oestrus stage, from 5 individual the ratio was 11 : 4, in those at the metoestrus stage from 7 individuals the ratio was 7 : 1, and in the eggs from the dioestrus stage from 4 individuals the ratio was 4 : 0 (Table 2).

A suggestion is made that the disintegration of granulosa cells and egg body is due to the influence of the sex stimulating hormone. This may be proved by the investigation on the effects of the sex stimulating hormones on the abnormal mature ova on the sexual cycle of mice, the details being described in the latter section.

The atretic follicles in the mammalian ovary have been studied from the morphological view point by many investigators (KINGERY 1914, LOEB 1911, ASAMI 1920, ALLEN 1923, BRANCA 1925, ENGLE 1927, ALLEN, KOUNTZ & FRANCIES 1925, EREUD & VEDDER 1938, PLISKE 1940, SIGEMORO 1947, PINCUS & ENZMANN 1947, HISAW 1948, ISHIDA 1953, 54). ALLEN, KOUNTZ & FRANCIES (1925) reported the cyclic destruction of ovarian ova in the oestrous cycle. ASAMI (1920) ENGLE (1927), are a quantitative study of follicular atresia in the mouse, Engle (1927) stated that all of the atretic follicles are divided into two general types. Type I refers to small primary follicle, with from two to six or eight rows of cells in the granulosa, but in which the antrum follicles has not yet formed. Type II refers to all follicles which have an antrum, these usually ranging between 250μ and 750μ, although those with one layer are occasionally found. The results of observation on the atretic follicles at four stages of the oestrous cycle showed that there was a cyclic variation, both in number of pseudomaturation spindles and in the total number

of atretic follicles. The destruction is at its highest peak during the first day of the dioestrus and in its lowest on the second day. The curves showing the number of atretic follicles do not indicate the same type of variation at four stages of early pregnancy. This seems to suggest a rather low ovarian activity: it is comparable to the condition at pseudopregnancy. PINCUS & ENZMANN (1947) studied atresia of ovarian eggs in the rabbit. They reported that four definite types of atretic follices were found and the percentage of atresia among the young oocyte is low (10%). In the follicles of larger size about 60 percent was atretic ovary and the hormone administration affected only the largest follicles and their ova. SIGEMORO (1947) studied abnormal follicles in rats, and reported that the widely encountered features of atresia were observed in the sign occuring in the egg nucleus. Recently, ISHIDA (1953, 54) made some morphological observations of atretic ova in adult rats and some other domestic animals and reported that the primary follicles seemed to undergo destruction, showing their ova which were shrinking. The first sign of degeneration of ova in the secondary and Graafian follicles was the appearance of a pseudomaturation spindle.

(3) Polyovular follicles of mice

Extensive literature regarding polyovular follicles was presented in the reviews by HARTMAN (1926), ENGLE (1927), DEDERER (1934), TAKEWAKI (1937), LANE (1938), MAKINO & SIGEMORO (1946) and SIGEMORO (1947) in the mature and immature mammals. HARTMAN (1926) seems to be the first who studied the mechanism of polyovular follicles in the majority of mammals, including the maruspials and man. He expressed the view in connection with the polyovular follicle and twining. ENGLE (1927) reported 18 polyovular follicles based on one hundred ovaries in the mouse, and TAKEWAKI (1937) recorded a five-ova-containing follicle in the mouse. But they did not give any detailed morphology of polyovular follicles. DEDERER (1934), LANE (1938), and SIGEMORO (1947) reported the polyovular follicle in immature and mature rats. SIGEMORO (1947) made a detailed study with the Norway rat and reported that the total number of polyovular follicles in 120 mature rat ovaries from 60 individuals was found to be 375; among of them 316 follicles (84%) were biovular, triovular follicles were 47 (13%); 9 follicles (2%) contained four ova and 3 follicles (1%) included five ova. He described that the polyovular follicles were not less common in the rat than in other mammals.

In the present study working with mice, 70 to 120 days of age (mature mice), it has been revealed that the polyovular follicles is rather rare in occurrence; 80 polyovular follicles were found to occur in 60 ovaries under study. Of them, 62 follicles (78%) were biovular; 13 follicles (16%) were triovular; 4 follicles (5%) were tetraovular, 1 follicle (1%) included five ova (Table 2). When observed regarding the oestrous cycle, the ovaries from fourteen individuals contained 44 polyovular follicles at the proestrus stage, those from 5 individuals contained 9 at the oestrus stage, those from 7 individuals showed 18 at the metoestrus stage and those from 4 individuals included 9 at the dioestrus stage. The average number of the polyovular follicles observed in twenty-five individuals of S-strain mice was found

Table 1. Number of normal, abnormal mature follicles and polyovular follicles in normal mature ovaries of mice.

Material	Oestrous cycle	Number of individual	Normal mature follicle			Abnormal mature follicle			Polyovular follicle		
			Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left		Right	Left		Right	Left	
Thirty mature mice (*Mus musculus*, Siwss albinos)	Proestrus	14	50	59	109	25	33	58	22	22	44
	Oestrus	5	10	10	20	7	8	15	4	5	9
	Metoestrus	7	15	13	28	4	4	8	13	5	18
	Dioestrus	4	0	0	0	3	1	4	4	5	9
	Total	30	75	82	157	39	46	85	43	37	80

Table 2. Type of abnormal mature follicles and polyovular follicles in normal mature ovaries of mice.

Material	Oestrous cycle	Number of individual	Type of abnormal follicle			Type of polyovular follicle				
			Abnormality in granulosa cells	Abnormality in egg body	Total	Biovular	Triovular	Teraovular	Fiveovular	Total
Thirty mature mice (*Mus musculus*, Swiss albinos)	Proestrus	14	49	9	58	33	7	3	1	44
	Oestrus	5	11	4	15	6	3	0	0	9
	Metoestrus	7	7	1	8	15	2	1	0	18
	Dioestrus	4	4	0	4	8	1	0	0	9
	Total	30	71	14	85	62	13	4	1	80

to be 2.7, with a range from 1 to 6.

The majority of the polyovular follicles was small in size with no antrum. It seems probable that the polyovular follicles are in the process of degeneration without establishing the antrum folliculi (Figs. 53-56). Only three biovular follicles were found in the stage of antrum formation. One of them showed an ovum advancing to the maturation division, while the other remained without the first maturation division (Figs. 63-65). There were also two other examples in which the first maturation spindles was formed in the ovum (Fig. 62). The evidence presented seems to suggest that polyovular follicles originate from fusion of the previously separated follicles, or arise through abnormal nuclear division, though there are a few polyovular follicles which resulted from the separation of an original ovum. They

seem to be the process of disintegration without advancing to the ovulation course.

Concerning the origin of polyovular follicles, HARTMAN (1926) enumerated three possible ways of formation; 1) by division of polyovular ova, 2) by concrescence of previously separated follicle, and 3) by persistent union of ova in the egg tube.

ISHIDA (1953) reported that the atretic ova of rat ovaries began to atrophy through three types of degeneration process. One type deals with that the ovum undergoes symmetrical cell division and then their cytoplasm degenerates showing irregular masses. He suggested that polyovular ova were in the process of degeneration without establishing the antrum folliculi as shown in Fig. 53. Recently, KENT (1959) informed that the occurrence of polyovular follicles and polynuclear ova were more common in immature animals than in mature ones, and he assumed that the feature may be due to alleviation of a deficient estrogen condition in young animals. The relation between the formation of polyovular follicles and the hormonal function in the ovary will be described in the next chapter.

According to the views of the present author, the polyovular follicles may be produced through the following three types of abnormal processes: 1) polyovular follicles may be formed by concresence of previously separated follicles, 2) polyovular ova may be produced by abnormal nuclear division, and 3) polyovular follicles may be arised by separation or fragmentation of the original ovum. Type 1 and 2 seem to be common in occurrence, though there are a few examples which are suggestive of the possibly of their normal ovulation course. But all ova of type 3 are doomed to atrophy in the ovary.

(4) Polyovular follicles in immature mice

The review of the extensive literature regarding polyovular follicles and polynuclear ova in the immature mammals was provided by LANE (1938), DAWSON (1951) and KENT (1959). The polyovular follicles were more common in occurrence in immature animals than in the mature individuals. LANE (1938) studied on the abnormal follicles in one hundred ovaries from sixty rats which varied in age from 15 to 64 days and reported that five follicles (0.018%) contained binuclear ova; thirteen follicles (0.045%) were biovular and three follicles (0.011%) were triovular. Only two of those atypical follicles had progressed in development to the stage of formation of the antrum. KENT (1959) investigated the polyovular follicles in young hamsters, 41 days of age, by treating them with estradiol monobenzoate in order to reduce the incidence of polyovular follicles and polynuclear ova. According to his results, the incidence of polyovular follicles showed a reduction of 18 percent.

The present study with immature mice, 10-20 days of age (7.0-9.0 gram in body weight), has made it clear that the polyovular follicles and polynuclear follicles are rather rare in occurrence. In 20 ovaries from a series of ten normal mice, 13 polyovular follicles and 1 nuclear ova were found in total. Of them, 10 follicles were biovular and 3 follicles were triovular (Table 3). All of them were in the process of atretic degeneration without establishment of the antrum folliculi. The

Table 3. Number of polyovular follicles from twenty ovaries of immature mice.

Mouse Number	Body weight in gram	Right ovary				Left ovary			
		Biovular	Bi-nuclear	Tri-ovular	Tetra-ovular	Bi-ovular	Bi-nuclear	Tri-ovular	Tetra-ovular
No. 1	8.5	0	0	0	0	0	0	0	0
No. 2	8.0	0	0	0	0	2	0	0	0
No. 3	8.5	1	0	0	0	0	0	0	0
No. 4	9.0	1	0	0	0	0	0	0	2
No. 5	7.0	1	0	0	0	0	0	0	0
No. 6	8.0	0	1	0	0	0	0	0	0
No. 7	8.5	0	0	0	0	1	0	0	1
No. 8	8.0	0	0	0	0	0	0	0	0
No. 9	7.0	1	0	0	0	1	0	0	0
No. 10	9.0	1	0	0	0	1	0	0	0
Total		5	1	0	0	5	0	0	3
		6				8			
Average Number		14							

number of polyovular follicles in the immature mice was 1.5 in an average. On the other hand, mature mice, 70 to 120 days of age, weighting 25 to 28.5 gram, showed a value 2.7, with a range from 1 to 6. Generally, the polyovular follicles were found to be rather rare in occurrence in both mature and immature mice.

Part II. Effects of sex stimulating hormones on abnormal follicles of mice

In the previous study, the author has made some cytological studies on abnormal mature follicles contained polyovular follicles in mouse ovaries during different phases of the oestrous cycle. Based on the results of observations, the suggestion may be possible that the disintegration may takes place in both the egg body and the granulosa cells under the influence of sex stimulating hormones on the follicular apparatus. Taking the above view in mind, the effects of sex stimulating hormones derived from the anterior pituitary gland, placenta gland and pregnant mare serum of horses, are to be examined in abnormal mature ova during the sexual cycle of mice in the present study.

LANE (1935 and 38) reported the influence of pituitary gonadotrophic hormones on the follicular apparatus of female rats which varied in age from 15 to 64 days. In his study of ovaries from animals treated with various hormone preparations (Aminiotin, F.S.H., L.H.), the conclusion was made that the influence of hormones on the atypical follicles materially altered under these experimental conditions. PINCUS and ENZMANN (1947) stated that the percentage of atretic ova in the rabbit young oocytes is low (10%). They indicated that among follicles of larger size about 60 percent were atretic at any one time, and that the hormone administration affected the largest follicles and their ova. PAYNE and HELLBAUM (1955) reported the possibility of a normal follicular maturation in the absence of the pituitary.

KENT (1959), in the young female golden-hamster, has pointed out that the reduction in number of polyovular follicles and polynuclear ova were obtained by treating with estradiol monobenzoate. The above feature seems to be due to allevation of a deficient estrogen condition in the young animal. However, no particular attention has been paid by any author on the influence of hormones on the abnormal follicles in mature animals.

There are many papers which deal with polyovulation under the effect of anterior pituitary hormones or pregnant mare serum or oestrogen in certain vertebrates (PINCUS 1943, ROBINSON 1949, GATES 1956, GREEN 1956, HUNTER, ADAM & ROBINSON 1955, RAESIDA & LAMOND 1956, OKIGAKI 1958, SATO 1959 and some others). The course of normal polyovulation under the influence of sex stimulating hormones has not yet been solved.

The following work was undertaken to dertermine whether or not the administration of anterior pituitary sex gland stimulating hormones, and the sex gland stimulating hormones of placenta and pregnant mare serum of horses would reduce the number of abnormal mature ova and polyovular ova in mature female mice.

(1) Effects of mixture of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormones of placenta.

Twenty-five mice received the injection of mixed hormones "Synaphorin"[1] (Teikoku Hormone MFG. Co.) had ovaries which were heavily weighted than normal ovaries. It was reported that "Synaphorin" contains follicle stimulating as well as luteinizing hormones, and stimulates the growth of primary and secondary sex glands of both sexes, or engage in the up-keep of their functions.

Microscopical observations have revealed that the abnormal mature ova proceeding beyond the maturation stage are smaller in number in hormone treated mice than in the untreated mice. The atretic follicles of larger size which advance towards the maturation course were very abundant in number in the untreated mature ovaries as shown in Part I. Thirty untreated individuals were found to have in total 157 normal mature ova, 85 abnormal mature ova, and 80 polyovular ova at four stages of the oestrous cycle. Fourteen individuals observed at the proestrus stage were found to possess 109 normal, 58 abnormal, and 44 polyovular ova. Five individuals at the oestrus stage were found to have 20 normal, 15 abnormal, and 9 polyovular ova. Seven individuals at the metoestrus stage showed 28 normal, 8 abnormal, and 18 polyovular ova. Four individuals at the dioestrus stage were observed to have no normal, 4 abnormal, and 9 polyovular ova.

On the other hand, twenty-five mice which received the injection of "Synaphorin" showed in their ovaries 202 normal mature ova, 45 abnormal mature ova, and 48 polyovular ova in total. Seventeen individuals at the proestrus stage were observed to have 156 normal, 30 abnormal, and 29 polyovular ova. Two individuals at the oestrus stage possessed 19 normal, 2 abnormal, and 5 polyovular ova. Four individuals at the metoestrus stage were found to contain 20 normal, 10 ab-

1) "Synaphrin" is a mixture of two kinds of hormones: a sex gland stimulating hormone taken from the anterior pituitary gland and a sex stiumulating hormone taken from human placenta.

normal, and 5 polyovular ova. Three individuals at the dioestrus stage showed 7 normal, 3 abnormal, and 9 polyovular ova (Table 4).

The control data from 17 untreated mouse ovaries, showed 144 normal, 83 abnormal, and 37 polyovular ova in total. Five individuals at the proestrus stage were formed to have 56 normal, 26 abnormal, and 16 polyovular ova. Five individuals at the oestrus stage were observed to have 30 normal, 19 abnormal, and 8 polyovular ova. Two individuals at the metoestrus stage were found to show 9 normal, 8 abnormal, and 2 polyovular ova. Five individuals at the late dioestrus stage were observed to present 49 normal, 30 abnormal, and 11 polyovular ova at the oestrus stages (Table 8).

Abnormal mature ova showed the chromosomes laying on the equatorial plate of the first spindle, having no nuclear membrane and antrum. Some ova showed the second polar spindle at metaphase accompanied by the first polar body in the follicle with a little antra. Some others showed the completion of the second division offering the second polar body, though no antrum follicle has been formed. There occur some chromosome abnormalities and the abberant first division showing a terapolar spindle. In the extreme case, the ovum contained many vacuoles in its ooplasm. The abnormal mature ova were abundant in number in untreated ovaries. The evidence here presented indicated that the abnormal ova decreased in number in treated ovaries in comparison with those in untreated ovaries, probably under the influence of sex stimulating hormones on mature ova.

In the atretic ova which showed advance towards the maturation division, the disintegration took place either in the granulosa cells or in the egg body itself in the process of degeneration. Thirty untreated mice which showed the former type of abnormal ova were 71 in number and those of the latter type were 14 in total as reported in the Part I (Table 2).

Twenty-five mice treated with the anterior pituitary hormone (A.P.H.) showed 35 abnormal ova of the former type, 10 such ova of the latter type in total (Table 5). Seventeen control mice showed 71 abnormal ova of the former type, and 12 of the latter type (Table 8).

From the results of the above experiments, it is evident that the decrease in number of abnormal ova showing immature condition of granulosa cells is remarkable, and further that in the A.P.H. treated mice the reduction of polyovular ova was not striking. The results of the present study with mice, 70 to 120 days of age has shown that polyovular ova were rather rare in occurrence in a normal condition. Out of them, 32 follicles (67%) contained biovular, 15 follicles (31%) were triovular, and 1 follicle (2%) was tetraovular. Sixteen individuals observed at the proestrus stage showed 20 biovular, 8 triovular and 1 tetraovular, 2 individuals at the oestrus stage were found to show 4 biovular and 1 triovular, 4 individuals at the metoestrus stage showed 3 biovular and 2 triovular, and 3 individuals at the late dioestrus stage were observed to possess 5 biovular and 4 triovular (Table 5). The majority of polyovular follicles (92%) were in the process of degeneration, though there were four examples of biovular follicles (8%) which are suggestive of

Table 4. Number of normal, abnormal mature follicles and polyovular follicles in A.P.H. treated mouse ovaries.

Material	Oestrous cycle	Number of individual	Normal follicle			Abnormal follicle			Polyovular follicle		
			Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left		Right	Left		Right	Left	
Twenty-five mature mice (*Mus musculus*, "Swiss albinos")	Proestrus	16	89	67	156	11	19	30	18	11	29
	Oestrus	2	9	10	19	1	1	2	3	2	5
	Metoestrus	4	9	11	20	8	2	10	3	2	5
	Late Dioestrus	3	3	4	7	2	1	3	4	5	9
	Total	25	110	92	202	22	23	45	28	20	48

Table 5. Type of abnormal mature follicles and polyovular follicles in A.P.H. treated mouse ovaries.

Material	Oestrous cycle	Number of individual	Type of abnormal follicle			Type of polyovular follicle				
			Abnormality in granulosa cells	Abnormality in egg body	Total	Biovular	Triovular	Tetraovular	Fiveovular	Total
Twenty-five mature mice (*Mus musculus*, "Swiss albinos")	Proestrus	16	24	6	30	20	8	1	0	29
	Oestrus	2	1	1	2	4	1	0	0	5
	Metoestrus	4	7	3	10	3	2	0	0	5
	Late Dioestrus	3	3	0	3	5	4	0	0	9
	Total	25	35	10	45	32	15	1	0	48

the possibility of their maturation.

Seventeen control mice in this experiment showed 28 follicles (76%) which contained biovular, 4 follicles (11%) which showed triovular, 3 follicles (8%) which were tetraovular, and 2 follicles (5%) which contained five-ovular ova (Table 9).

The data obtained from this observation, have shown that the polyovular ova were small in average number in A.P.H. treated mice than in the untreated mice. It was rather difficult to determine whether or not the administration of anterior pituitary hormone and the sex gland stimulating hormones of placenta would reduce the number of polyovular follicles (Text-figs. 1-2).

(2) Effects of the mixture of the anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormone from placenta and pregnant mare

serum of horses.

The following experiments were undertaken in order to determine whether or not the administration of pregnant mare serum of horses (P.M.S.) would reduce the number of abnormal mature ova and polyovular follicles in mouse ovaries than in A.P.H. treated ovaries.

It was found that the ovaries of 25 P.M.S. treated mice were more heavily weighted than normal untreated ovaries. The hormone used here as P.M.S. was "Synaphorin" (Teikoku Hormone MFG., Co.). The results of the application of Synaphorin indicated that abnormal ova proceeding further beyond the maturation division were very small in number in P.M.S. treated mice than in untreated mice. Twenty-five treated mice showed in their ovaries 308 normal mature ova, 46 abnormal mature ova, and 32 polyovular ova in total. Nine individuals examined at the proestrus stage showed 162 normal, 22 abnormal, and 16 polyovular ova. Seven individuals at the oestrus stage contained 93 normal, 11 abnormal, and 8 polyovular ova. Four individuals at the metoestrus stage were found to have 25 normal, 10 abnormal, and 3 polyovular ova. Five individuals at the late dioestrus and proestrus stage were observed to show 28 normal, 3 abnormal, and 5 polyovular ova (Table 6).

Seventeen mice which reserved as control were observed to possess 144 normal, 83 abnormal, and 37 polyovular ova in total. The data here obtained indicated a remarkable reduction in number of abnormal ova in P.M.S. treated mice than in A.P.H. treated mice (Text-figs. 1-2). Particulary, the decrease in number of abnormal mature ova showing an immature condition of granulosa cells is remarkable.

Abnormal mature ova observed in 25 P.M.S. treated mice showed a disintegration taking place either in the granulosa cell or in the egg body itself. The abnormality of the former type were found in 39 examples, that of the latter type were in 7 examples in total. Seventeen control mice showed 71 abnormal ova of the former type and 12 ova of the latter type (Table 7).

In the polyovular ova observed here, 25 follicles (78%) contained biovular ova, 6 follicles (19%) contained triovular ones, and 1 follicle (3%) showed five ova. The majority of ova (91%) were in the process of degeneration, though there were 3 examples of biovular follicles (9%) which were suggestive of the possibility of their maturation.

Based on the results of the present study, it is evident that the polyovular follicles slightly decreased in number in P.M.S. treated mice as compared with untreated mice. It is difficult to state from the above data that the hormones affected the reduction of polyovular follicles.

On the basis of the results of the present observations, the conclusion is possible that the reduction in number of abnormal mature ova may be explicable by the effect of sex stimulating hormones. Also the evidence presented in the two experiments mentioned above has indicated that an abnormal mature ova showed a remarkable decrease in number in P.M.S. treated mice in comparison with A.P.H.

Table 6. Number of normal, abnormal mature follicles and polyovular follicles in P.M.S. treated mouse ovaries.

Material	Oestrous cycle	Number of individual	Normal follicle			Abnormal follicle			Polyovular follicle		
			Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left		Right	Left		Right	Left	
Twenty-five mature mice (*Mus musculus*, "Swiss albinos")	Proestrus	9	88	74	162	11	11	22	8	8	16
	Oestrus	7	45	48	93	6	5	11	5	3	8
	Metoestrus	4	12	13	25	6	4	10	2	1	3
	Late Dioestrus	5	12	16	28	1	2	3	1	4	5
	Total	25	157	151	308	24	22	46	16	16	32

Table 7. Type of abnormal mature follicles and polyovular follicles in P.M.S. treated mouse ovaries.

Material	Oestrous cycle	Number of individual	Type of abnormal follicle			Type of polyovular follicle				
			Abnormality in granulosa cells	Abnormality in egg body	Total	Biovular	Triovular	Tetraovular	Fiveovular	Total
Twenty-five mature mice (*Mus musculus*, "Swiss albinos")	Proestrus	16	19	3	22	10	5	0	1	16
	Oestrus	2	9	2	11	7	1	0	0	8
	Metoestrus	4	9	1	10	3	0	0	0	3
	Late Dioestrus	3	2	1	3	5	0	0	0	5
	Total	25	39	7	46	25	6	0	1	32

treated mice (Text-figs. 1-2).

Recently many papers have appeared regarding polyovulation under the effect of anterior pituitary hormone or oestrogen or pregnant mare serum in certain vertebrates. RUNNER & PALM (1953) observed artificial polyovulation in immature mice which received injections of cholionic gonadotropin and pregnant mare serum gonadotropin. Also, PINCUS (1940), GATES (1956), GREEN (1956) observed polyovulation in mature mice which received injections of the anterior pituitary gonadotrophic hormone. SATO (1958) and OKIGAKI (1959) published the results of preliminary observations on hormone induced polyovulation and superfecundity in mature mice of D-240 strain which treated with the anterior pituitary gonadotrophic hormone and pregnant mare serum gonadotropin. ROBINSON (1949), RASEIDA &

Table 8. Number of normal, abnormal mature follicles and polyovular follicles in control mouse ovaries.

Material	Oestrous cycle	Number of individual	Normal follicle			Abnormal follicle			Polyovular follicle		
			Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left		Right	Left		Right	Left	
Seventeen mature mice (*Mus musculus*. "Swiss albinos")	Proestrus	5	23	33	56	11	15	26	10	6	16
	Oestrus	5	16	14	30	9	10	19	6	2	8
	Metoestrus	2	5	4	9	5	3	8	1	1	2
	Late Dioestrus	5	21	28	49	18	12	30	6	5	11
	Total	17	65	79	144	43	40	83	23	14	37

Table 9. Type of abnormal mature follicles and polyovular follicles in control mouse ovaries.

Material	Oestrous cycle	Number of individual	Type of abnormal follicle			Type of polyovular follicle				
			Abnormality in granulosa cells	Abnormality in egg body	Total	Biovular	Tri-ovular	Tetra-ovular	Five-ovular	Total
Seventeen mature mice (*Mus musculus*, Swiss albinos)	Proestrus	5	23	3	26	13	0	2	1	16
	Oestrus	5	16	3	19	6	2	0	0	8
	Metoestrus	2	5	3	8	1	0	1	0	2
	Late Dioestrus	5	27	3	30	8	2	0	1	11
	Total	17	71	12	83	28	4	3	2	37

LAMOND (1956) and some others recorded the effect of estrogen and pregnant mare serum of horses in higher vertebrates. According to them, the single application of P.M.S. produced no effective on polyovulation. SATO (1959) has made some observation on hormone induced polyovulation and superfecundity in mature mice, and reported that the number of eggs ovulated was 23 to 135 in treated mice, being 58.5 on an average. The number of eggs at the natural ovulation of mice was 8.2 on an average. OKIGAKI (1959) observed natural and artificial ovulation in mature and immature rats, and reported that one mature rat discharged 40 eggs, 31 from the left ovary and 9 from the right ovary through the injection of the gonadotrophic hormone. Control untreated rats observed ovulated 8 to 10 eggs in the majority of cases, being 8.8 on an average.

The data obtained in the present study are presented in Table 10 in which the average numbers of normal ova, abnormal ova and polyovular ova in normal and hormone treated mice are given. In untreated ovaries 5.2 normal ova, 2.8 abnormal ova, and 2.7 polyovular ova were observed, while A.P.H. treated mice showed 8.1 normal ova, 1.8 abnormal ova, and 1.9 polyovular ova, and P.M.S. treated mice presented 12.3 normal ova, 1.8 abnormal ova, and 1.3 polyovular ova. Untreated control mice furnished 8.5 normal, 4.9 abnormal, and 2.2 polyovular ova.

From the results of this study, the conclusion is possible that the reduction in number of abnormal mature ova may be explicable by the effect of sex stimulating hormones. Also, the evidence presented in the above two experiments has indicated that the abnormal ova significantly decrease in number in P.M.S. treated mice in comparison with A.P.H. treated mice. It is presumed that abnormal mature ova which failed to ovulate may undergo ovulation after their growth under the influence of sex stimulating hormones, and that the hormones seem not to affect upon atretic ova in young oocytes or in primary follicles. Further, the results of observations indicated that the hormones did not affect the reduction of polyovular follicles, so far as the present experiments have gone in mature mice.

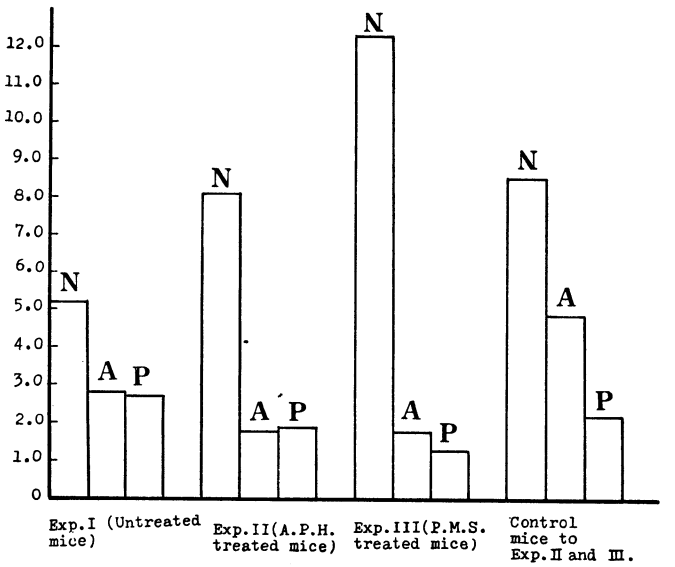
The number of polyovular follicles was observed in Swiss albinos showing that the polyovular follicles are rather rare in occurrence. The number of polyovular follicles was studied in A.P.H. treated and non- treated Swiss albinos. The untreated mice showed 2.7 polyovular ova in average, while treated mice showed 1.9 polyovular ova. In P.M.S. treated mice, polyovular ova were 1.3. From the above findings it can be stated that the hormone treated mice indicate a slight decrease in number of polyovular ova, but the effect of hormones is not remarkable on the reduction in number of polyovular follicles. KENT (1959) reported the reduction of polyovular follicles and polynuclear ova following the application of estradiol monobenzoate (Estrogen) in the immature golden hamster. According to him, polyovular follicles and polynuclear ova reduced 17 to 18 percent as compared with control value. Generally, immature mammalian animals were found to have many polyovular follicles and polynuclear ova, while in mice the polyovular follicles are rather rare in occurrence. Taken into consideration the above fact, the effect of the sex stimulating hormone on polyovular follicles may not be conclusive in the present status of investigation.

PINCUS & ENZMANN (1947) assumed that maturation of atretic ovarian eggs in the rabbit ovary, was found in small atresia of young oocytes (10%), and that the larger follicles at 60 percent were found to be atretic. According to them, the hormone administration affected only the largest follicle and their contained ova.

From the results of observations described in Part I and II, it is apparent that the atretic follicles of larger size in which the ova showed advance towards the maturation course are very abundant in number in ovaries of mature mice, being 35% in total. It is most probable that the atretic follicles are in the process of degeneration, and that the disintegration takes place either in the egg body or in the granulosa cells. From this evident it is presumable that there is a relationship between the

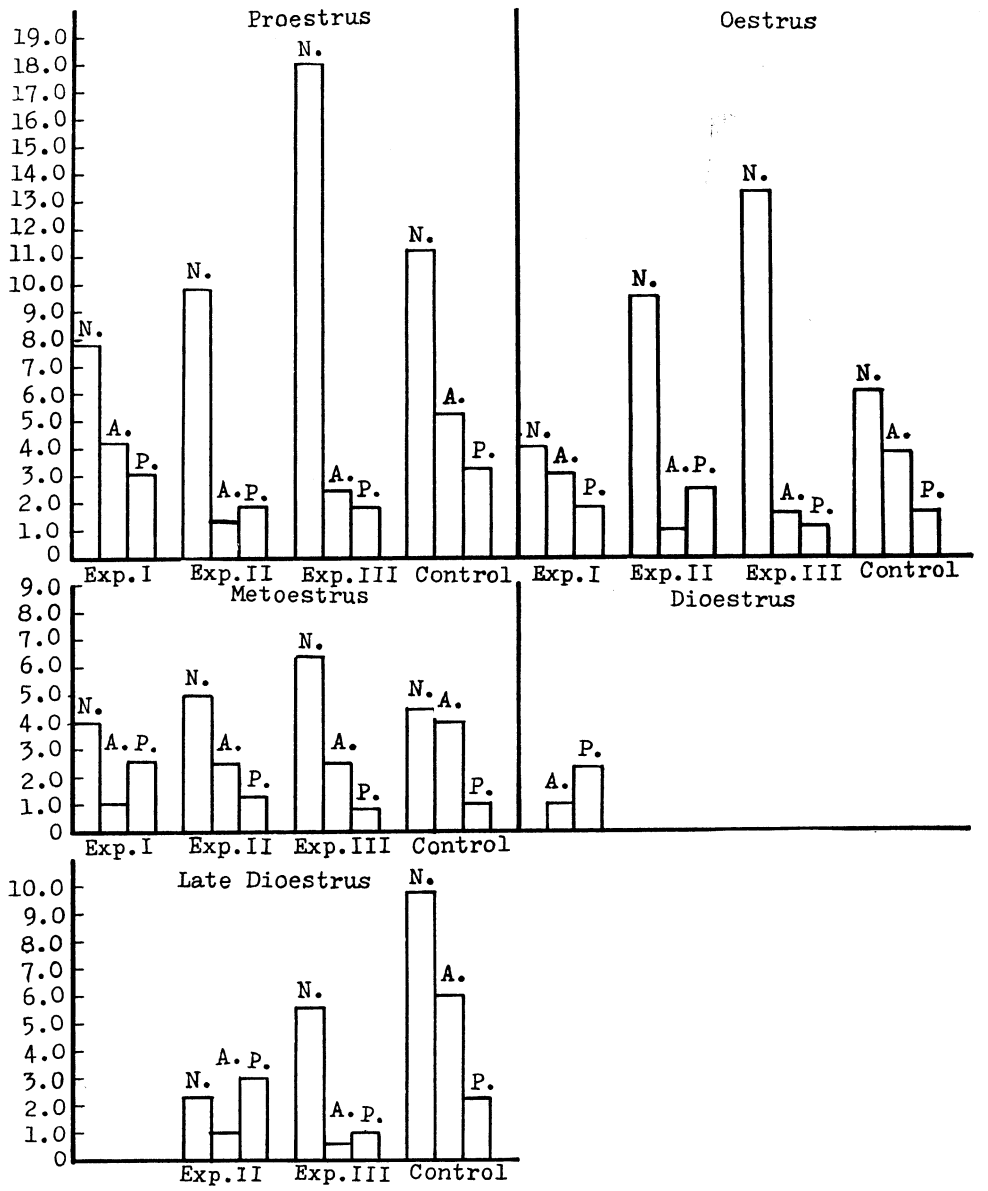
Table 10. Average number of normal, abnormal mature follicles and polyovular follicles in mouse ovaries at three experiments.

Method	Oestrous cycle	Number of individual	Average number of normal follicle	Average number of abnormal follicle	Average number of polyovular follicle
Exp. I (Untreated mice)	Proestrus	14	7.8	4.1	3.1
	Oestrus	5	4.0	3.0	1.8
	Metoestrus	7	4.0	1.1	2.6
	Dioestrus	4	0	1.0	2.3
	Total	30	5.2	2.8	2.7
Exp. II (A.P.H. treated mice)	Proestrus	16	9.8	1.3	1.8
	Oestrus	2	9.5	1.0	2.5
	Metoestrus	4	5.0	2.5	1.3
	Late Dioestrus	3	2.3	1.0	3.0
	Total	25	8.1	1.8	1.9
Exp. III (P.M.S. treated mice)	Proestrus	9	18.0	2.4	1.8
	Oestrus	7	13.3	1.6	1.1
	Metoestrus	4	6.3	2.5	0.8
	Late Dioestrus	5	5.6	0.6	1.0
	Total	25	12.3	1.8	1.3
Control (Exp. II and Exp. III)	Proestrus	5	11.2	5.2	3.2
	Oestrus	5	6.0	3.8	1.6
	Metoestrus	2	4.5	4.0	1.0
	Late Dioestrus	5	9.8	6.0	2.2
	Total	17	8.5	4.9	2.2



N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 1. Average number of normal, abnormal mature follicles and polyovular follicles in the mouse ovaries at three experiments.



N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 2. Average number of normal, abnormal mature follicles and polyovular follicles in mice taken at different phases of the oestrous cycle.

maturation of the Graafian follicles and the effects of the sex stimulating hormone in the largest follicle: the abnormal ova preceding further beyond the maturation course are very few in number in hormone treated mice than in the untreated mice. Especially, P.M.S. treated mice indictated the remarkable decrease in number of abnormal ova. Further, the decrease in number of abnormal ova showing an im-

mature condition of grnulosa cells is remarkable. Based on the results of the present study the assumption is possibly made that abnormal mature ova which failed to ovulate may undergo ovulation under the effect of the sex stimulating hormones.

Part III. Number of Aberrant follicles in relation to the strain of mice

The above investigations have dealt with cytological studies carried out with the ova derived from 66 ovaries which were taken from a series of mature unmated mice of Swiss-albinos at different phases of the oestrous cycle, with special reference to the maturation and degeneration ova in mature ovaries.

Part III is devoted to the cytological research on the number of aberrant follicles in relation to mouse strain. The strains of mice used here are Swiss albinos, D-240 albinos, dd/Ma albinos and B-72 albinos. They are 70 to 120 days of age, weighting about 20 to 30 gm. The work was carried out during from late summer to late autumn through active breeding seasons in the year. The work was aimed to determine whether or not the four mouse strains are different in the number of normal, abnormal and polyovular ova.

The full grown ovarian egg containing the resting nucleus is approximately spherical in shape and lies in an eccentric position in the Graafian follicle. Such a mature follicle is found occupying a position at the periphery of the ovary. The ovum is surrounded by the zona pellucida and the latter again by the corona radiata. Surrounding the corona radiata there are several layers of cells, stratum granulosum which are arranged lining surface of the follicle and enclosing a huge cavity filled up with the fluid, liquor folliculi. The size of the mouse egg are from 51 to 82μ in S-strain, 75-82μ in dd/Ma strain, 78-89μ in B-72 strain, 73-80μ in D-strain mice under the fixed condition. The mature follicle are 217-290μ in diameter in S-strain, 286–340μ in B-72 strain, 286–350μ in dd/Ma strain, 243–357μ in D-strain in diameter. The size of the mouse egg therefore nearly approximates in four different strains.

Microscopical observations revealed that the normal and abnormal mature ova proceeding prior to the maturation course and polyovular ova are abundant in four strains of mice. Swiss albinos showed in their mature ovaries 157 normal mature ova, 85 abnormal mature ova and 80 polyovular ova in total. Fourteen individuals observed at the proestrus stage showed 109 normal, 58 abnormal, and 44 polyovular follicles. Five individuals at the oestrus stage indicated 20 normal, 15 abnormal, and 9 polyovular ones. Seven individuals at the metoestrus stage presented 28 normal, 8 abnormal, and 18 polyovular ones. Four individuals at the dioestrus stage showed no normal, 4 abnormal, and 9 polyovular follicles.

D-240 mice showed in their mature ovaries from 30 individuals showed 129 normal, 86 abnormal, and 56 polyovular follicles in total. Six individuals at the proestrus stage presented 43 normal, 19 abnormal, and 10 polyovular ones. Seven individuals at oestrus stage showed 58 normal, 36 abnormal, and 11 polyovular ones. Eighteen individuals at the metoestrus stage indicated 26 normal, 19 abnormal, and 14 polyovular ones. Seven individuals at the dioestrus stage showed no normal, 9

abnormal, and 17 polyovular ones. Two individuals at the late dioestrus stage provided 2 normal, 3 abnormal, and 2 polyovular follicles.

dd/Ma mice furnished in their mature ovaries from twenty specimens 118 normal, 69 abnormal, and 36 polyovular follicles in total. Five individuals at the proestrus stage showed 26 normal, 15 abnormal, and 8 polyovular follicles. Six individuals at the oestrus stage indicated 58 normal, 23 abnormal, and 17 polyovular ones. Four individuals at the metoestrus stage presented 25 normal, 14 abnormal, and 5 polyovular ones. One individual at the dioestrus stage showed no normal, 2 abnormal, and no polyovular ones. Four individuals at the late dioestrus stage furnished 9 normal, 15 abnormal, and 6 polyovular follicles.

B-72 mice showed in their mature ovaries from twenty specimens 87 normal, 37 abnormal, and 20 polyovular follicles in total. Four individuals at the proestrus stage showed 24 normal, 14 abnormal, and 5 polyovular ones. Six individuals at the oestrus stage indiacted 42 normal, 12 abnormal, and 7 polyovular ones. One individual at the metoestrus stage presented 5 normal, 3 abnormal, and 2 polyovular ones. Four individuals at the dioestrus stage showed no normal, 3 abnormal, and 4 polyovular ones. Five individuals at the late dioestrus stage provided 16 normal, 5 abnormal, and 2 polyovular follicles (Table 11).

In abnormal mature ova, the disintegration took place either in the granulosa cells or in the egg body itself. In D-strain mice, the abnormal ova of the former type were found in 82 examples, those of the latter type being observed in 4 examples in total. In dd/Ma mice the former type ova were 60 and the latter type ones were 9 in total. In B-72 mice, the former type ova were 33 and the latter type ones were 4. The results from S-strain mice are given in Part I.

Next, the average number of normal, abnormal and polyovular ova were observed in four mouse strains. In S-strain mice the average numbers were 5.2 for normal, 2.8 for abnormal, and 2.7 for polyovular ova. In D-strain mice, they were 4.3 for normal, 2.9 for abnormal, and 1.8 for polyovular ova. In dd/Ma mice, they were 5.9 for normal, 3.5 for abnormal, and 1.8 for polyovular ova. In B-72 mice, they were 4.4 for normal, 1.9 abnormal, and 1.0 polyovular ova. The data obtained for different stage of the oestrous cycle are given in Table 12.

Based on the data obtained from the above observations, it is evident that the number of normal and abnormal mature ova showed no remarkable difference in four strains of mice.

The polyovular follicles were observed in four strains of mice, 70 to 120 days of age. The results revealed that the polyovular follicles were rather rare in occurrence and not different by strain.

In S-strain mice 80 polyovular follicles were found to occur in 60 ovaries. Of them, they were 62 follicles (78%) which were biovular, 13 follicles (16%) which were triovular, and 5 follicles (6%) which were tetraovular. Only three biovular follicles were found in the stage of normal antrum formation, and the other follicles are in the process of degeneration without the establishment to the antrum follicle.

In D-strain mice 54 polyovular follicles were found to occur in 60 ovaries. Of

them, there were 42 follicles (78%) which were biovular, 7 follicles (13%) which were triovular, and 5 follicles (9%) which were tetraovular. All are the atretic follicles with no antrum.

In dd/Ma mice 36 polyovular follicles were found to occur in 40 ovaries. Of them, there were 28 follicles (78%) which were biovular, 3 follicles (8%) which were triovular, 3 follicles (8%) which were tetraovular and 2 follicles (6%) which contained five ova. Two biovular follicles were found in the stage of the normal antrum formation and one of them seems to suggest that biovular follicles originate from the previously separated follicles and other follicles seem to arise through abnormal nuclear division in the second maturation division.

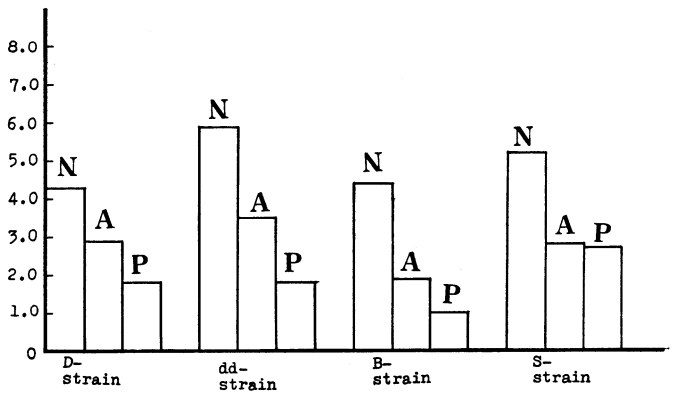
In B-strain mice, 20 polyovular follicles were found to occur in 40 ovaries. Of them, there were 18 follicles (90%) which were biovular, and 2 follicles (10%)

Table 11. Number of normal, abnormal mature follicles in mature ovaries at three mouse strains.

Material	Oestrous cycle	Number of individual	Normal follicle			Abnormal follicle			Polyovular follicle		
			Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left		Right	Left		Right	Left	
D-240 albinos strain	Proestrus	6	18	25	43	8	11	19	5	5	10
	Oestrus	7	31	27	58	20	16	36	6	5	11
	Metoestrus	8	13	13	26	10	9	19	9	5	14
	Dioestrus	7	0	0	0	5	4	9	10	7	17
	Late Dioestrus	2	0	2	2	1	2	3	1	1	2
	Total	30	62	67	129	44	42	86	31	23	54
dd/Ma albinos strain	Proestrus	5	15	11	26	9	6	15	7	1	8
	Oestrus	6	26	32	58	13	10	23	8	9	17
	Metoestrus	4	15	10	25	11	3	14	3	2	5
	Dioestrus	1	0	0	0	2	0	2	0	0	0
	Late Dioestrus	4	3	6	9	7	8	15	4	2	6
	Total	20	59	59	118	42	27	69	22	14	36
B-72 albinos strain	Proestrus	4	13	11	24	5	9	14	2	3	5
	Oestrus	6	26	16	42	6	6	12	5	2	7
	Metoestrus	1	2	3	5	3	0	3	1	1	2
	Dioestrus	4	0	0	0	2	1	3	2	2	4
	Late Dioestrus	5	7	9	16	1	4	5	1	1	2
	Total	20	48	39	87	17	20	37	11	9	20

Table 12. Average number of normal, abnormal mature follicles and polyovular follicles in mature ovaries at three mouse strains.

Method	Oestrous cycle	Number of individual	Average number of normal follicle	Average number of abnormal follicle	Average number of polyovular follicle
D-240 albino strain	Proestrus	6	7.2	3.2	1.7
	Oestrus	7	8.3	5.1	1.6
	Metoestrus	8	3.3	2.4	1.8
	Dioestrus	7	0	1.3	2.4
	Late Dioestrus	2	1.0	1.5	1.0
	Total	30	4.3	2.9	1.8
dd/Ma albino strain	Proestrus	5	5.2	3.0	1.6
	Oestrus	6	9.7	3.8	2.8
	Metoestrus	4	6.3	3.5	1.3
	Dioestrus	1	0	2.0	0
	Late Dioestrus	4	2.3	3.8	1.5
	Total	20	5.9	3.5	1.8
B-72 albino strain	Proestrus	4	6.0	3.5	1.3
	Oestrus	6	7.0	2.0	1.2
	Metoestrus	1	5.0	3.0	2.0
	Dioestrus	4	0	0.8	1.0
	Dioestrus Late	5	3.2	1.0	0.4
	Total	20	4.4	6.1	1.0



N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 3. Average number of normal, abnormal mature follicles and polyovular follicles in the mouse ovaries at four strains.

which were tetraovular. All of them are in the process of degeneration without establishing the antrum follicle (Table 13).

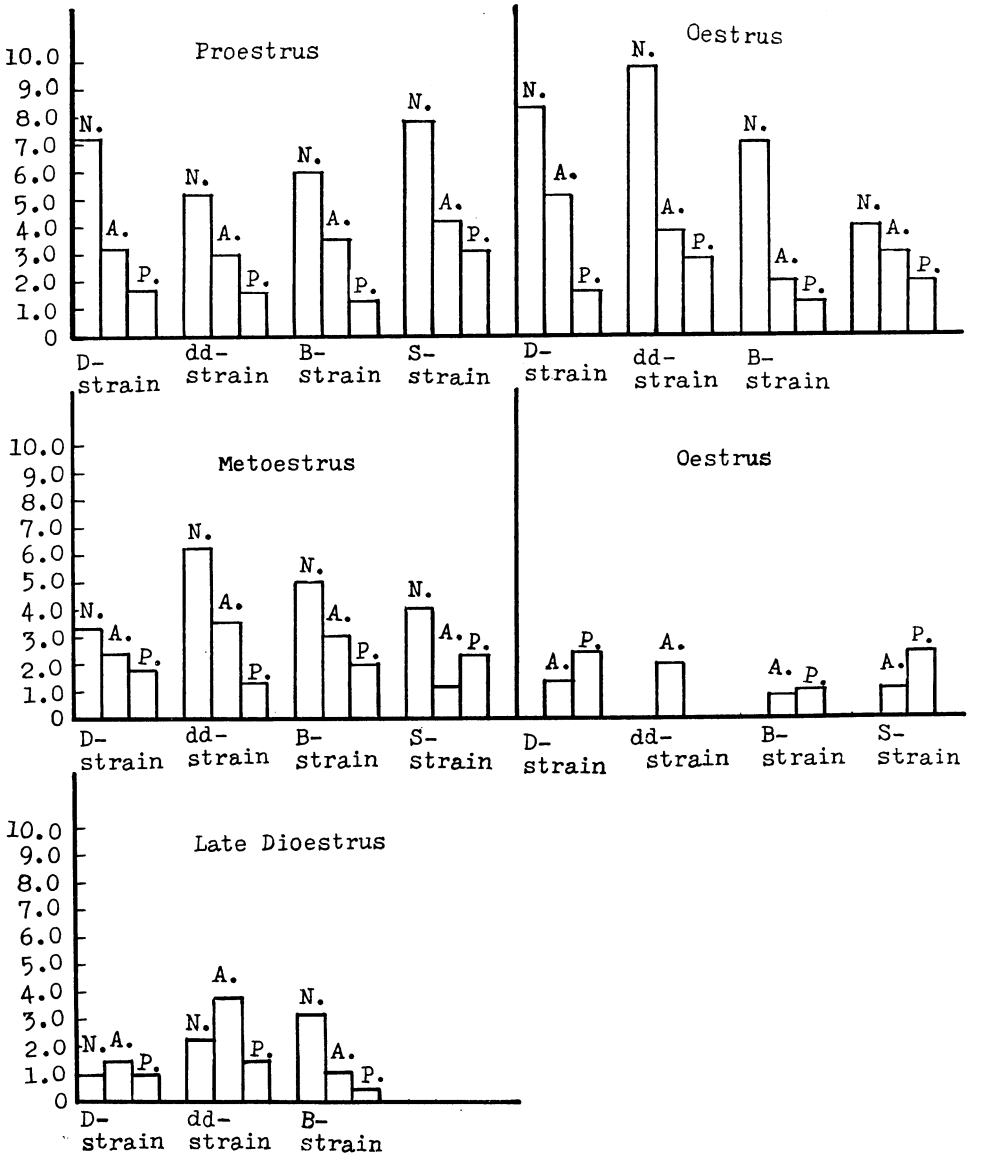
The data herein presented were based on observations with the ovaries derived from four mouse strains: they show no remarkable difference in number of normal, abnormal mature ova and polyovular ova by mouse strain. The features above

Table 13. Type of abnormal mature follicles and polyovular follicles in mature ovaries at three mouse strains.

Material	Oestrous cycle	Type of abnormal follicle			Type of polyovular follicle				
		Abnormality in granulosa cells	Abnormality in egg body	Total	Biovular	Tri-ovular	Tetra-ovular	Five-ovular	Total
D-240 albinos strain	Proestrus	19	0	19	8	1	1	0	10
	Oestrus	35	1	36	9	2	1	0	11
	Metoestrus	19	0	19	12	1	0	0	14
	Dioestrus	6	3	9	11	3	3	0	17
	Late Dioestrus	3	0	3	2	0	0	0	2
	Total	82	4	86	42	7	5	0	54
dd/Ma albinos strain	Proestrus	13	2	15	6	0	1	1	8
	Oestrus	19	4	23	13	2	1	1	17
	Metoestrus	11	3	14	3	1	1	0	5
	Dioestrus	2	0	2	0	0	0	0	0
	Late Dioestrus	15	0	15	6	0	0	0	6
	Total	60	9	69	28	3	3	2	36
B-72 albinos strain	Proestrus	12	2	14	5	0	0	0	5
	Oestrus	11	1	12	5	0	2	0	7
	Metoestrus	2	1	3	2	0	0	0	2
	Dioestrus	3	0	3	4	0	0	0	4
	Late Dioestrus	5	0	5	2	0	0	0	2
	Total	33	4	37	18	0	2	0	20

mentioned are referable rather clearly to Text-figs. 3–4.

Some mention may be made on the seasonal variation in number of normal and abnormal ova. SIGEMORO (1944) studied the seasonal variation in number of abberant follicles from a series of sixty mature rats. The data presented by him indicated that the degenerating follicles in the ovaries represent no significant variation by season through the year. MAKINO, SIGEMORO & KOBAYASHI (1943) have studied seasonal variation of the normal follicles in female Norway rats, and examined the number of mature ova in ovaries through a year, reporting that the maximum number was found in the specimens secured in January and Febrary. The data presented in this study are insufficient to make any conclusion, since the present study



N. Normal mature follicle; A. Abnormal mature follicle; P. Polyovular follicle

Text-fig. 4. Average number of normal, abnormal mature follicles and polyovular follicles in four mouse strains at different phases of the oestrous cycle.

was carried out from late summer to late autumn.

Part IV. Some histochemical observations on normal and abnormal ova in mouse ovaries

A considerable amount of literature has been published pertaining to the histochemical studies of glycogen, and nucleic acids in mammalian ova by many authors. TOGARI (1927), BRANDENBURG (1938) and HARTER (1948) have dealt with the

cytochemical demonstration of glycogen in rodent ovaries. They stated that the normal and atretic ova generally contained a large amount of glycogen. ISHIDA (1953, 1954) studied glycogen in the ovaries of several mammals, stated that normal ova observed in the secondary and Graafian follicles showed a extreme difference in glycogen content. Especially, rat and hamster ova contained glycogen in a large amount. He has found that atretic ova of rats were different remarkably in glycogen content; some ova contained no glycogen, but some others contained in a small or large amount.

VINCENT & DORNFELD (1948), JONES-SEATON (1950) and ISHIDA (1953) studied the contents of nucleic acids (RNA) in rodents eggs. According to them, a lsmall amount of RNA was observed in different stages of the normal oestrous cycle of rats. ISHIDA (1953) studied RNA contents in ovaries of several mammals such as rats, hamsters, guinea-pigs, rabbits, goats, sheeps and cattles, and found no remarkable difference among them.

In the present study, the author has undertaken the study on the localization of glycogen and RNA in normal and abnormal ova in the ovaries of mature mice derived from Swiss albinos and D-240 albinos, 70 to 120 days in age. The results are as shown in the following. The work was carried out in 1960 and the results were partly reported.

(1) Glycogen content in ova

Use was made of four individuals of S-strain mice at four oestrous stages and two individuals of D-strain mice at the proestrus and oestrus stages. With the use of the PAS method (Periodic acid-Schiff method), the glycogen was demonstrated as red-purple granules. Seventy to eighty percent of normal ova from the primary follicles having one or more cell layers with no antrum were colored moderately red-purple in the surface (Fig.74). A few ova at the oestrus stage showed no existence of glycogen. A large amount of glycogen was observed in the majority of secondary normal ova in the follicles with a prominent antrum (Fig. 75), while a few others (10%) contained no glycogen (Fig. 76).

The atretic ova of large size proceeding beyond the maturation course contained glycogen without excetion (Figs. 77–78).

ISHIDA (1952) studied the localization of glycogen in the primary follicles in the ovaries of normal mature and immature rats, and reported that normal ova in primary follicles contained no glycogen, while secondary follicles were found to contain a large amount. TOGARI (1927), BRANDENBURG (1938), HARTER (1948) and some others, reported that all ova in secondary and Graafian follicles contained glycogen. ISHIDA (1954) studied glycogen contents in atretic ova in rats and hamsters undergoing fragmentation or cleavage during atresia, and reported that a large amount of glycogen was observed in atretic ova. But, in some other animals such as cattles, sheeps, pigs, goats, rabbits and guinea-pigs only a little or none of glycogen was found, except a large amount of glycoprotein.

The data obtained from this experiment indicated that the primary and second-

ary normal ova in mature mouse ovaries contained a varying amount of glycogen, in a few individuals the ova contained no glycogen, and that the atretic ova of larger size contained always glycogen.

The results of investigations have shown that there is no difference in content of glycogen by mouse strain, and further that there is also no remarkable difference in content of glycogen by different stages of the oestrous cycle of mice.

(2) RNA content in ova

RNA contents were studied in four individuals of S-strain mice at four stages in the oestrous cycle, with the use of a thionin staining method. Normal ova observed in both primary and secondary follicles showed a weak reaction to the test for RNA. This seems to indicate that follicles contain a small amount of RNA. A few atretic ova proceeding beyond the maturation course, 8 percent showed a reaction indicating the presence of a small amount of RNA.

JONES-SEATON (1950) reported that rat ova at every stage contained a small amount of RNA, and that with the development of the ova RNA increased in the cytoplasm. ISHIDA (1954) informed that normal follicles at every stage of the oestrous and atretic follicles at the early stage usually contained a small amount of RNA and that RNA showed no increase during the course of development of the ova.

The results of the present experiment are in agreement with those shown by ISHIDA (1954): RNA showed no remarkable increase during the development of ova.

(IV) DISCUSSION

The present investigations have dealt with the morphology of the atretic follicles found in mouse ovaries, particularly on cytological features of abnormal mature ova proceeding beyond the maturation and polyovular ova found in the Graafian follicles of mature ovaries. The papers relating to atretic follicles have been published by many authors. ASAMI (1920) in the rabbit, ENGLE (1927) in the mouse, LANE (1938) in the immature rat, PLISKE (1940) in the ground squirrel and SIGEMORO (1947) in the Norway rat reported the morphology and structure of atretic ova occurring in the primary and secondary follicles. It was recoginized that a great many ova are produced in the mammalian ovary during the oestrous cycle, but the majority of them undergo degeneration, only a few attaining maturation course, and developing into functional ova. These authors did not inquire into the nature and behavior of atretic follicles in detail, and many questions have remained not answered on the meaning of their occurrence. The present author has undertook cytological observations from both morphological and cytochemical standpoints on the nature of atretic follicles, with the use of mouse ovaries derived from mice of different strains at 70 to 120 days of age. It was observed that the atretic follicles of larger size which seem to advance beyond the maturation course are very abundant in number in normal mature ovaries.

There were found several atretic follicles of larger size in which the ovum

showed the second polar body after the completion of the second maturation division, though the antrum folliculi has not been formed (Fig. 42). YAMANE (1930, 1935a & b, 1937) reported that insemination is an important anticident for the completion of the second division. The above abnormal examples showed the second polar body without insemination of spermatozoa. To the author's view, the second maturation division may proceed without insemination in the ovary under certain abnormal conditions. On the other hand, ASAMI (1929), BRANCA (1925), EREUD & VEDDER (1938) and PLISKE (1949), emphasized that the degeneration of abnormal follicles may cause by the disintegration of granulosa cells. It is probable from the author's results that the atretic ova may be produced by the influence of the hormonal secretion. Especially, the occurrence of aberrant follicles of large size which advance to the maturation course may justify the above view involving the influence of sex stimulating hormones.

Morphologically, the follicles are divided into two general types. Type 1 refers to the small primary follicles with several rows of granulosa cells, but having no antrum folliculi. Type 2 denotes the follicles which advance to the maturation course and have an prominent antrum in each. The sex stimulating hormone application is effective to only the largest follicles belonging to type 2.

The most important hormones produced by the mammlian ovary are those known as female hormones. Probably, the follicle stimulating hormones of the anterior pituitary seem to induce rapid growth of a Graafian follicle; the growth may be partly achieved by mulitiplication of follicles cells and partly by the hormonal secretion into the antrum of the follicular fluid. The pituitary hormones stimulate the gonad in two ways. First, they cause to secrete their own hormones (oestrogen in the ovary), and second they work together with these hormone to induce the maturation and escape of the germ cells. It is possible to consider that the gonadotrophic hormones of the anterior pituitary concerned for the most part with the separation of these hormones into follicles stimulating (F.S.H.) and ovulating or luteinizing (L.H.) fractions. This also applies to the other gonadotrophic hormones such as the luteinizing hormone obtained from the urine of pregnant woman (P.U.) and the follicles stimulating hormone which is found in the blood serum of the mare between the 45th and 100th day of pregnancy (P.M.S.).

Next, the author's study has been extented to analyse the effect of hormones on the atretic follicles with particular concern to the reduction of those follicles, with the application of anterior pituitary sex gland stimulating hormone (A.P.H.), the sex gland stimulating hormones of placenta (Cholionic gonadotrophin) and pregnant mare serum of horses (P.M.S.). The effect of sex stimulating hormones on abnormal mature ova of mice is rather remarkable. Especially, in the P.M.S. treated mice the remarkable decrease occurred in number of abnormal mature ova. Further, the decrease in number of abnormal ova showing immature condition of granulosa cells is remarkable. On the other hand, many atretic primary follicles were observed in the ovaries under the condition of hormone treatment. This evidence indicates that the hormones are not effective on the atretic primary follicles.

However, the present investigations have failed to produce any conclusive results on the question that the administration of any hormone would affect for the reduction of the atretic mature follicles during the normal oestrous cycle. In general, there are individual differences in mice which received injection of different hormone preparations.

LANE (1938) dealt with aberrant ovarian follicles in immature rats, and reported that the incidence of atypical follicles was not materially altered under the experimental condition in animals treated with various hormone preparations. PINCUS & ENZUMANN (1947) studied the growth, maturation and atresia of ovarian eggs of rabbits. According to them, the percentage of atresia among the young oocyte is low (10%); the larger follicles belonging to the secondary type showing about 60 percent are atretic at all stages of the reproductive cycle; they stated that the hormonal effect are on the secondary atretic follicles in the rabbit.

Numerous workers have shown how, by injection of hormones of various kinds, the number of ova may be considerably increased. Microscopical observations of artificially induced polyovulation through the injection of various hormones were reported by PINCUS (1940), PARKES (1943) in the rabbit, RUNNER & PALM (1953) in the immature mouse, GATES (1956) & GREEN (1956) in the mouse, OKIGAKI (1958) in the mature rat and SATO (1959) in the mature and immature mouse, and some others. On the other hand, ROBINSON (1949), HUNTER, ADAM & ROWSON (1955), RASIDA & LAMOND (1956) and others reported polyovulation in mature and immature domestic animals, under the administration of hormones such as anterior pituitary sex stimulating hormones or sex gland stimulating hormones or placenta or pregnant mare serum of horses or oestrogen. Their experiments have provided no evidence for effective results on polyovulation when single injection of P.M.S. hormone was made. But, most of the above results have offered no important analytical evidence for the mechanism of polyovulation under the influence of sex stimulating hormones.

Based on the data obtained from the present observations, the following assumption is possible that abnormal mature ova which may be failed to ovulate in untreat state may undergo ovulation under the effect of sex stimulating hormones, while such hormones may exert no effects on the primary atretic follicles having one or more cell layers with no antrum.

The widespread occurrence of the polyovular follicle has been reported in many kinds of mammals by HARTMAN (1926), ENGLE (1927), LANE (1938), DEDERER (1934), TAKEWAKI (1937), LANE (1938), SIGEMORO & MAKINO (1946), and SIGEMORO (1947). According to them, the aberrant forms of polyovular follicles are generally more common in immature and in older animals than in mature ones. But, SIGEMORO (1947) dealt with morphological observations of abnormal follicles in sixty mature wild rats, and reported that the polyovular follicles were abundant in number in the mature ovaries, being 275 in total.

According to the results of the present study which was based on mice at 70 to 120 days of age, polyovular follicles were few in number in occurrence; 80 poly-

ovular follicles were found to occur in 60 ovaries. Of them, almost 78 percent were biovular, in accordance with the results by the other authors. The author observed the ovaries of immature mice at 10 to 20 days of age, and found that the polyovular follicles are rather rare in occurrence; in 20 ovaries from a series of ten normal mice, 13 polyovular follicles and 1 binuclear ova were found in total. The data from the present observations indicated that the polyovular follicles were less common in mature and immature mice than in other mammals in general.

The present author is of opinion that the polyovular follicles may be formed through one of the following three processes: 1) polyovular follicles presented by concresence of previously separated follicles as shown in Fig. 63, 2) polyovular ova presented by abnormal nuclear division as shown in Fig. 62, and 3) polyovular follicles arised by separation or fragmentation of the original ovum as shown in Fig. 53. Type 1 and 2 seem to be common in occurrence. They may be in process of degeneration, though there are a few examples which possibly suggest their normal ovulation course. Type 3 may be doomed to atrophy in the ovary.

Concerning the origin of the polyovular follicles, HARTMAN (1926) enumerated three possible ways of formation; 1) by division of polyovulation ova, 2) by concrescence of previously separated follicles, and 3) by persistent union of ova in the egg tube.

KENT (1959) reported that occurrence of polyovular follicles and polynuclear ova were rather common in immature animals. He assumed that polyovular follicles may be produced due to alleviation of a deficient estrogen condition in the young golden-hamster. He showed that polyovular follicles and polynuclear ova was reduced 16 to 18 percent of the control value through daily injections of estradiol monobenzoate in seame oil.

The results of the present study indicated that polyovular follicles were slightly reduced in number in P.M.S. treated mice than in the untreated mice. But, no conclusive statement was made whether or not the administration of the sex stimulating hormones would also reduce the number of polyovular ova.

Further, evidence has been presented by the author that there is no remarkable difference in number of normal and abnormal mature ova and polyovular follicles among the following four strains of mice; Swiss albinos, D-240 albinos, dd/Ma albinos and B-72 albinos. In the four strains of mice, abnormal ova which advance towards the maturation course were very abundant in number in mature ovaries. Also, polyovular ova were rather rare in occurrence.

The oestrous cycle was observed in four mouse strains, and the following results were obtained that there is a variation in their cycle. It is noticeable that a few S-strain and D-strain mice showed some abnormality in sexual cycle. They showed an abnormally long dioestrus stage.

Some histochemical studies were undertaken on the normal and abnormal ova as regard to the charge in occurrence and localization of glycogen and nucleic acids. Recently, ISHIDA (1953) reported that the atretic ova of rat ovaries began atrophy through the following three types of degeneration process, (1) the nucleus of the

ovum showed the piknosis and its cytoplasm shrunk without fragmentation or cleavage, (2) the ovum underwent symmetrical cell division until about 2 to 8 cell stages, and then their cytoplasm became irregular and finally disintegrated, and (3) the cytoplasm of the ovum showed irregular cell division by which irregular cell masses were formed. He stated that the three degeneration types occurred in parallel condition to the appearance of glycogen in ovaries. The ova of rats and hamsters undergoing fragmentation or cleavavge during atresia were generally found to contain a large amount of glycogen, while those of cattles, sheeps, pigs, goats, rabbits and guinea-pigs undergoing shrinkage during atresia contained a little or none of glycogen. The present study with mice has resulted in that atresia ova have been found to contain at various stages glycogen without exception. Some atretic ova proceeding the maturation course contained a small amount of glycogen, though sometimes a large amount of glycogen was found to occur. In the present study, no positive evidence has so far been obtained to show that there is any relationship between the charge in amount of glycogen and the formation of atretic ova.

(V) SUMMARY

The data obtained in the present investigations were described being separated into four parts.

Part I is devoted to the cytological studies of normal, abnormal mature ova and polyovular follicles in mature mice, *Mus musculus* (Swiss albinos) during the normal sexual cycle. It was found that the atretic follicles of large size in which the ova were in a course of maturation were very abundant in number in ovaries of mature mice. It seems probable that the course of degeneration takes place in both egg body and granulosa cells, and that the results affect the hormone secretion in the mature ovary. The polyovular follicles were found to be rather rare in occurrence both mature and immature mice.

In Part II is described an cytological effect of sex stimulating hormones on large abnormal follicles and ova in *Mus musculus* (Swiss albinos). The experiments were carried out by two ways. The first dealt with the effect of the mixture of anterior pituitary sex gland stimulating hormone and sex gland stimulating hormones from placenta upon the fate of abnormal follicles. The second was to examine the effect of the mixed hormones as above and the pregnant mare serum of horse upon abnormal follicles.

On the basis of the results of the present observations, the conclusion is possible that the reduction in number of abnormal mature ova may be explicable by the effect of sex stimulating hormones. Also, the evidence presented in the two experiments has indicated that abnormal mature ova showed a remarkable decrease in number in pregnant mare serum treated mice. Especially, the hormone adminstration probably affects only the follicles of larger size. Further, the number of abnormal ova showing an immature condition of granulosa cells is remarkable. From the results of the present study, it is presumed that abnormal mature ova which

failed to ovulate may undergo ovulation under the effect of the sex stimulating hormones.

Part III describes the results of the cytological investigation of some comparative features of normal, abnormal mature ova and polyovular follicles in mature ovaries of the following mouse strains: Swiss albinos, D-240 albinos, B-72 albinos and dd/Ma albinos. There is no remarkable difference in number of normal, abnormal mature ova and polyovular follicles by mouse strain.

Part IV gives accounts of the histochemical observations of normal and abnormal ova in mature ovaries of mice studied at four stages of the oestrous cycle. The occurrence and localization of glycogen and RNA were the subjects mainly dealts with in ovaries of *Mus musculs* (Swiss albinos and D-240 albinos). The data derived from this experiment indiacted that the primary and secondary normal ova in mature ovaries contained a varying amount of glycogen, that in a few individuals the ova contained no glycogen, and further that the atretic ova of large size contained always glycogen. Normal ova observed in both primary and secondary follicles showed a weak reaction to the test for RNA. This seems to suggest that the follicles contain a small amount of RNA. A few atretic ova preceding beyond the maturation course showed a reaction indicating the presence of a small amount of RNA.

(VI) LITERATURE CITED

ALEEN, E. 1923. Ovogenesis during sexual maturity. Am. J. Anat. **31**: 439–480.

————, W. S. Kountz and B. F. Francis. 1925. Selective elimination of ova in the adult ovary. Am. J. Anat. **34**: 445–468.

ASAMI, G. 1920. Observations on the folliclular atresia in the rabbit ovary. Anat. Rec. **18**: 323–343.

BRANCA, A. 1925. 'Lovocyte atrésique et son involution. Arch. Biol. **35**: 325–335.

BRANDENBURG, W. 1938. Das Glykogen im Eierstock der Ratte. Zeitsch. Mik-anat. Forsch. **43**: 581–594.

BULLOUGH, W. S. 1951. Vertebrate sexual cycles. IV. Hormones and reproduction. Methuen's Monographs on Biological subjects. 45–79.

DAWSON, A. B. 1951. Histogenetic interrelationships of oocytes and follicle cells. A possible explanation of the mode of center polyovular follicles in the immature rat. Ibid. **110**: 181–197.

DEANE, H. W. 1952. Histochemical observations on the ovary and oviduct of the albino rat during the estrus cycle. Am. J. Anat. **91**: 363–413.

DEDERER, P. H. 1934. Polyovular follicles in the rat. Anat. Rec. **60**: 393–403.

DEMPSY, D., R. O. Greep & H. W. DEANE. 1949. Changes in the distribution and concentration of alkaline phosphatases in tissue of the rat after hypophysectomy for gonadectomy and after replacement therapy. Endocrinol. **44**: 88–103.

Engle, M. T. 1927. A quantitative study of follicular atresia in the mouse. Am. J. Anat. **39**(2): 187–203.

EREUD, J. & A. VEDDER. 1938. The structure of rat ovaries under varying condition. Acta Neerlandica Morphologiae. **2**: 71–87.

GATES, A. H. 1956. Vitability and development capacity of eggs from immature mice with gonadotrophins. Nature. **177**: 754–755.

GREEN, J. A. 1956. Functional responses of the immature mouse ovary to different levels of equine pituitary gonadotrophin. Anat. Rec. **126**: 195–212.

HARTMAN, C. G. 1926. Polyovular ova and polyovular follicles in the oppossum and other mammals, with special reference to the problem of fecundity. Am. J. Anat. **37**: 1–18.

HARTER, B. T. 1948. Glycogen and carbohydrate-protein complex in the ovary of the white rat during the oestrous cycle. Anat. Rec. **102**: 349–367.

HISAW, F. L. 1948. Development of the graafian follicle and ovulation. Physiol. Review **27**: 95–119.

HUNTER, G. L., C. E. ADAM & L. E. ROWSON. 1955. Inter-breed ovum transfer in sheep. Jour. Agric. Sci. **46**: 46–78.

ISHIDA, K. 1952. Histological studies of rat ova with special reference to glycogen of them after ovulation. Tohoku J. Agric. Res. III (I): 39–49.

———— 1953. Morphological and histochemical studies of atretic ova. Tohoku J. Agric. Res. IV (I): 29–41.

———— 1954. Morphological and cytochemistry of ovaries of domestic animals. Tohoku J. Agric. Res. IV (3–4): 197–211.

JONES-SEATON. A. 1950. Cytoplasmic organization of the rodent egg with special reference to ribonucleic acid basophilia. Biol. Abst. 1951, **25** (14039). (Arch. Biol. 61: 291–444.)

KENT, H. A. 1959. Reduction of polyovular follicles and polynuclear ova by estradiol monobenzoate. Anat. Rec. **134**: 455–461.

KINGERY, H. 1914. So-called parthenogensis in the white mouse. Biol. Bull. **27**: 240–245.

LANE, CH. E. 1935. The follicular apparatus of the ovary of the immature rat and some of the factors which influence it. Anat. Rec. **61**: 141–153.

———— 1938. Abberent ovarian follicles in the immature rat. Anat. Rec. **71**: 243–245.

LOEB, L. 1911. The parthenogensis development of ova in the mammalian ovary and the origin of ovarian treratoma and chorioepitheliomata. J. Am. Med. **56**: 1327–1330.

MAKINO, S. 1914. Studies on the murine chromosomes, I. Cytological investigations of mice, included in the genus *Mus*. J. Fac. Sci. Hokkaido Imp. Univ. Ser. IV (Zool. 7): 305–380.

————, E. SIGEMORO and H. KOBAYASHI. 1943. Some oecological observations of Norway rat, *Rattus norvegicus* in Sapporo (In Japanese). Sapporo Nat. Hist. Soc. **17** (3): 173–184.

———— and E. SIGEMORO. 1946. On the polyovular follicles found in ovaries of the Norway rat, *Rattus norvegicus* (In Japanese). Seibutu Suppl. **1**: 63–67.

———— 1959. Artificial polyovulation and superfecundity in mice. (In Japanese). Science **29** (8): 419–421.

NAKAMURA, T. 1957. Cytological studies on abnormal ova in mature ovaries of mice observed at different phases of oestrous cycle. J. Fac. Fish. Anim. Husb. Hiroshima Univ. **1** (3): 343–356.

———— 1959a. The second polar body of the ovum found in mature mouse ovaries. (In Japanese). Medicine and Biology **51** (3): 100–102.

———— 1959b. Cytological effects of P.M.S. hormones on abnormal mature ova of mice (In Japanese). Zool. Mag. **68** (10): 358–361.

———— 1960a. Some cytological effects of sex stimulating hormones on abnormal mature ova of mice. J. Fac. Fish. Anim. Husb. Hiroshima Univ. **2** (2): 359–364.

———— 1960b. Some histochemical studies on normal and abnormal ova of mature mice. Jap. Jour. Genet. **35** (11): 357–360.

OKIGAKI, T. 1958. Ovulation in vitro in the rat ovary. J. Fac. Sci. Hokkaido Univ. VI (Zool. 14): 39–44.

———— 1959. Some microscopical observations on natural and artificial ovulation in mature and immature albino rats (*Rattus norvegicus*) (In Japanese). Jap. Jour. Genet. **34**: 15–22.

PAYNE, R. W. & A. D. HELLBAUM. 1955. The effect of estrogen on the ovary of the hypophysectomized rat. Endocrinol. **57**: 193–199.

PARKES, A. S. 1943. Introduction of superovulation and superfecundation in rabbits. Endocrinol. **3**: 268–280.

————, *et al.* 1952. Marshall's physiology of reproduction. **3** (11).

PLISKE, E. G. 1940. Studies on the influence of the zona pellucida in atresia. J. Morph. **67**: 321–346.

PINCUS, G. 1940. Superovulation in rabbits. Anat. Rec. **77**: 1-8.

————, and E. V. Enzmann. 1947. The growth maturation and atresia of ovaries eggs in the rabbit. Physiol. Review **27**: 351-383.

RUNNER, M. N. & J. PALM, 1953. Transplantation and survival of unfertilized ova of the mouse in relation to postovulatory age. J. Exp. Zool. **124**: 303-316.

RAESIDA, J. I. & D. R. LAMOND. 1956. Effects of progesterone and pregnant mare serum administration in the anoestrous ewe. Aust. J. Agric. Rec. **7** (6): 591-607.

SATO, A. 1959. Preliminary reports on artificial superfecundity in mature and immature mice. Yamashina's Institute for Ornothology and Zoology **14**: 531-534.

SIGEMORO, E. 1942. On the seasonal variation in number of mature ova in rats (In Japanese). Biology and Medicine **5** (8): 448-450.

———— 1947. Morphological observations of abnormal follicles in mature ovaries of Norway rat, *Rattus norvegicus*. J. Fac. Sci. Hokkaido Univ. Ser. VI (9): 233-241.

SOBBOTA, J. & G. BURCKHARD. 1910. Reifung und Befruchtung des Eies der Weissen Ratte. Anat. Anz.. **433**: 45-85.

STOKARD, C. R. & G. N. PAPNICOLAOU. 1917. The existence of a typical oestrous cycle in the guinea pig, with a study of its histological changes. Am. J. Anat. **22**: 225-283.

TAKEWAKI, K. 1937. A follicles containing five ova found in the mouse (In Japanese). Zool. Mag. **49**: 231-233.

TOGARI, CH. 1927. On the appearance of glycogen in the female reproductive glands of rodents with special reference to their histology. Folia Anat. Jap. **5**: 429-489.

VINCENT, W. S. & E. J. DORNDELD. 1948. Localization and role of nucleic acids in the developing rat ovary. Am. J. Anat. **83**: 437-469.

YAMANE, J. 1930. The proteolytic action of mammlian spermatozoa and its bearing upon the second maturation division of ova. Cytologia **1**: 393-403.

———— 1935a. Kausal-analytishe Studien über die Befruchtung des Kanincheneies. I. Die Dispersion der Follikelzellen und die Ablösung der Zellen der Corona radiata des Eies durch Spermatozoen. Cytologia **6**: 233-255.

———— 1935b. Kausal-analytishe Studien über die Befruchtung des Kanicheneies. II. Die Isolierung der auf Eizytoplasma auflösend wie Kenchen aus den Spermatozoen. Cytologia **6**: 474-483.

———— 1937. Kausal-analytishe Studien über die Befruchtung des Kanincheneies. III. Polyspermie Befruchtung der Eizellen und mono und polysperme Befruchtung der zweiten Polzelle bei der künstlichen Besamung in vitro. Cytologia **37**: 583-605.

(VII) EXPLANATION OF PLATES 1-13

Plate 1.

Figs. 1-6. Photomicrographs of Graafian follicles showing the normal course of maturation in the mature mice.

Fig. 1. Graafian follicles containing a full grown egg nearing maturity.

Figs. 2-4. Ova showing the disintegration of nucleoli.

Figs. 5-6. Ova nearing the first maturation division after the disappearance of the nuclear membrane. Visible well-defined bivalent chromosomes are found in the nucleus.

Plate 2.

Figs. 7-12. Ova showing the normal first maturation division at the side and polar view. Graafian follicles are found to have a prominent antrum.

Plate 3.

Figs. 13-18. Ova showing the normal first and second maturation division.

Figs. 13-14. Side view of the first division at metaphase in the egg.

Figs. 15–16. Ova showing the second polar spindle at metaphase, accompanied by the first polar body.
Figs. 17–18. Ova just before rupture. The maturation process in the ova stops at the metaphase stage of the second division.

Plate 4.

Figs. 19–24. Abnormal Graafian follicles with a few antra showing the maturation process.
Figs. 19–20. Abnormal mature ova nearing first maturation division.
Figs. 21–24. Abnormal ova with a few antra showing the first division.

Plate 5.

Figs. 25–30. Abnormal Graafian follicles showing the first maturation division, the antrum has not been fully formed.

Plate 6.

Figs. 31–36. Abnormal ova with a few antra showing the first maturation spindle. The atretic follicles are in the process of degeneration and the disintegration takes place in the granulosa cells.

Plate 7.

Figs. 37–42. Abnormal ova showing the second maturation division.
Figs. 37–41. Ova showing the second polar spindles at metaphase accompanied by the first polar body in the follicles with a few antra.
Fig. 42. Abnormal ovum showing the first and second polar bodies.

Plate 8.

Figs. 43–48. Ova showing the abnormal maturation division with a prominent antrum.
Figs. 43–44. Chromosome abnormalities observed in mature ova.
Figs. 45–46. Abnormal ova showing piknosis of chromosomes at the first maturation division.
Figs. 47–48. Unshed ova are doomed to atrophy.

Plate 9.

Figs. 49–54. Multinucleate ova and polyovular follicles.
Figs. 49–50. Ova showing the abnormal maturation division, the ova performed irregular cell division or cell fragmentation.
Figs. 51–52. Ova containing poly-nuclei.
Figs. 53–54. Polyovular follicles. The ova are doomed to atrophy.

Plate 10.

Figs. 55–56. Polyovular follicles.
Fig. 57. Ovum showing the irregular two cell fragmentation.
Figs. 58–59. Abnormal ova showing vacuoles in the cytoplasm.
Fig. 60. Mature ovum showing the metaphase of the first division with tetra-polar spindles.

Plate 11.

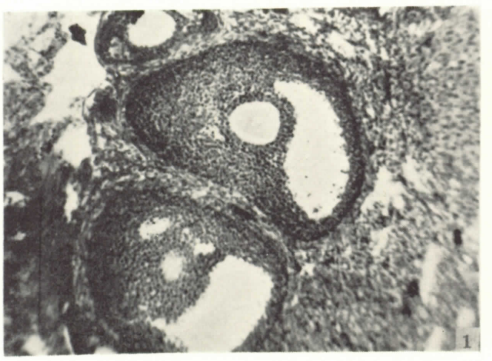
Fig. 61. Ovum showing tetra-polar spindle.
Fig. 62. Normal biovular follicle.
Figs. 63–65. Biovular follicles containing one ovum advancing towards the maturation, but other ovum is prior to maturation division.
Fig. 66. Biovular follicle arise by formation of the cell fragmentation.

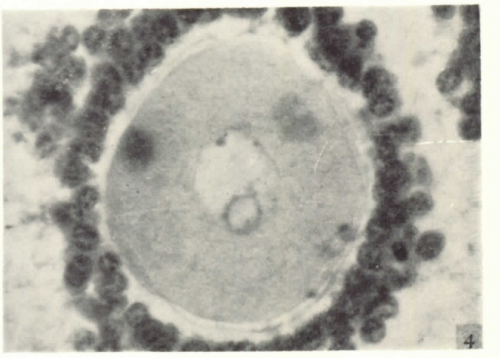
Plate 12.

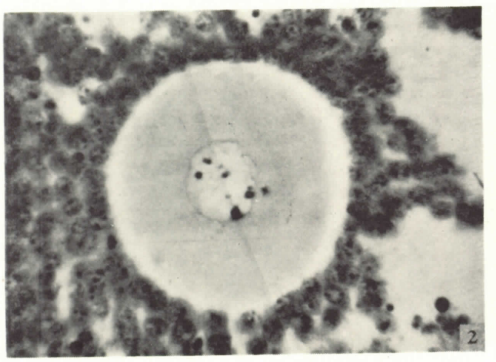
Figs. 67–72. Ova of the immature mice.
Fig. 67. Many immature follicle contained ova in prior to maturation course in the ovaries.
Fig. 68. Bi-nucleate follicle.
Figs. 69–70. Unshed ova with no antrum showing the first maturation division.
Fig. 71. Degenerating ovum are doomed to atrophy.
Fig. 72. Abnormal polyovular follicles.

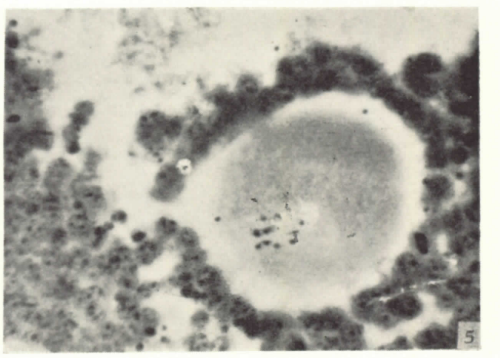
Plate 13.

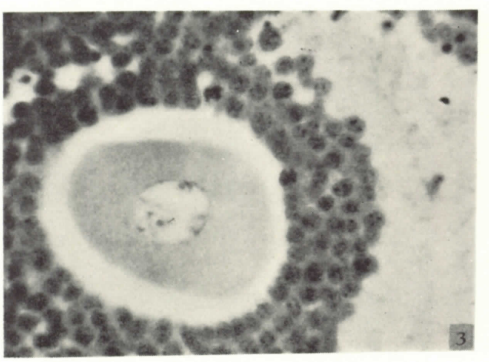
Fig. 73–78. Section of mature ovaries for the observation of glycogen content with the use of the PAS method.
Fig. 73. The ova contained a varying amount of glycogen granules.
Fig. 74. Primary follicles show a large or moderate amount of glycogen in the cytoplasm of ova.
Figs. 75–76. Secondary follicles show a small amount of glycogen in the cytoplasm of ova.
Figs. 77–78. Atretic ova show a large amount of glycogen granules.

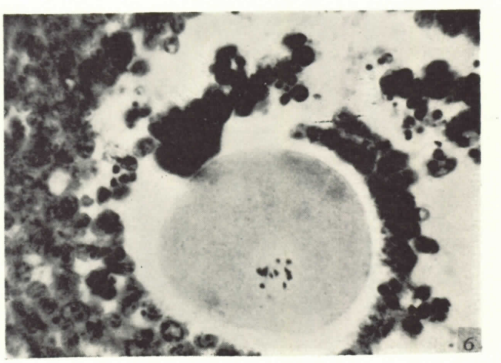












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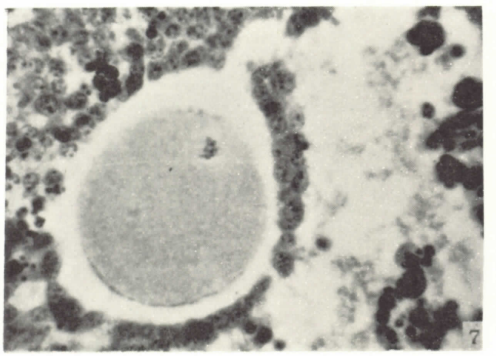

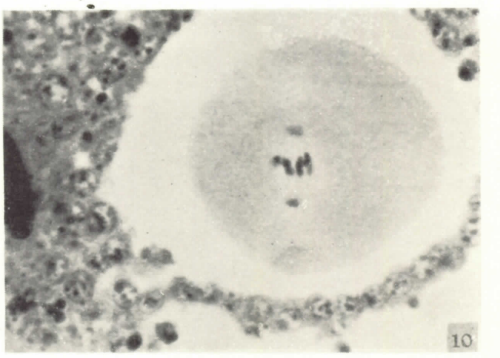

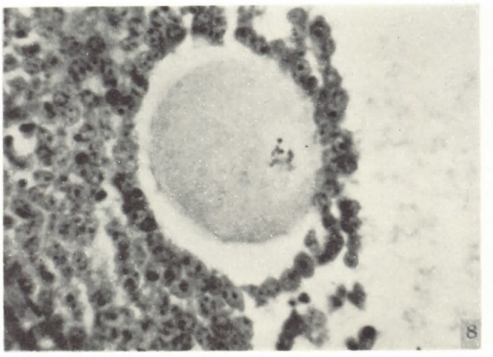

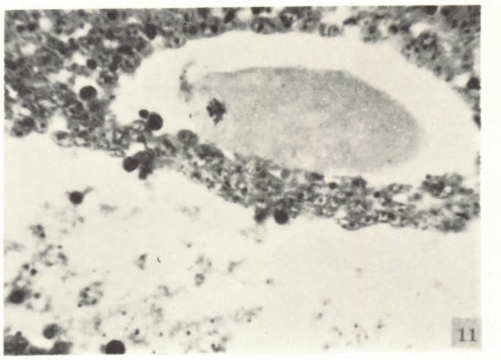

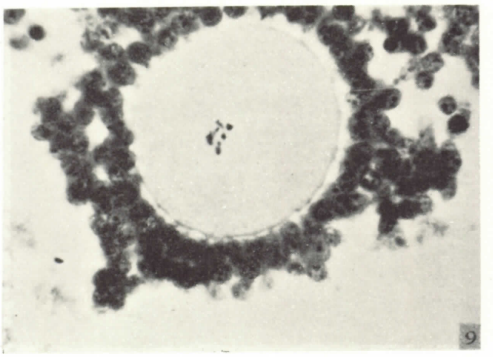

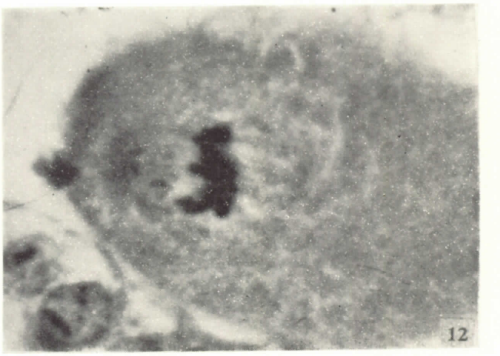

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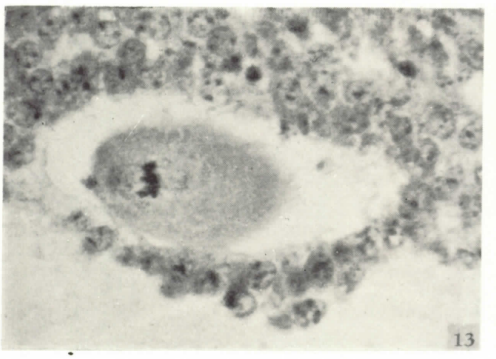
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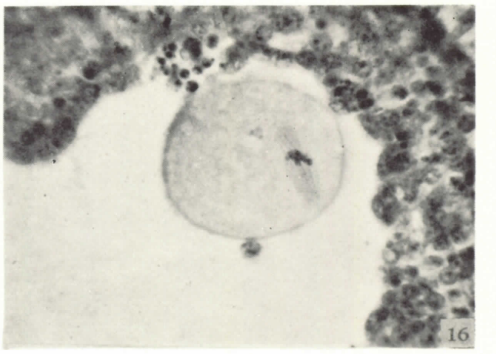
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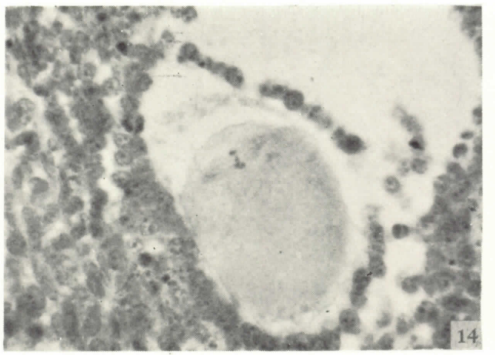
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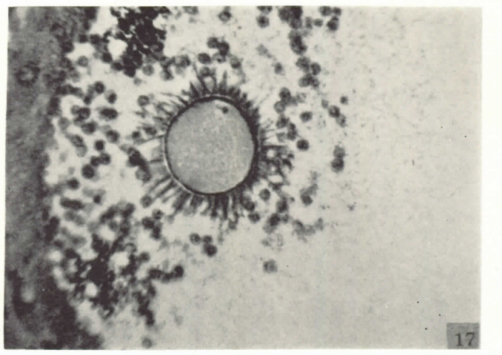
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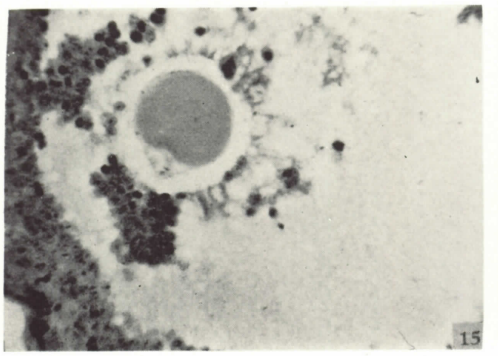
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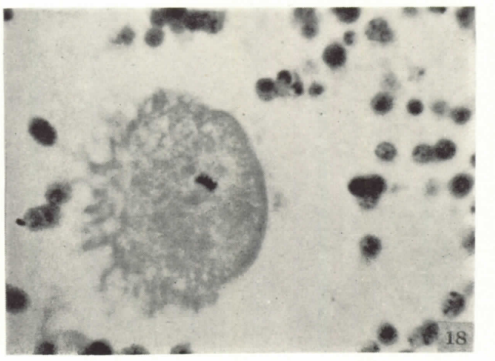
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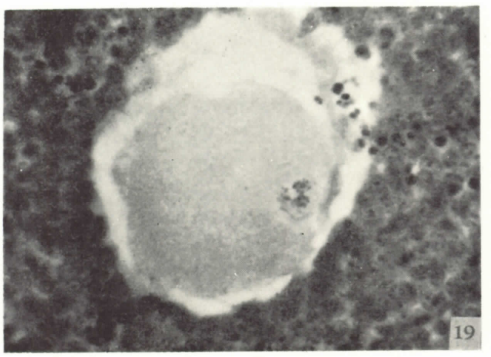

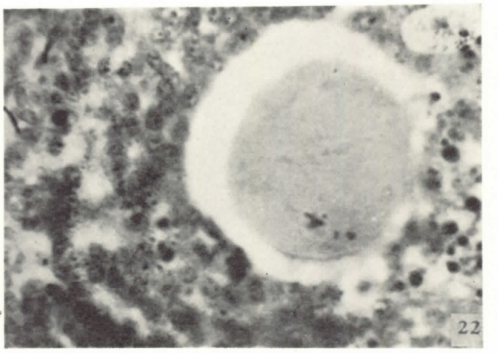

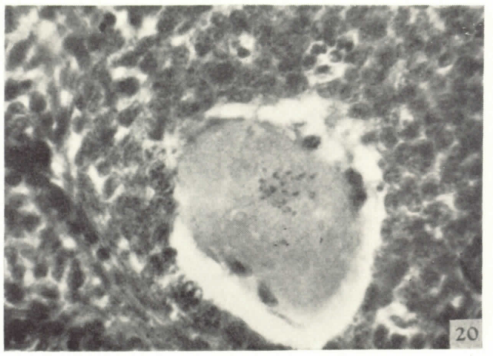

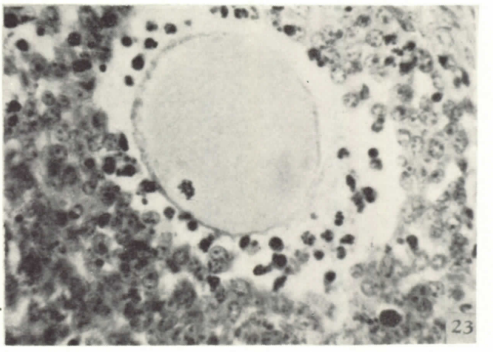

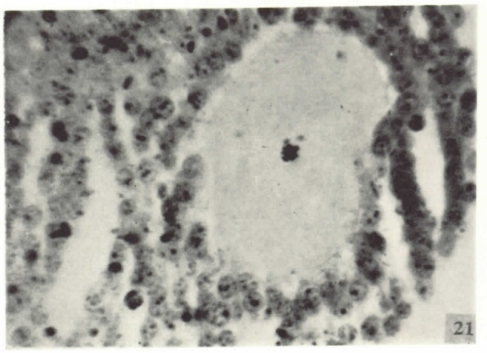

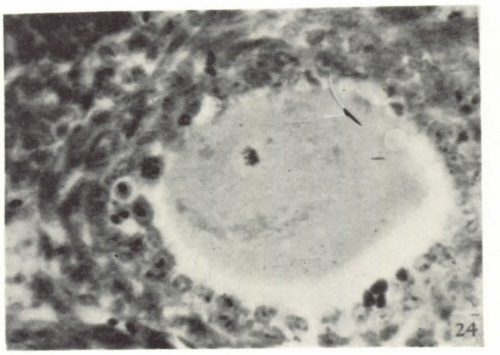

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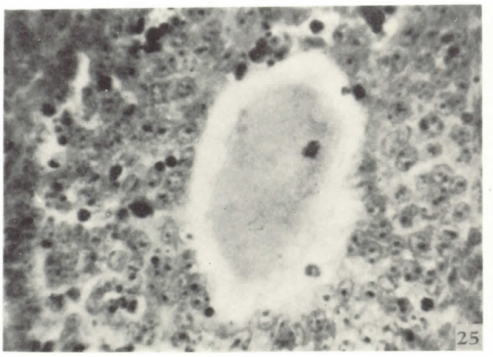

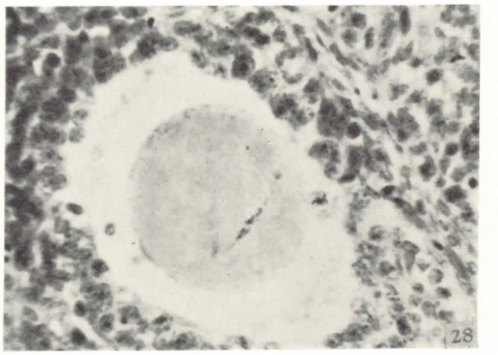

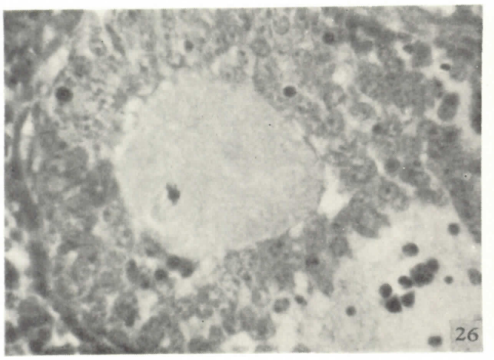

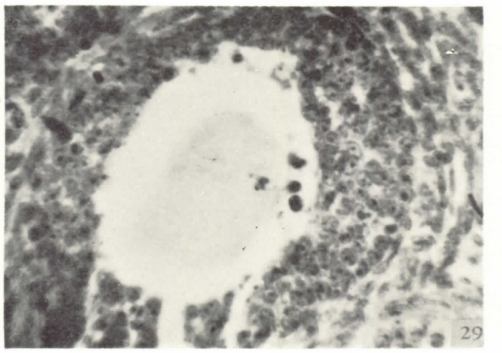

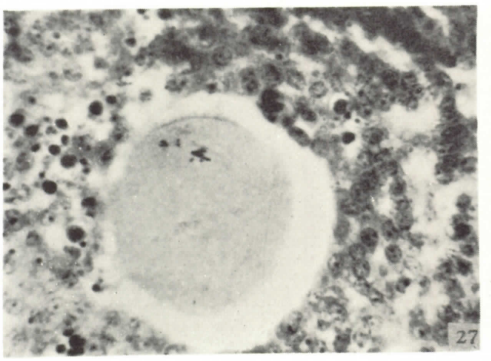

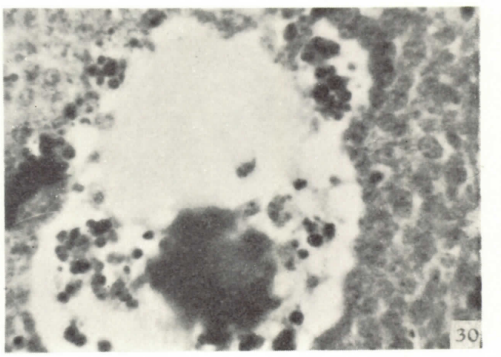

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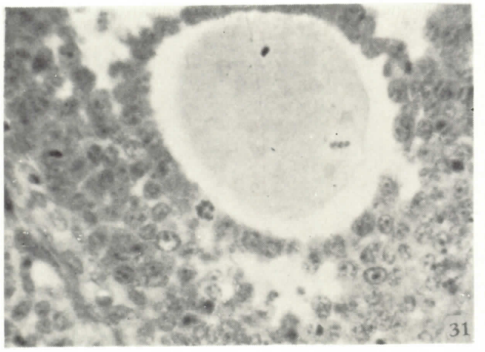

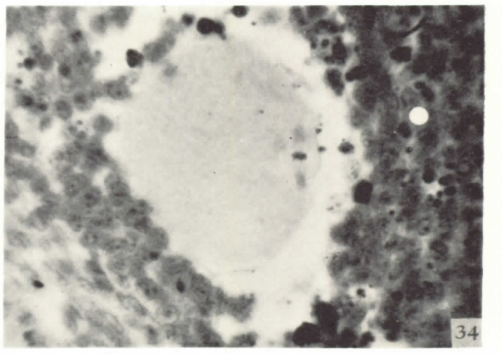

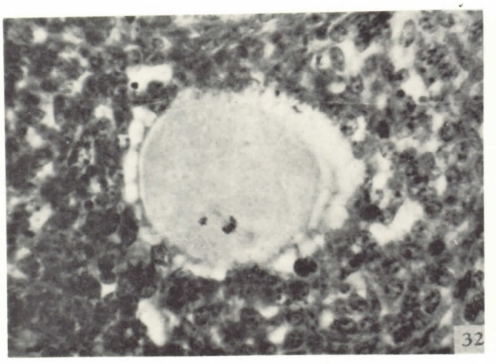

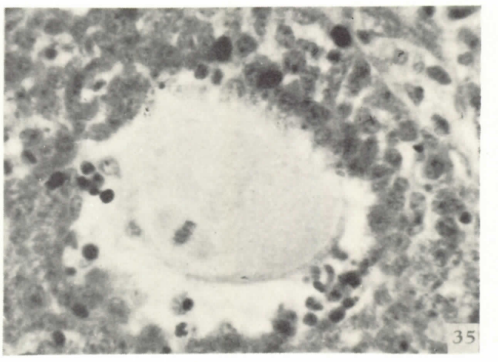

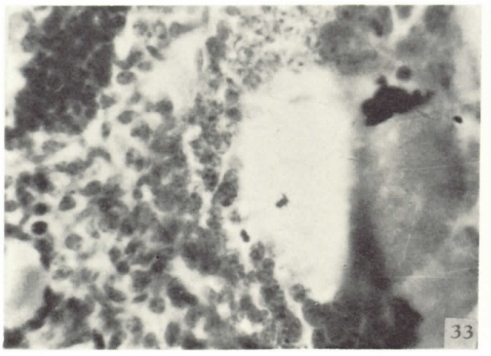

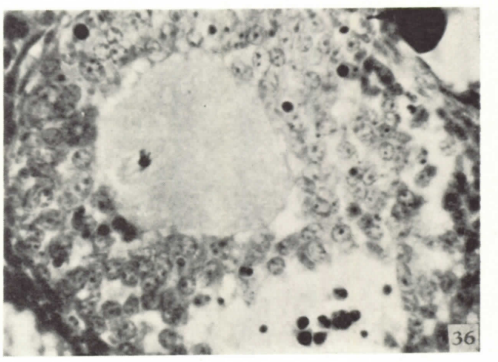

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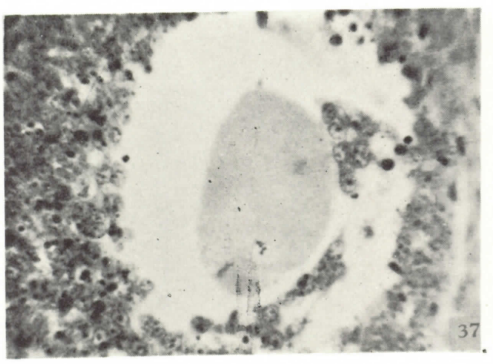

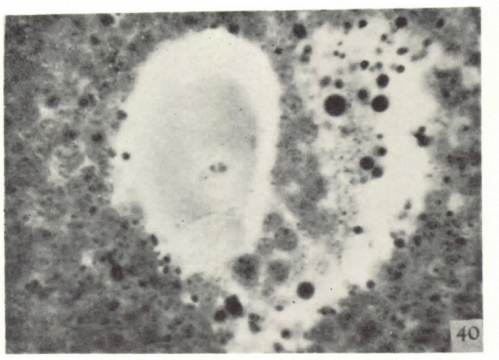

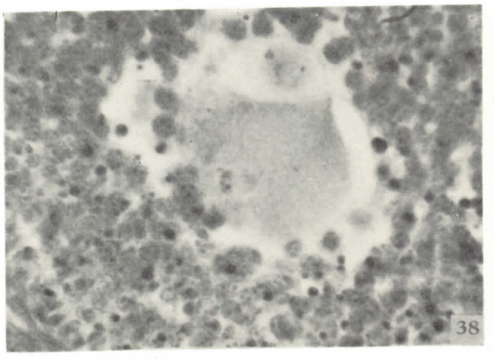

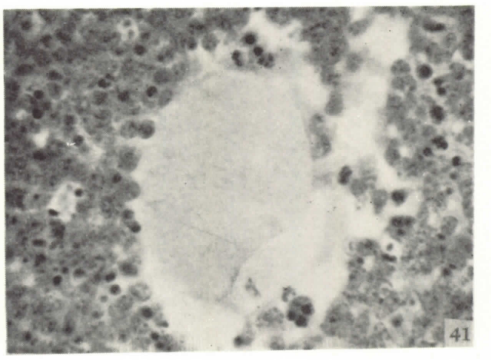

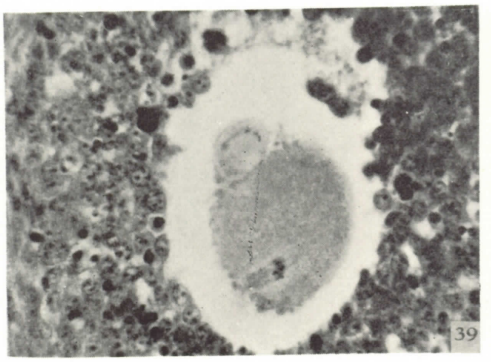

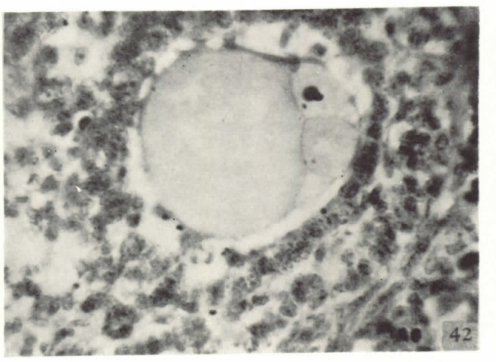

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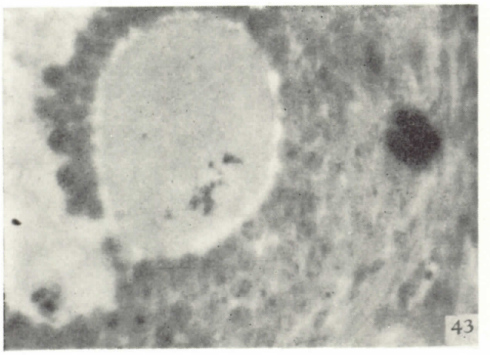

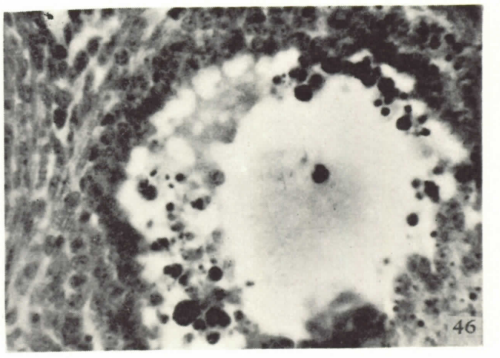

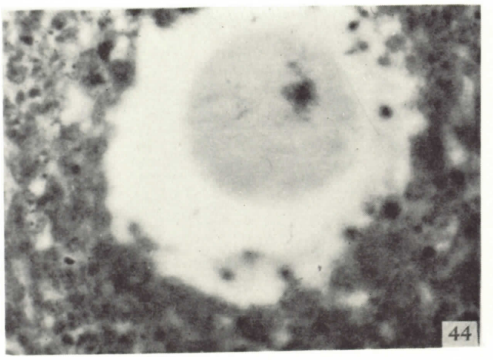

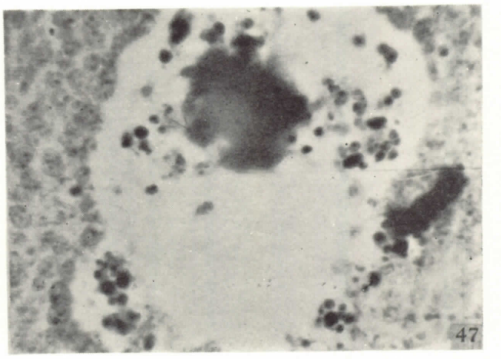

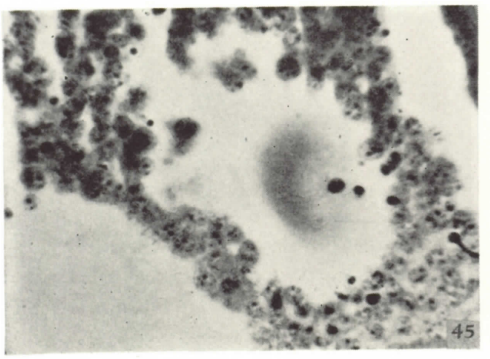

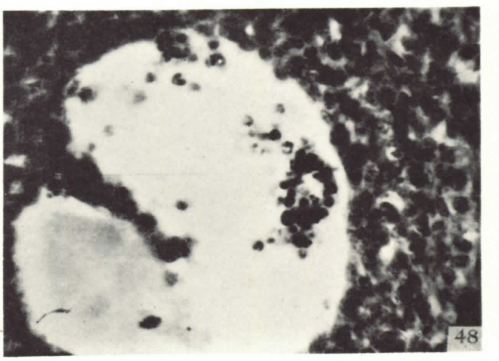

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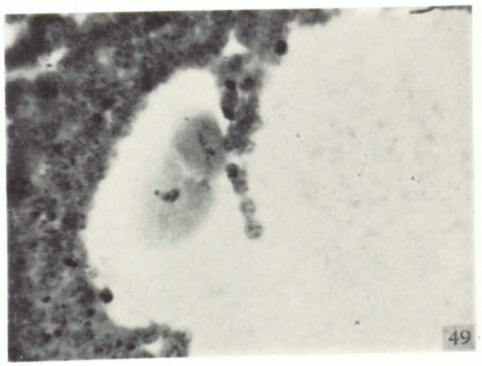

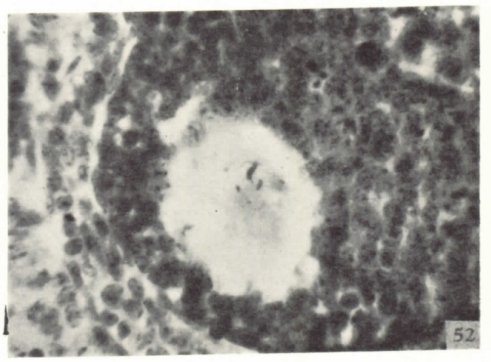

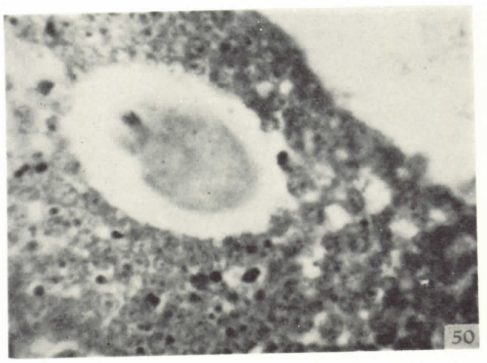

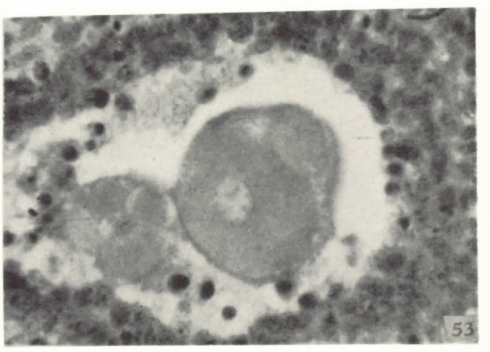

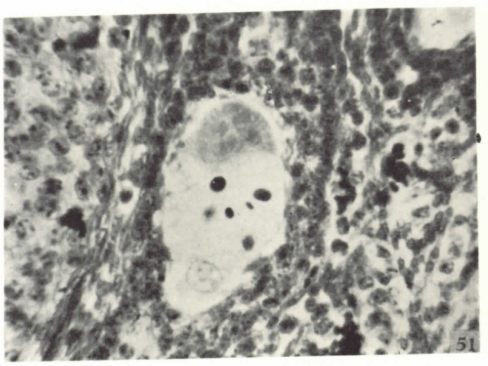

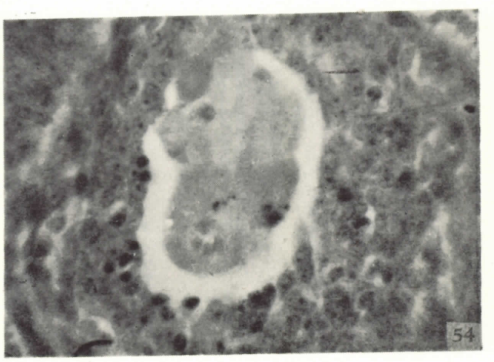

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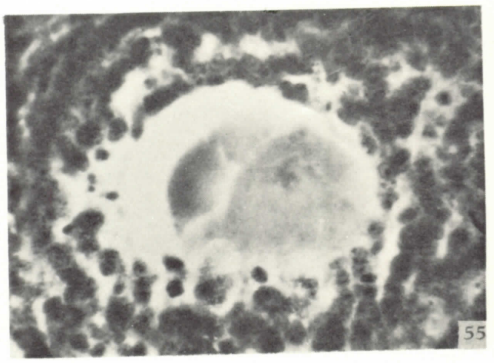

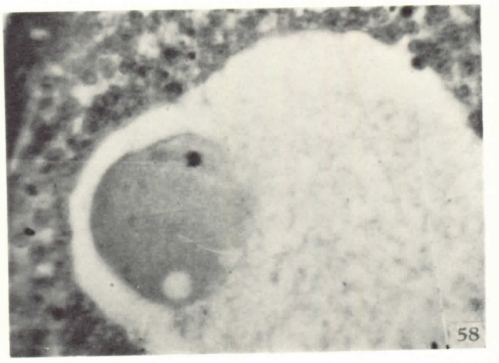

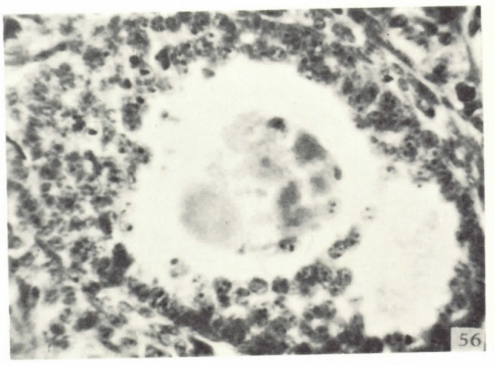

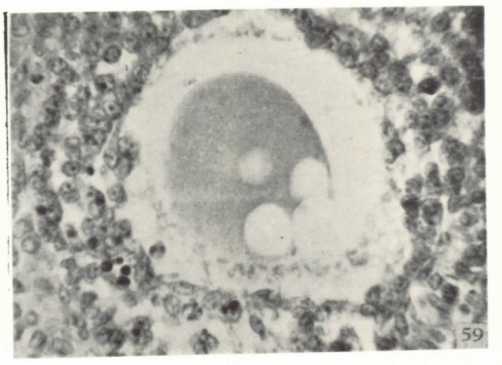

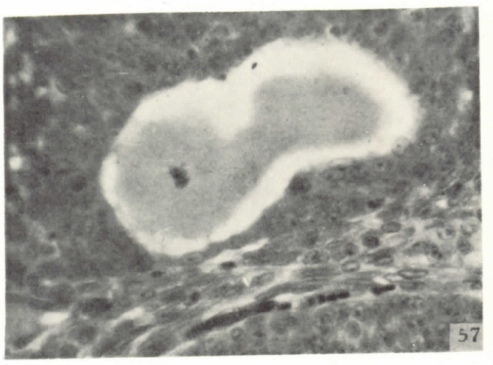

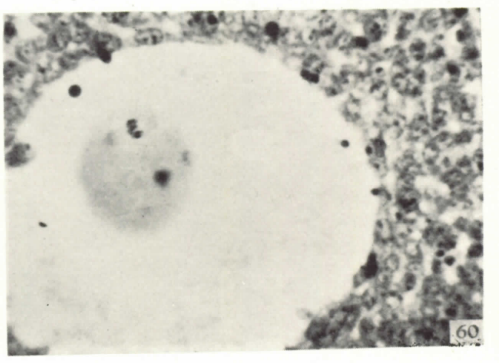

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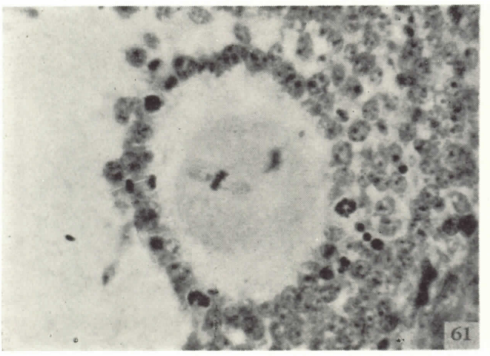

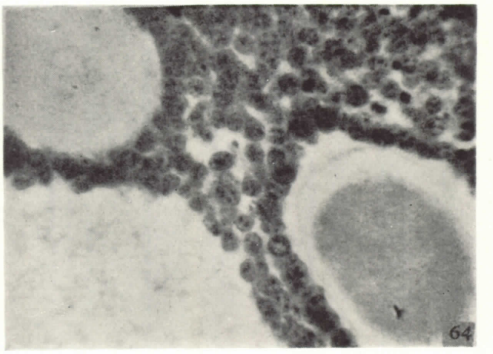

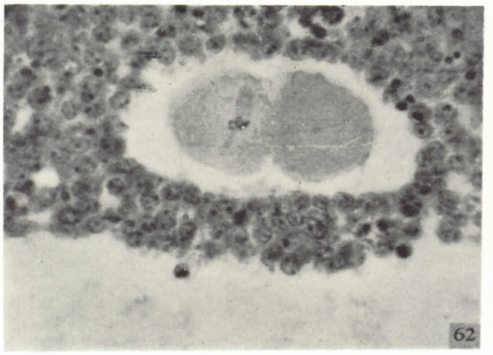

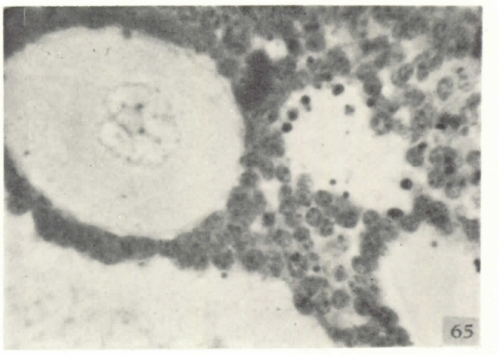

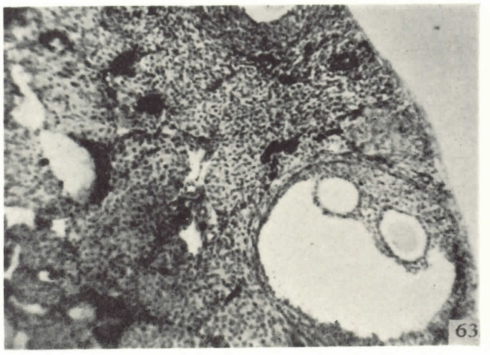

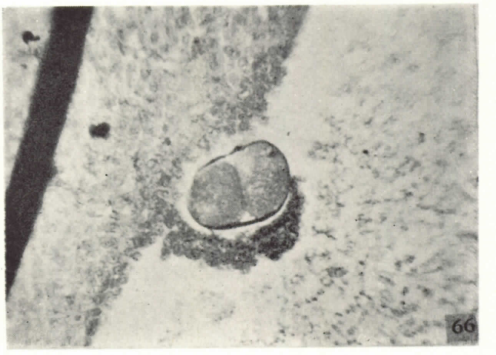

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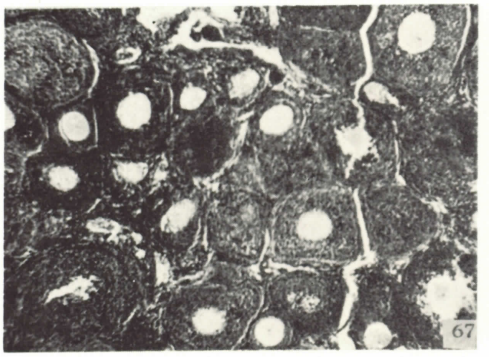

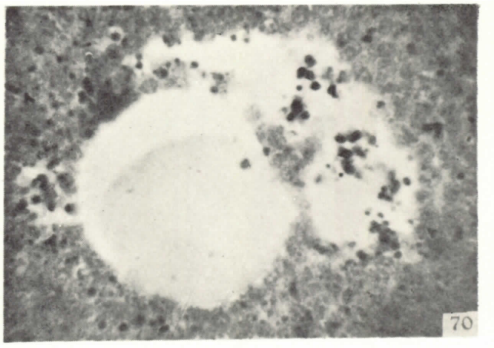

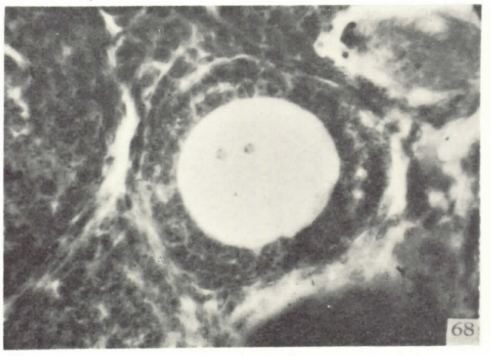

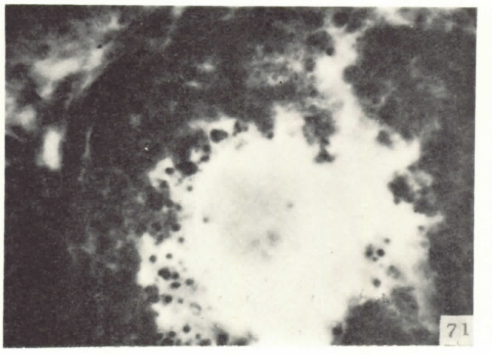

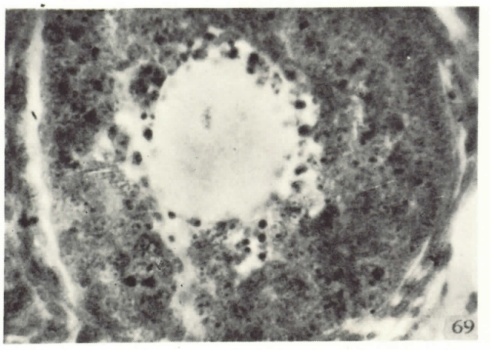

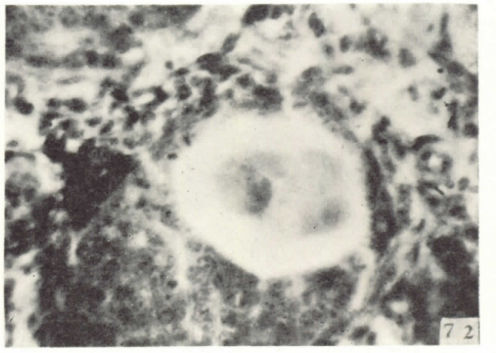

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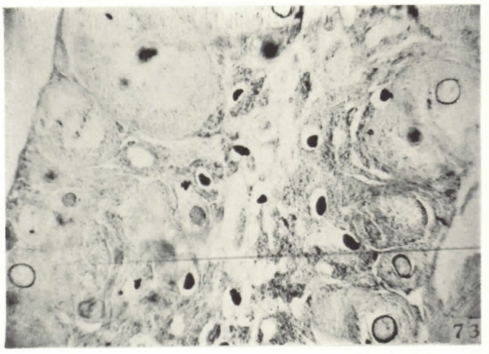

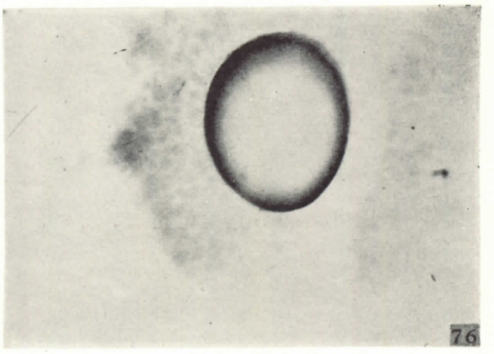

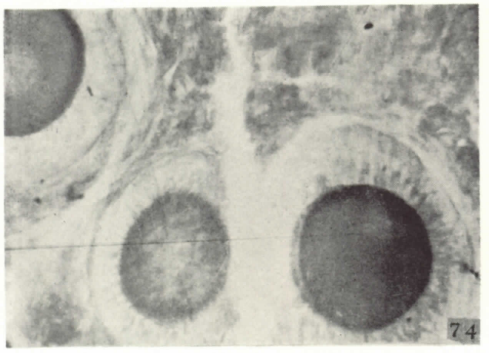

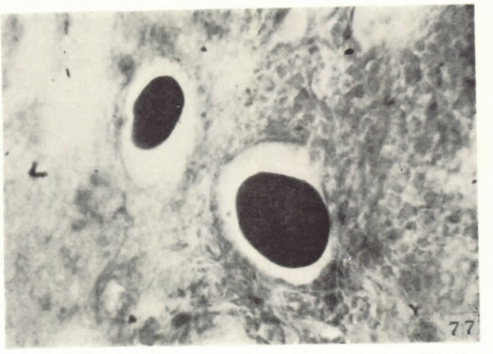

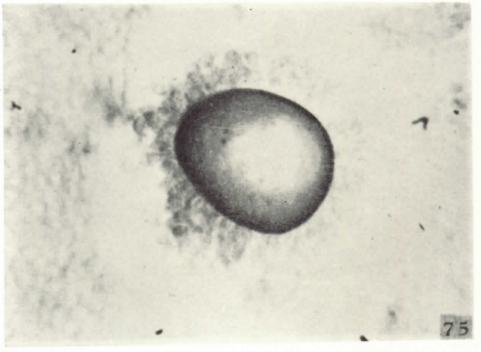

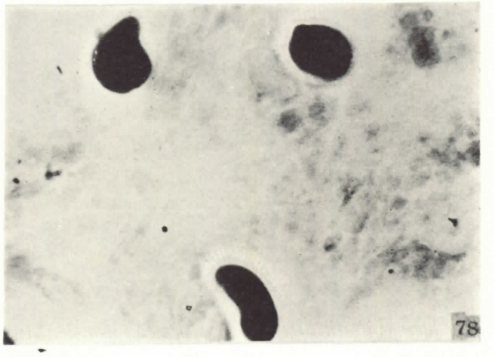

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