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By

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Introduction

Morphological studies of the atretic ova in the ovary have been made by many investigators (Pincus (1936), Kingsbury (1939), Allen (1950), Rennels (1951), Pederson (1951), and etc.). The most interesting facts in their reports are the cell division of the ovarian ova during the early stage of atresia and the invasion of certain cells through the zona pellucida into the ova.

Recently, histochemical studies of the atretic ova in the ovary have been performed by many investigators. Harter (1948) studied on the polysaccharides in the rat ovary and reported that the histochemical nature of these substances in the atretic ova differed from that in the normal ones. Vincent (1948) studied the interrelationship of the DNA and RNA in the ova and in the follicular cells and reported that the RNA did not decrease during their degenerating processes owing to the transformation of DNA to RNA. Deane (1951) reported the appearance of the polysaccharides and phosphatases in the rat ova during their atresia. Further, he (Deane, 1952) discussed the intersignificance among polysaccharides, DNA, RNA and phosphatases in the atretic ova of the rat.

These mentioned investigations do not describe either detailed morphological or histochemical observations.

In the previous paper (1952), the author reported that some ova in the adult rat had no glycogen, but others contained a small to large amount of it, and the atretic ova also possessed glycogen.

In the present investigation I have dealt with the prosperity and decay of the polysaccharides, nucleic acids and phosphatases in the ovarian ova of the rat during the course of the atresia together with morphological observations of the atretic ova, and also with the morphology and histochemistry of the cells penetrating into the atretic ova, as the second step of the investigation concerning the physiological significanec of the chemical substances in the ovary.

Materials and Methods

As materials, ovaries were taken from 50 adult rats. 95 per cent alcohol was used as fixing fluid. All materials were embedded in celloidin and sectioned 20 μ thick. The staining methods were as follows: For the demonstration of polysaccharides, the sections were stained by Best's carmine method, Bauer-Feulgen's method, periodic acid-Schiff method (PAS) modified by Lillie. For acid polysaccharides, the metachromatic reaction of thionin was used. The identification of glycogen was made by means of salivary test at 37°C in an incubator. For DNA, Feulgen's nuclear reaction, for RNA pyronin-methylgreen and thionin were used. RNA was confirmed by ribonuclease treatment. For the demonstration of alkaline and acid phosphatase, Gomori's revised method was employed by using sodium glycerophosphate as a substrate.

Morphology and histochemistry of the cells penetrating into the atretic ova was made by the same histochemical procedures as mentioned above and by haematoxylin and eosin staining.

Results

1. *Morphological observations of atretic ova.*

The atresia of the ova was found in the follicles of various stages, primary, secondary and Graafian follicles.

When the primary follicles were doomed to destruction the ova shrunk and degenerated. The follicular cells showed a tendency to engulf the debris of the ova, but they also degenerated quickly, afterwards the small follicular cavities in the stroma were closed entirely with the connective tissue.

In the secondary and the Graafian follicles the ova and the follicular cells showed various signs of degeneration. With the increase in size of the follicles undergoing atresia, the histological pictures became more complicated and variable as compared with those of the primary follicles. At the begining of the restrogression the cells near the cavity of the follicle were affected. The peripheral cells adjacent to the basement membrane, as well as the cumulus, remained alive for a considerable time and even showed mitosis. The first sign of the restrogression was the disarrangement of the follicular cells. Afterwards, these cells showed the various degenerative changes as described below.

(1) First type of the degeneration: The nucleus became globular in various sizes and the cytoplasm disappeared after shrinkage, leaving the nucleus in the follicular fluid. The globular nuclei were Feulgen positive, although some of them were stained faintly. Some nuclei became often larger than the follicular cells apparently by fusing together. These nuclei disappeared by shrinkage sooner or later (Fig. 16, b).

(2) Second type of the degeneration : The cytoplasm of the follicular cells hypertrophied and became polygonal and round. Frequently, one or more vacuoles of various size appeared in the cytoplasm. The nuclei remained morphologically normal for a long time, but finally disappeared, when the cytoplasm also dissolved completely (Fig. 16, c). The cells undergoing such degenerative process certainly penetrated into the atretic follicles as will be discussed in Sections 2 and 3 where these cells were described as "the cells transformed from the follicular cells".

Soon after the destruction of the follicular cells the hypertrophied theca interna cells, which surrounded the cavity of the atretic follicle, developed interstitial glands, which were irregularly scattered in the stroma.

At the early stage of atresia, the degenerating ovum which lost its corona radiata and floated freely in the follicular fluid often showed signs of an atypical development : Maturation spindle (Pseudomaturation spindle) appeared near the surface of the ovum (Figs. 1, 11). This aspect was only found in the ovum of the fully grown Graafian follicle.

Afterwards, the ovum with the maturation spindle as described above began atrophy apparently through the three kinds of degenerating processes as follows :

(1) The nucleus of the ovum showed the so-called piknosis, and its cytoplasm shrunk, as it is, without fragmentation or cleavage (Figs. 11, 12).

(2) The ovum performed an equal cell division from about 2 to 8 cells stages, and their cytoplasm transformed into irregular cell masses which finally disintegrated leaving only the zona pellucida (Figs. 2, 3, 4, 8, 9, 10).

(3) The cytoplasm of the ovum performed an irregular cell division and irregular cell masses of various sizes were formed inside the zona pellucida. Some of them contained one or more condensed chromatins. The cells transformed from the follicular cells penetrated into these cell masses through the zona pellucida. Finally these cell masses disappeared completely (Figs. 6, 7, 8, 9, 10).

In each case described above, after the disappearance of the cell masses, the zona pellucida remained twisted for a long time in the central cavity of the interstitial glands, but finally it disappeared completely.

The ratio of the ova which showed equal cell division and the ones which showed irregular cell division (fragmentation) is given in Table 1.

As shown in Table 1, among the atretic ova observed, the ova showing equal cell division amounted to 18.5 per cent and the ones showing cell fragmentation were 81.5 per cent of the total . The number of the ova showing equal cell division in the early stage exceeded those in the later stage. On the other hand, the ova of fragmentation division containing invaded cells amounted to 38.9

Table 1. Atretic ova showing equal cell division and cell fragmentation, and number of cells penetrating into them.

Stages of cell division of atretic ova	Total ova counted	Ova showing equall cell division				% of ova showing equal cell division	Ova showing cell fragmentation				% of ova showing cell fragmentation
		Ova without invaded cells	Ova with invaded cells	Sum	% of ova with invaded cells		Ova without invaded cells	Ova with invaded cells	Sum	% of ova with invaded cells	
1 cell	116						82	34	116	29.3	
2 cells	75	26	3	29	10.3	34.7	29	17	46	37.2	65.3
3 cells	49	5	0	5	0	6.1	24	20	44	45.5	93.9
4 cells	25	4	0	4	0	16.0	12	9	21	42.9	84.0
5 cells	21	3	0	3	0	14.3	7	11	18	61.1	85.7
6 cells	14	1	0	1	0	7.1	7	6	13	46.2	92.9
7 cells over	27	0	0	9	0	0	13	14	27	51.9	100.0
Total	327	39	3	42	7.1	18.5	174	111	285	38.9	81.5

per cent of the total in the average. On the contrary, the ova of equal cell division had no invaded cells. As is generally known, this equal cell division is distinctly a parthenogenetic development. The cell fragmentation, however, is not considered as a parthenogenetic development, but merely a destruction of cytoplasm because of the fact that each fragment has no definite configuration though it contains condensed chromatin-like substances (Figs. 6, 7, 8, 9).

Various kinds of cells penetrated into the atretic ova through the zona pellucida. The details of the penetrating of these cells are given in Table 2.

Table 2. Kinds of cells penetrating into the atretic ova.

Stage of cell division of atretic ova	Total ova counted	Ova without invaded cells	Ova with cells					Total ova with invaded cells	% of ova with invaded cells
			Ova with follicular cells	Ova with cells transformed from follicular cells	Ova with mast cells	Ova with connective tissue cells	Ova with interstitial gland cells		
Ova with pseudomaturation spindle	65	65	0	0	0	0	0	0	0
1 cell	116	82	1	29	0	2	2	34	29.3
2 cells	75	55	1	14	0	3	2	20	26.7
3 cells	49	29	1	18	1	0	0	20	40.8
4 cells	25	16	0	8	0	1	0	9	36.0
5 cells	21	10	0	7	0	2	2	11	52.4
6 cells	14	3	0	5	0	1	0	6	42.9
7 cells over	27	13	0	10	0	2	2	14	51.9
Zona pellucida only	21	6	0	8	0	4	3	15	71.4
Total	413	284	3	99	1	15	11	129	31.2

As shown in Table 2, the ova with invaded cells were about 31.2 per cent of the total. The ratio of invaded cells was higher in the later stage of atresia than in the early stage. Five kinds of invaded cells were histochemically demonstrated as follows; follicular cells, cells transformed from the follicular cells, mast cells, connective tissue cells, interstitial gland cells. Among them, the cells transformed from the follicular cells were most numerous.

Pincus (1936) mentioned that the pseudomaturation spindle occurred as the result of pituitary hormone action and the subsequent atresia of the ova containing them occurred because these ova were not liberate and fertilized. He also stated that the pseudomaturation spindle did not appear in the hypophysectomized animals although atersia had. Recently, Rennels (1951) reported that pseudomaturation spindle appeared in the onset of atresia of the rat ova. Allen (1930) stated that the cytoplasm of human ova was vacuolated during the course of atresia, and further that the follicular cells penetrated into the cytoplasm of the atretic ova through the zona pellucida. On the contrary, however, Pederson (1951) studied the atretic follicles of the rats and reported that all follicular cells restrogressed without exception in the early stage of the atresia and there was no evidence of the follicular cells penetrating into the ova.

In the present investigation of the atretic ova of the rat the invaded cells were divided into five forms, follciular cells, cells transformed from the follicular cells, mast cells, connective tissue cells, interstitial gland cells ; the second form was most numerous.

2. *Histochemical observations of atretic ova.*

As the next step of this investigation, polysaccharides, nucleic acids, and phosphatases in the atretic ova were demonstrated histochemically.

The normal ova in the primary follicles of the adult rats contained no polysaccharides, phosphatases and RNA. The atretic ova in the primary follicles also did not contain these substances. In the secondary and Graafian follicles of the adult rats, the normal ova, each of which differed in glycogen and phosphatase contents, but was the same in RNA content, were found. In these follicles the atretic ova were also found and their histochemical pictures were complicated ; namely, polysaccharides, nucleic acids and phosphatases were demonstrated as given in Table 3.

(1) Polysaccharides.

Stained with Best's carmine fluid, the red coloured granules of polysaccharides were demonstrated in the cytoplasm of the atretic ova. (Figs. 1 to 10). Using the PAS method, they were demonstrated in red-purple granules. In this case the zona pellucida was stained diffusely (Fig. 13). Since the Best's carmine and PAS reactive substances in the cytoplasm of the atretic ova disappeared by means of a salivary test, they are glycogen. On the contrary, however, the PAS reactive substance in the zona pellucida was not digested by saliva, accordingly it may be considered as glycoprotein as has been stated by Harter (1948).

As shown in Table 3, the atretic ova which differed extremely in glycogen content were found ; namely, some ova contained no glycogen, but others

Table 3. Histochemical aspects of atretic ova.

Methods	Normal ova	Atretic ova						
		Pseudo-maturation spindle	1 cell	2 cells	3 and 4 cells	5 cells	Trace of cytoplasm	Zona pellucida only
Best's carmine	− to +++	− to +++	− to +++	+++	+++	+++	− to +++	−
PAS	− to +++	− to +++	− to +++	+++	+++	+++	− to +++	+++
Metachromasia (Thionin)	−	−	−	−	−	−	−	−
(Salivary test)	−	−	−	−	−	−	−	−
Thionin	+	± to +	−	−	−	−	−	−
Pyronin-methyl-green	+	± to +	−	−	−	−	−	−
(RNA-ase)	−	−	−	−	−	−	−	−
Alkaline phosphatase	− to +	− to +	− to ++	++	+++	+++	− to +++	−
Acid phosphatase	+	+	+	+	+	+	+	−
Eosin	+	+	+	+	+	+	+	−

possessed it in small or in large amounts. The former followed the course of (1) as described in Section 1 : The nuclei of the ova showed piknosis and their cytoplasm shrunk without fragmentation or cleavage. The latter followed the course of (2) and (3) : The ova performed equal cell division and then their cytoplasm disintegrated leaving only the zona pellucida (2), or the cytoplasm of the ova performed irregular cell division and thus had irregular cell masses, in which the cells transformed from the follicular cells penetrated through the zona pellucida (3). In the ova which followed the course of (2) and (3), glycogen granules were found during the course of atresia. The glycogen granules became coarses with the progress of atresia and finally disappeared with the disintegration of the cell masses. Acid polysaccharides were not demonstrated in the atretic ova.

(2) Nucleic acids.

An intense accumulation of DNA occurred in the pseudomaturation spindle which appeared near the surface of the ova in the onset of the atresia, and in the condensed chromatin that appeared in the irregular cell masses in the later stage of it.

As shown in Table 3, RNA occurred in the cytoplasm of the normal ova without exception, afterwards it almost disappeared in the stage of pseudo-maturation formation.

(3) Phosphatases.

As shown in Table 3, alkaline phosphatase reaction occurred in the cytoplasm of the ova during the course of the atresia (Fig. 14). The amount of it differed in different ova agreeing with the amount of glycogen : In the ova containing a large amount of glycogen, alkaline phosphatase reaction was high, though in the ova containing no glycogen, its reaction was negative. Acid phosphatase reaction also occurred in the cytoplasm of the atretic ova (Fig. 15).

The glycogen in the atretic ova has been already been reported by many investigators (Brandenburg (1938), Harter (1948), Deane (1952), Ishida (1952)). However, there are no detailed descriptions of the glycogen in the ova during their degenerating processes. In the present investigation, glycogen was found in the cytoplasm of the ova during the course of the atresia. Probably, it has an important role for the cleavage and the fragmentation of the ova.

Vincent (1948) reported that the RNA in the cytoplasm of the rat ova disappeared in the course of the atresia, coinciding nearly with the results obtained in the present investigation. Davidson (1947) claimed that the RNA content in the young tissue was very high. Caspersson (1947) demonstrated unequvocally that protein synthesis was associated with an increase in RNA, and that intense protein synthesis was characteristic of cells during rapid growth and secretion. As to the fact that no RNA was found in the later stage of the atresia, it is considered that no more protein synthesis occurs in that stage.

Although histochemical studies of the atretic ova of mammals have been made by many investigators no one has reported on the appearance of phosphatase in them. In the present investigation both alkaline and acid phosphatase were found in the atretic ova of the rat.

The atretic ova of the rat, containing glycogen, perform either cell fragmentation or equal cell division as a parthenogenetic development. It is clear that the energy for these processes is given by decomposition of the glycogen, because glycogen and phosphatase are always found in the ova which represent a parthenogenesis throughout the course of the atresia.

3. *Histochemical observations of invaded cells.*

Various kinds of cells penetrated into the atretic ova as already mentioned in Section 1. Histochemical studies of these cells were made with the results given in Table 4.

As shown in Table 4, the PAS reactive substance did not appear in the normal follicular cells. However, it appeared in the cells of the early stage which were transformed from the follicular cells, and subsequently disappeared with shrinkage of them. This PAS reactive substance was glycogen, because it was digested by saliva. Metachromatic reaction of thionin was negative during the

Table 4. Histochemical natures of the cells penetrating into the atretic ova.

Methods	Follicular cells	Cells transformed from follicular cells			Mast cells	Connective tissue cells	Interstitial gland cells
		early stage	middle stage	later stage			
Best's carmine	−	−	−	−	+++	−	−
PAS	−	−	+	−	+++	−	−
Metachromasia (Thionin)	−	−	−	−	+++	−	−
Thionin	+++	++	+	−	+++	−	−
Alkaline phosphatase	−	−	+	−	+++	−	−
Acid phosphatase	−	−	−	−	+	−	−
Aldehyde fuchsin	−	−	−	−	+++	−	−
Eosin	−	−	−	−	+	+	−
Neutral red	−	−	−	−	+++	−	−

course of degenerative process. A great amount of DNA was always contained in the nuclei which became globular in various sizes. A great amount of RNA was contained in the normal follicular cells, but it decreased gradually with their transformation process, and finally disappeared completely. Alkaline phosphatase activity did not occur in the normal follicular cells. However, it appeared in the cytoplasm of the cells transformed from the follicular cells, agreeing with the appearance of glycogen. No acid phosphatase activity was found in both the normal and transformed cells.

Besides the normal follicular cells and the cells transformed from them, mast cells, connective tissue cells, and interstitial gland cells penetrated into the atretic ova. As shown in Table 4, no histochemical views were found in these cells with the exception of the mast cells, in which glycogen, acid polysaccharide, RNA, alkaline and acid phosphatase were detected in large amount.

Eosinophilic leucocytes, neutrophilic leucocytes, lymphocytes were investigated as controls ; the first contained a small amount of PAS reactive polysaccharide and of alkaline phosphatase, the second, a large amount of glycogen and of alkaline phosphatase, and the third, a small to large amount of RNA. None of them were found in the atretic ova.

Deane (1951, 1952) reported that although the normal follicular cells of the rat was normally lacking in glycogen and alkaline phosphatase, the atretic follicular ones displayed a large amount of them. In the present investigation, both the normal follicular cells and the atretic follicular ones contained neither glycogen nor phosphatase, but when they transformed their shape to penetrate into the atretic ova, a large amount of the substances were found in their cytoplasm.

Vincent (1948) reported that RNA in the follicular cells of the rat did not decrease during the degenerative process. In the present investigation, however, RNA in the follicular cells disappeared completely in the early stage of the degenerative process.

Allen et al. (1930) studied the atretic ova of human and reported that many follicular cells penetrated into the atretic ova. In the present investigation, various kinds of the invaded cells, such as normal follicular cells, cells transformed from the follicular cells, mast cells, connective tissue cells, and interstitial gland cells were found in the atretic ova as already mentioned. Furthermore, in these cells various kinds of histochemical substances, such as glycogen, RNA, and alkaline phosphatase were found.

Summary

The results obtained in this investigation are summarized as follows :

1. When the primary follicles were doomed to destruction the ova shrunk and degenerated. The first sign of degenerating ova in the secondary and Graafian follicles was the appearance of pseudomaturation spindle. Afterwards, the ova began to atrophy through three kinds of degenerating process as follows :

(a) The nucleus of the ovum showed the piknosis, and its cytoplasm shrunk.

(b) The ovum performed equal cell division, and then their cytoplasm became irregular cell masses and finally disappeared.

(c) The cytoplasm of the ovum performed irregular cell division by which were formed irregular cell masses. Various kinds of cells penetrated into these cell masses.

2. Various kinds of cells penetrated into the atretic ova ; follicular cells, cells transformed from the follicular cells, mast cells, connective tissue cells, interstitial gland cells. Among them, the second kind were most numerous.

3. Atretic ova which differed extremely in glycogen content were found ; some ova contained no glycogen, but others contained it in a small or in a large amount. The former followed the course of (a), and the latter the course of (b) and (c).

4. An intense accumulation of DNA occurred in the pseudomaturation spindle and in the condensed nuclei. RNA in the cytoplasm of the atretic ova almost disappeared in the early stage of atresia.

5. The amount of alkaline phosphatase differed from the different ova, agreeing with the amount of glycogen. Acid phosphatase reaction also occurred in the cytoplasm of atretic ova.

6. The degenerative processes of the follicular cells were as follows :

(a) The nuclei became globular in various sizes, and then their cytoplasm

shrunk and finally disappeared.

(b) The cytoplasm of the follicular cells hypertrophied and became polygonal or round. Frequently, one or more vacuoles appeared in the cytoplasm.

7. The cells transformed from the follicular cells contained glycogen, alkaline phosphatase and RNA.

Acknowledgement: Before leaving the subject, I wish to express my hearty thanks to Prof. Dr. Y. Toryu for his valuable guidance and critism throughout the entire course of this work.

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Plate 1
Explanation of Figures

Fig. 1. An atretic ovum with pseudomaturation spindle.
Fig. 2. An atretic ovum in two cells stage.
Fig. 3. An atretic ovum in three cells stage.
Fig. 4. An atretic ovum in four cells stage.
Fig. 5. An atretic ovum without cell division. The cells transformed from the follicular cells are seen in the atretic ovum.
Fig. 6. An atretic ovum in fragmentation division. No follicular cells are seen in the cytoplasm.
Fig. 7. An atretic ovum in fragmentation division. Many follicular cells are seen in the cytoplasm.
Fig. 8. An atretic ovum in fragmentation division. Many cells transformed from the follicular cells are seen in the cytoplasm.
Fig. 9. An atretic ovum in fragmentation division. The cells transformed from the follicular cells and the connective tissue cells are seen in the cytoplasm.

Plate 1

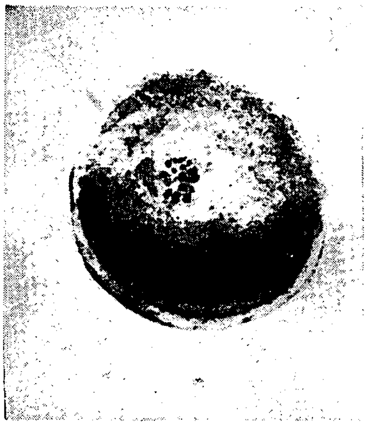
Fig. 1.

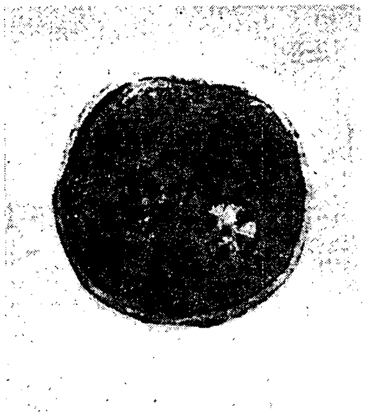
Fig. 2.

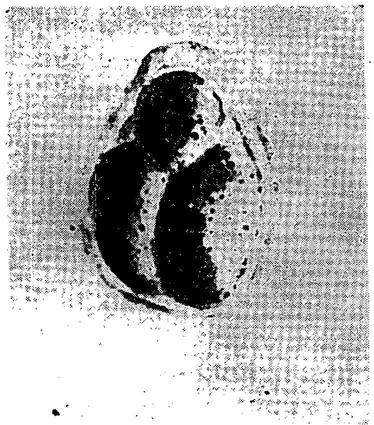
Fig. 3.

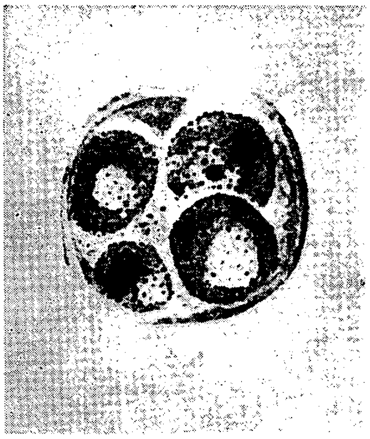
Fig. 4.


Fig. 5.

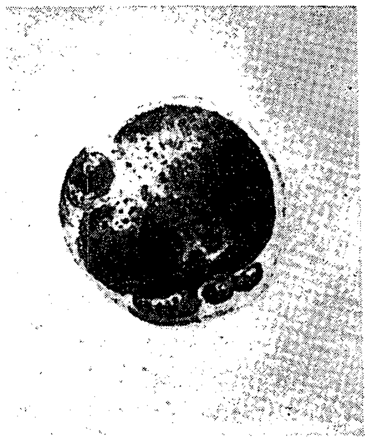
Fig. 6.


Fig. 7.


Fig. 8.


Fig. 9.

Plate 2

Explanation oi Ffgures

Fig. 10. An atretic ovum in the later stage of atresia.
Fig. 11. An atertic ovum with pseudomaturation spindle.
Fig. 12. An atretic ovum with shrunken cytoplasm.
Figs. 1–12 : Best's carmine stain. × 625.
The ova in Figs. 1–10 contain a large amount of glycogen, and those in Figs. 11 and 12 do not.
Fig. 13. An atretic ovum. PAS stain. × 625. The ovum contains a large amount of glycogen.
Fig. 14. An atretic ovum. Alkaline phosphatase reaction. Gomori's method. × 625. Intense reaction occurs in the cytoplasm.
Fig. 15. An atretic ovum. Acid phosphatase reaction. Gomori's method. × 625. Intense reaction occurs in the cytoplasm.
Fig. 16. Follicular cells in degenerating process. Thionin stain × 1000.
a. Normal follicular cells containing a large amount of RNA.
b. Follicular cells in various stages of degeneration.
c. Follicular cells in various stages of transformation.

Plate 2

Fig. 10.

Fig. 11.

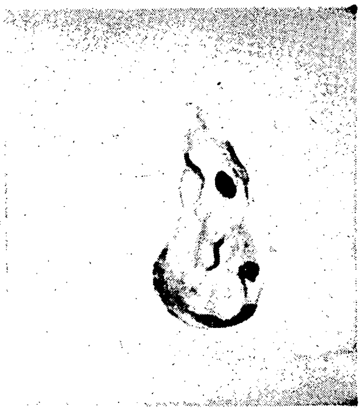

Fig. 12.

Fig. 13.

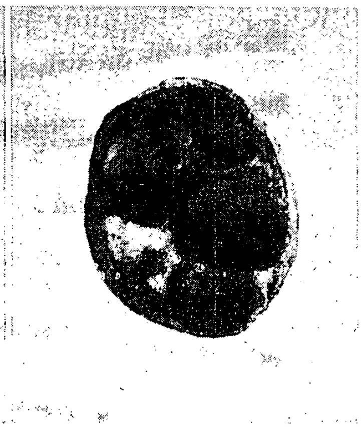

Fig. 14.

Fig. 15.

Fig. 16.