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By

Kazuo ISHIDA

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Introduction

Cytochemical studies of glycogen, nucleic acids, phosphatases in the mammalian ovaries have been performed by many investigators following the recent progress of cytochemistry, but there are only several who have studied on these chemical substances in the ovaries of domestic animals as rodents, especially rats.

Togari (1927) studied the appearance of glycogen in the ovaries of rodents as rabbits, mice, rats and guinea-pigs. He stated that all tissues in the adult ovaries usually contained no glycogen except the ova. Brandenburg (1938) and Harter (1948), who made similar studies in the ovaries of rats, reported that a large amount of it was contained in the normal and atretic ova. Deane (1951) studied the histochemical characteristics of ovarian follicles in the ovaries of the rats and reported that little glycogen was detectable in normal follicles in which only the ova contained a large amount of it, but in the atretic follicles it occurred within the follicular cells as well, especially those of the cumulus oöphorus. Ishida (1952, 1953) performed histochemical studies of the rat ovaries and stated that in the normal and atretic follicles the ova which differed extremely in glycogen content were found ; some ova contained no glycogen, but others contained it in a small or a large amount.

Vincent (1948) and Jones-Seaton (1950) studied the distribution of RNA in the ovaries of rats and reported that a small amount of it was always contained in the ova at various stages of development.

Dempsey, Greep and Deane (1949) reported that alkaline phosphatase was demonstrable in the Graafian follicles, corpora lutea, interstitial glands and blood vessels of the rat ovaries. Kaizuka (1949) studied the appearance of alkaline phosphatase in the ovaries of rabbits and obtained results similar to those authors.

Quen (1952) and Ishida (1953) reported that lipase was demonstrated in the ova, theca folliculi, corpora lutea and blood vessels of the rat ovaries.

In the present investigation I have dealt with the appearance and localization of glycogen, RNA, phosphatase and lipase in the ovaries of domestic animals and of rodents. I have also studied the histological differences in their ovaries.

Materials and Methods

Ovaries were taken from three cattle (dioestrus), one sheep (dioestrus), five pigs (prooestrus, oestrus, metoestrus and dioestrus), three goats (dioestrus), five rabbits (prooestrus, oestrus, metoestrus and dioestrus), one guinea-pig (dioestrus), seven rats (prooesturs, oestrus, metoesturs and dioestrus) and four hamsters (oesturs and dioestrus). Sheep, goat, rabbit, guinea-pig, rat and hamster ovaries were obtained from the specimens kept in our laboratory, but the cattle and pig ovaries were obtained from the specimens killed at the Sendai Slaughter House.

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 and periodic acid-Schiff method (PAS) modified by Lillie. The identification of glycogen was made by means of the salivary test at 37°C in an incubator. For RNA, methylene blue, toluidine blue or thionin were used. RNA was confirmed by ribonuclease treatment. For alkaline phosphatase, Gomori's revised method was employed by using sodium glycerophosphate as a substrate. For the demonstration of lipase, Gomori's revised method was employed by using Emasol 4130 (Kao Soap Co. Ltd.) as a substrate. For the histological observation of the ovaries, haematoxylin and eosin stain, Van Gieson connective tissue stain was employed.

Results

I. *Histological Observations*

At the first step of this investigation, the histological features of the ovaries of both domestic animals and rodents were examined comparatively. The results are given in Tables 1, 2, 3 and 4.

Size of ova: The diameter of the ova in the well developed Graafian follicles, excluding the zona pellucida, was measured. As shown in Table 1, remarkable differences in the size of the ova were not found among the animals used except for the guinea-pig and rats, the estimate being about 90 to 130 micron in cattle, sheep, pigs, goats, rabbits and hamsters and about 70 to 80 micron in guinea-pigs and rats.

Table 1. Histological features of the ovaries of domestic animals and of rodents.

Animals	Size of ova in Graafian follicles (μ)	Vacuoles of Call-Exner in Graafian follicles	Aspects of atretic ova	Interstitial glands	Mast cells	Stroma
Cattle	120 to 130	absent	shrinkage	not developed	abundant	abundant
Sheep	90 to 120	〃	〃	〃	absent	〃
Pigs	90 to 120	abundant	〃	〃	〃	moderate
Goats	90 to 100	absent	〃	〃	abundant	abundant
Rabbits	100 to 120	abundant	〃	developed	absent	little
Guinea-pigs	70 to 80	absent	〃	〃	〃	moderate
Rats	65 to 80	〃	Fragmentation or shrinkage	〃	〃	little
Hamsters	90 to 100	〃	〃	〃	〃	〃

Vacuoles of Call-Exner : As shown in Table 1, the vacuoles of Call-Exner in the Graafian follicles were abundantly present in the ovaries of the pigs and rabbits, but scarcely in those of the other animals. It is generally considered that these vacuoles probably represented new centers of secretion of the follicular fluid.

Atretic ova : I (1953) have already reported that in the atretic follicles of the rats the ova followed the processes of cell fragmentation and shrinkage during the course of atresia. In the present investigation, I have observed the atretic processes in the ova of domestic animals and rodents with the following results : As shown in Table 1, only the ova of rats and hamsters followed the processes of cell fragmentation (Figs. 1 and 2) and shrinkage during the course of atresia, but only the process of shrinkage (Figs. 3, 4, 5 and 6) in such animals as cattle, sheep, pigs, goats, rabbits and guinea-pigs. The cytoplasm of the ova finally disappeared leaving the zona pellucida. A possible interpretation among these features of the atretic ova will be discussed in the "Section II" in connection with the histochemical demonstration of glycogen in the atretic ova.

I (1953) have also reported that five kinds of cells such as follicular cells, cells transformed from the follicular cells, mast cells, connective tissue cells and interstitial gland cells penetrated into the atretic ova of the rats through the zona pellucida. In the present investigation, a few invaded cells, which were demonstrated as follicular cells, cells transformed from the follicular cells and connective tissue cells, were found.

Interstitial glands : In the early stage of atresia, the interstitial cells formed a layer around the follicular cavity. Later, this layer was broken up into

separated cell clusters. As shown in Table 1, in cattle, sheep, pigs and goats, they soon degenerated and disappeared without further development. On the contrary, however, in rabbits, guinea-pigs, rats and hamsters, they were developed in the interstitial glands as stated previously (1953).

Mast cells and stroma : As shown in Table 1, the mast cells were abundant in the ovaries of cattle and goats, but almost absent in those of sheep, pigs, rabbits, guinea-pigs, rats and hamsters. The stroma was abundant in the ovaries of cattle, sheep and goats, moderate in those of pigs and guinea-pigs, and few in those of rabbits, rats and hamsters.

Follicular cells in atretic follicles : I (1953) have reported that follicular cells of the rats showed two types of degenerative changes as follows : (1) First type ; the nucleus became globular in various sizes and the cytoplasm disappeared after shrinkage, leaving the nucleus in the follicular fluid. These nuclei also disappeared sooner or later. (2) Second type ; the cytoplasm of the follicular cells hypertrophied and became polygonal or round. The nuclei remained normal for a long time, but finally disappeared when the cytoplasm also dissolved completely. In the present investigation, I have studied the degenerative changes of the atretic follicles of the domestic animals and rodents. The results are given in Tables 2, 3 and 4.

Table 2. Appearance of the atretic follicular cells of the first type.

Animals	Stages of the follicular atresia		
	early	middle	later
Cattle	abundant	absent	absent
Sheep	moderate	〃	〃
Pigs	little	〃	〃
Goats	moderate	〃	〃
Rabbits	little	〃	〃
Guinea-pigs	〃	〃	〃
Rats	abundant	〃	〃
Hamsters	〃	〃	〃

As shown in Table 2, the atretic follicular cells of the first type were only found during the early stage of the follicular atresia in which the connective tissue cells or theca interna cells did not yet develop. The appearance of the atretic follicular cells of the first type differed in different animals ; they appeared abundantly in cattle, rats and hamsters, moderately in sheep and goats, and scantly in pigs, rabbits and guinea-pigs. No morphological differences in the atretic follicular cells of the first type were found among the animals used.

Table 3. Appearance of the atretic follicular cells of the second type.

Animals	Stage of the follicular atresia		
	early	middle	later
Cattle	little	little	absent
Sheep	moderate	〃	〃
Pigs	abundant	moderate	little
Goats	moderate	little	absent
Rabbits	abundant	absent	〃
Guinea-pigs	〃	〃	〃
Rats	little	〃	〃
Hamsters	〃	〃	〃

As shown in Table 3, the atretic follicular cells of the second type of rodents such as rabbits, guinea-pigs, rats and hamsters were only found during the early stage of the follicular atresia, those of the cattle, sheep and goats during the early to middle stages of atresia, and those of pigs during the early to later stages of atresia. The appearance of the atretic follicular cells of the second type differed in different animals ; they appeared abundantly in pigs, rabbits and guinea-pigs, moderately in sheep and goats, and scantly in cattle, rats and hamsters.

Morphological and cytochemical features of the atretic follicular cells of the second type differed extremely in different animals as shown in Table 4.

Table 4. Cytochemical features of the atretic follicular cells of the second type.

Animals	Stage of the follicular atresia		
	early	middle	later
Cattle	Glycogen	Glycoprotein	disappeared
Sheep	〃	〃	〃
Pigs	〃	〃	〃
Goats	Glycogen to Glycoprotein	disappeared	〃
Rabbits	Glycoprotein	〃	〃
Guinea-pigs	Glycogen to Glycoprotein	〃	〃
Rats	Glycogen	〃	〃
Hamsters	〃	〃	〃

The follicular cells of rats and hamsters showed the degenerating changes as follows : The cells hypertrophied and became polygonal or round. The nuclei remained normal for a long time, and finally disappeared when the cytoplasm

also dissolved completely. Frequently, one or more vaculoes of various sizes appeared in the cytoplasm. Glycogen was found in the cytoplasm of the cells of the early stages of atresia and subsequently disappeared with shrinkage of them. The cells of the cattle, sheep, pigs, goats, rabbits and guinea-pigs showed degenerating changes as follows : The cells hypertrophied and became polygonal or round. The nuclei remained normal for a long time, but afterwards showed the so-called piknosis, and the cytolpasm shrunk without fragmentation, and finally disappeared.

In cattle, sheep, pigs, goats and guinea-pigs, the follicular cells hypertrophied and contained glycogen during the early stages of atresia. Afterwards, the glycogen transformed into glycoprotein when the cytoplasm shrunk and the nuclei showed the piknosis. In such cells the glycoprotein stained intensely with PAS method (Figs. 4 and 5) and showed no metachromasia with thionin or toluidine blue. Finally, these cells completely disappeared. In the atretic follicular cells of the rabbits, the degenerating features differed from other animals in that the cells contained glycoprotein in place of glycogen during the early stage of atresia (Fig. 6).

Kingsbury (1939), Rennels (1951) and Deane (1951, 1952) reported that concomitant with the follicular atresia of the rat ovaries, the follicular cells showed a transient response to the histochemical test for glycogen, but these cells were soon lost and formed no part of the interstitial glands which arised by the hypertrophy of theca interna cells. In the present investigation using the ovaries of domestic animals and of rodents, the follicular cells formed no part of the interstitial glands, though their debris containing a large amount of glycoprotein remained near the central cavity of the glands which contained the twisted zona pellucida up to the later stage of their atresia.

II. *Histochemical Observations*

As the next step of this investigation, polysaccharides, RNA, alkaline phosphatase and lipase in the ovaries of the domestic animals and rodents were demonstrated histochemically. The results are as follows :

1. *Results obtained for polysaccharides*

Stained with Best's carmine fluid, polysaccharides were demonstrated in the red coloured granules. Using the PAS method, they were demonstrated in the red-purple granules. In this case the zona pellucida was also stained diffusely.

The distribution and the appearance of polysaccharides in the ovaries of domestic animals and of rodents are given in Tables 5 and 6. In Table 6 are given the data obtained from corpora lutea and interstitial glands.

Table 5. Distribution of polysaccharides in normal follicles of domestic animals and of rodents.

Animals	Total No. of animals used	Germinal epithelia	Ova in primary follicles	Ova in secondary and Graafian follicles	Follicular cells in secondary and Graafian follicles	Follicular fluid in secondary and Graafian follicles
Cattle	3	(+)	−	− to +	++*	(+++)
Sheep	1	−	−	− to ±	−	(+++)
Pigs	5	(+)	−	− to ++	−	(+++)
Goats	3	−	−	− to ±	−	(+++)
Rabbits	5	−	−	− to +	−	(+++)
Guinea-pigs	2	−	−	− to ±	−	(+++)
Rats	7	−	−	− to +++	−	(+++)
Hamsters	4	−	−	− to +++	−	(+++)

( ) .....Not digested by saliva.
* .....Follicular cells of cumulus oöphorus.

As shown in Table 5, the germinal epithelia of most animals used contained no polysaccharides except that the ones of cattle and pigs contained the PAS reactive substance. Since the PAS reactive substance just stated resists saliva, it is glycoprotein.

The ova in the primary follicles never contained polysaccharides.

Among the ova in the secondary and Graafian follicles of the domestic animals and rodents, extreme differences in glycogen content were found. Rat and hamster ova contained a large amount of it, pig ova in a moderate amount, and cattle, sheep, goat, rabbit and guinea-pig ova in a small amount. The ova in these follicles of each animals, however, differed extremely in glycogen content. Namely, some ova contained no glycogen, but others contained it in a small or a large amount.

The follicular cells in the secondary and Graafian follicles contained no glycogen except that those of cumulus oöphorus in the Graafian follicles of the cattle always contained it in a large amount.

Follicular fluid of all animals used reacted with the PAS method, but never with Best's carmine method as shown in Table 5.

As shown in Table 6, extreme differences in glycogen content of the atretic ova in the early stage were found among the animals used, as well as in that of the normal ova in secondary and Graafian follicles : Rat and hamster ova in these follicles contained it in a large amount, pig ova in a moderate amount, and cattle, sheep, goat, rabbit and guinea-pig ova in a small amount. The ova of each animal differed in glycogen content ; some ova contained no glycogen, and the others contained it in a small or a large amount. Afterwards, the ova

followed the processes of the cell fragmentation or shrinkage and finally disappeared : The ova of the rats and hamsters followed the processes of the cell fragmentation and shrinkage, and those of other animals such as cattle, sheep, pigs, goats, rabbits and guinea-pigs the process of shrinkage alone. It was also found that the ova which followed cell fragmentation usually contained a large amount of glycogen (Fig. 1), and the ova which followed shrinkage alone only a little amount of it and glycoprotein in various amounts (Figs. 4 and 5).

Table 6. Distribution of polysaccharides in atretic follicles of domestic animals and of rodents.

Animals	Ova in atretic follicles			Corpora lutea	Interstitial glands
	early stage	later stage			
		shrinkage	fragmentation		
Cattle	− to +	− to (++)	no fragment.	−	−
Sheep	− to ±	− to (++)	〃	−	−
Pigs	− to ++	− to (+++)	〃	−	−
Goats	− to ±	− to (++)	〃	−	−
Rabbits	− to +	− to (++)	〃	−	−
Guinea-pigs	− to ±	− to (++)	〃	+++	−
Rats	− to +++	− to (+++)	+++	−	−
Hamsters	− to +++	− to (+++)	+++	−	−

Corpora lutea usually contained no glycogen, except that those of guinea-pigs contained a large amount of it from the early stage of the hypertrophy of their cells to the early stage of the grown corpus luteum.

Interstitial gland cells never possessed glycogen.

Togari (1927) studied the appearance of glycogen in the ovaries of rodents such as rabbits, mice, rats and guinea-pigs and reported that glycogen appeared abundantly in the ova, corpora lutea and interstitial glands. Further, he stated that glycogen of the ova increased in amount gradually from the primary follicles up to the mature, being especially rich in the divided and the degenerating ova. He also stated that glycogen appeared richly in lutein cells at the early stage of their hypertrophy and existed up to the early stage of the grown corpus luteum. Brandenburg (1938) reported that glycogen appeared in the normal and atretic ova, hilum and blood vessels of the rats. Deane (1951) studied the histochemical characteristics of the ovarian follicles of the rat and claimed that little glycogen was detectable in healthy follicles by the McManus periodic acid-Schiff techinique ; only the ova contained it in a large amount. He also stated that in the atretic follicles glycogen occurred within the follicular cells as well, especially those of the cumulus oöphorus.

In the present investigation, a different amount of glycogen was contained in the ova of the domestic animals and rodents : Rat and hamster ova contained a large amount of glycogen, pig ova a moderate amount of it, and cattle, sheep, goat, rabbit and guinea-pig ova a small amount of it. Glycogen did not appear in the corpora lutea during the course of corpus luteum formation except that in those of the guinea-pig it appeared in a large amount during the early stage of their hypertropy and existed up to the early stage of the grown corpus luteum. Glycogen was not found in the interstitial gland cells during the course of development.

Harter (1948) studied the glycogen and carbohydrate-protein complex in the ovaries of the rats during the oestrous cycle and reported that glycoprotein was demonstrable in the germinal epithlia, ova, zona pellucida, follicular cells, corpora lutea, intercellular substances, basement membrane and atretic ova.

In the present investigation, glycoprotein which reacted with the PAS method and resisted saliva appeared in the zona pellucida, follicular fluid, stroma and blood vessels in the animals used and only in the germinal epithelia of the cattle and pigs, almost agreeing with the results obtained by Harter.

I (1953) have studied the histology and histochemistry of the atretic ova of the rats and found that the ova atrophied apparently through the shrinkage of cytoplasm and cell fragmentation, and in the former process contained no glycogen, but in the latter a large amount of it.

In the present investigation, the hamster ova performed cell fragmentation and accordingly irregular cell masses were found as well as the rat ova. The ova of other animals such as cattle, sheep, pigs, goats, rabbits and guinea-pigs showed only the shrinkage of cytoplasm without fragmentation division. It was noteworthy that the ova of the rats and hamsters undergoing the fragmentation or cleavage during atresia contained a large amount of glycogen, but the ones of other animals undergoing only shrinkage contained a little or none of it and a large amount of glycoprotein. This shows that glycogen is necessary to perform the cell fragmentation or cleavage during the degeneration of the ova.

2. *Results obtained for RNA*

RNA in the ovaries of the domestic animals and rodents was demonstrated with the routine techinique. The results were as follows :

As shown in Table 7, the germinal epithelia contained no RNA. The ova in the primary, secondary and Graafian follicles usually contained a small amount of RNA. Among the ova in these follicles, no differences in RNA content were found. The atretic ova in the early stage of degenerative process also possessed a small amount of RNA, but it soon disappeared.

Table 7. Distribution of RNA in the ovaries of domestic animals and of rodents.

Animals	Total No. of animals used	Germinal epithelia	Primary follicles		Secon. and Graaf. fol.		Atretic follicles		Corpora lutea	Interstitial glands
			Ova	Follicular cells	Ova	Follicular cells	Ova	Follicular cells		
Cattle	3	−	+	+	+	+	−to+	−to+	+	−
Pigs	5	−	+	+	+	++	−to+	−to++	+	−
Goats	3	−	+	+	+	+	−to+	−to+	+	−
Rabbits	5	−	+	+	+	+	−to+	−to+	+	−
Rats	7	−	+	+	+	+++	−to+	−to+++	+	−
Hamsters	4	−	+	+	+	+++	−to+	−to+++	+	−

Follicular cells in the primary follicles contained a small amount of RNA. Afterwards, the RNA increased gradually with the proliferation of the follicular cells, and reached a large amount of it in the follicular cells in the secondary and Graafian follicles. No visible variation in RNA content was found in different kinds of animals, though the rat and hamster possessed a relatively high content of it. In the early stage of degeneration of the atretic follicles, the RNA began to decrease and finally disappeared when the follicular cells completely disintegrated.

Corpora lutea usually contained a small amount of RNA in the developing stage. The amount of it did not differ in different animals.

Interstitial glands never contained RNA.

Vincent (1948) and Jones-Seaton (1950) studied the localization and the role of the nucleic acids in the rat ovaries and reported that with the growth of the ova RNA was developed in the cytoplasm, and the developing follicular cells showed a marked content of RNA that was highest in the innermost and outermost rows of the cells.

In the present investigation, the distribution of RNA in the ovaries of domestic animals and of rodents nearly coincided with their reports except that RNA in the ova did not increase throughout the course of development of the ova.

3. *Results obtained for alkaline phosphatase*

Alkaline phosphatase activity in the ovaries of domestic animals and of rodents was demonstrated as follows :

Treated with Gomori's revised method using the glycerophosphate as a substrate, black coloured precipitate of cobalt sulfite appeared in the ovaries, showing that the phosphatase reaction occurred in them. The results are given

Table 8. Distribution of alkaline phosphatase in the ovaries of domestic animal and of rodents.

Animals	Total no. of animals used	Germinal epithelia	Ova in primary follicles	Ova in secondary and Graaf. follicles	Follicular cells in secondary and Graafians follicle	Theca folliculi	Ova in atretic follicles	Follicular cells in atretic follicles	Corpora lutea	Interstitial glands
Cattle	3	−	−	±	−	+++	±	+	+	−to+
Pigs	5	−	−	−to++	−	+++	−to+++	++	+++	−to+
Goats	3	−	−	+	−	+	±	+	+	−
Rabbits	5	−	−	+	−	+	+	+	+++	−
Rats	7	−	−	−to+	−	+++	−to+++	+	+	±
Hamsters	4	−	−	−to+	−	+++	−to+++	+	+	±

in Table 8.

As shown in Table 8, alkaline phosphatase reaction occurred in the ova in the secondary, Graafian and atretic follicles, in the theca folliculi in the secondary and Graafian follicles, in the follicular cells in the atretic follicles, and in the corpora lutea and blood vessels.

In the normal ova in the secondary and Graafian follicles, the alkaline phosphatase activity differed in different ova, but agreed with the amount of glycogen : In the ova containing a large amount of glycogen, alkaline phosphatase reaction was high, and in the ova containing no glycogen, its reaction was negative. Alkaline phosphatase reaction also occurred in the atretic ova (Fig. 2). No differences in alkaline phosphatase reaction were found among the ova of different animals.

The relation between the alkaline phosphatase reaction and the degenerative process of the follicular cells was as follows : No reaction was found in the first type of the process, but it was found in its second type. Marked reaction was found in the follicular cells of pigs and hamsters, and weak reaction in those of cattle, goats, rabbits and rats.

Theca folliculi showed different alkaline phosphatase reaction in different animals ; those of cattle, pigs, rats and hamsters showed an intense reaction, but those of goats and rabbits a faint reaction. A similar relation was also recognized in corpora lutea ; those of pigs and rabbits showed an intense reaction, but those of other animals such as cattle, goats, rats and hamsters a faint reaction.

Interstitial gland cells also showed a faint or no reaction of alkaline phosphatase.

Dempsey, Greep and Deane (1949) reported that alkaline phosphatase was demonstrable in theca folliculi, corpora lutea, interstitial glands and blood

vessels of the rats. In the present investigation, however, alkaline phosphatase also occurred in the ova in the secondary, Graafian and atretic follicles, theca folliculi, corpora lutea, interstitial gland cells and blood vessels.

4. *Results obtained for lipase*

Lipase activity in the ovaries of the domestic animals and rodents was demonstrated with the routine techinique. Treated with Gomori's revised method using Emasol 4130, brown coloured precipitate of lead sulfite appeared in the ovaries, showing that the lipase reaction occurred in them. The results are given in Table 9.

Table 9. Distribution of lipase in the ovaries of domestic animals and of rodents.

Animals	Total No. of animals used	Gerninal epithelia	Ova in primary follicles	Ova in secondary follicles	Theca folliculi	Ova in atretic follicles	Corpora lutea	Interstitial glands
Cattle	3	–	–	– to ++	+	+++	+	–
Pigs	5	–	–	– to ++	+	+++	+	–
Goats	3	–	–	– to ++	+	+++	+	–
Rabbits	5	–	–	– to ++	+	+++	+	–
Rats	7	–	–	– to ++	+	+++	+	–
Hamsters	4	–	–	– to ±	+	+++	+	–

As shown in Table 9, lipase reaction occurred in the ova in the secondary, Graafian and atretic follicles, theca folliculi, and corpora lutea.

In the ova in the secondary and Graafian follicles, lipase activity differed in different ova; some ova showed no lipase reaction, but others a weak reaction. All atretic ova usually possessed an intense reaction (Fig. 3). Thus, no remarkable differences in the appearance of lipase were found among the animals.

Quen (1950) reported that lipase activity was abundant in the ovaries of the mice, being especially rich in the ova, follicular cells and corpora lutea. I (1953) have studied the lipase activity of the normal and atretic ova of the rats and reported that the ova which extremely differed in lipase activity were found, and that all atretic ova showed an intense lipase activity. Similar results were obtained in the present investigation on the cattle, pig, goat, rabbit and hamster ovaries.

Summary

The results obtained in this investigation are summarized as follows:

1. Among the normal ova in the secondary and Graafian follicles, extreme

differences in glycogen content were found : Rat and hamster ova contained it in a large amount, pig ova in a moderate amount, and cattle, sheep, goat, rabbit and guinea-pig ova in a small amount.

2. The ova of the rats and hamsters undergoing fragmentation or cleavage during atresia, contained a large amount of glycogen, but the ones of other animals such as cattle, sheep, pigs, goats, rabbits and guinea-pigs undergoing only shrinkage during atresia contained only a little or none of it and a large amount of glycoprotein.

3. The follicular cells showed two types of degenerative changes as follows : (1) First type : The nucleus became globular in various sizes and the cytoplasm disappeared after shrinkage, leaving the nucleus in the follicular fluid. These nuclei also disappeared sooner or later. (2) Second type : The cytoplasm of the follicular cells hypertrophied and became polygonal or round. The nuclei remained normal for a long time, but finally disappeared when the cytoplasm also dissolved completely.

4. In the domestic animals and rodents, the follicular cells of the first type of degeneration contained no glycogen during the course of atresia. In cattle, sheep, pigs, goats and guinea-pigs, the follicular cells of the second type of degeneration hypertrophied and contained glycogen during the early stage of atresia. Afterwards, the glycogen transformed into glycoprotein when the cytoplasm shrunk and the nuclei showed the piknosis, and finally the cells completely disappeared. In the atretic follicular cells of the rabbits, the degenerating features differed from the other animals in that the cells contained glycoprotein in place of glycogen during the early stage of atresia.

5. RNA was contained in the normal ova, but not in the atretic ova with no relation as to whether they performed cleavage during the atresia. The follicular cells and the corpora lutea contained RNA. No remarkable differences in RNA content were found in different animals.

6. Alkaline phosphatase reaction occurred in the normal and atretic ova, follicular cells in atretic follicles, theca folliculi, corpora lutea, interstitial gland cells and blood vessels. In the normal ova containing a large amount of glycogen, alkaline phosphatase reaction was high, and in the ova containing no glycogen, its reaction was negative.

7. Lipase reaction occurred in the normal and atretic ova, theca folliculi, and corpora lutea. No marked differences in the appearance of lipase were found in different animals.

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Explanation of Figures

Fig. 1. Atertic ovum of the hamster. × 310. Best's carmine stain. Glycogen granules are seen in the cytoplasm of the divided ovum.
Fig. 2. Atretic ovum of the hamster. ×310. Gomori's method. Intense alkaline phosphatase reaction occurs in the cytoplasm of the divided ovum.
Fig. 3. Atretic ovum of the pig. ×310. Gomori's method. Intense lipase reaction occurs in the cytoplasm of the shrunken ovum.
Fig. 4. Atretic follicle of the pig in the early stage. ×100. PAS stain. Glycoprotein is seen in the cytoplasm of the shrunken ovum and in the atretic follicular cells.
Fig. 5. Atretic follicle of the pig in the later stage. ×100. PAS stain. Glycoprotein is seen in the cytoplasm of the shrunken ovum and in the atretic follicular cells.
Fig. 6. Atretic follicle of the rabbit in the early stage. ×100. PAS stain. Glycoprotein is seen in the cytoplasm of the ovum and in the atretic follicular cells.

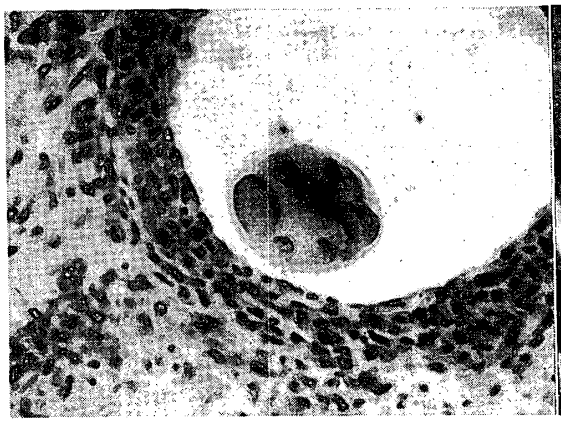

Fig. 1.

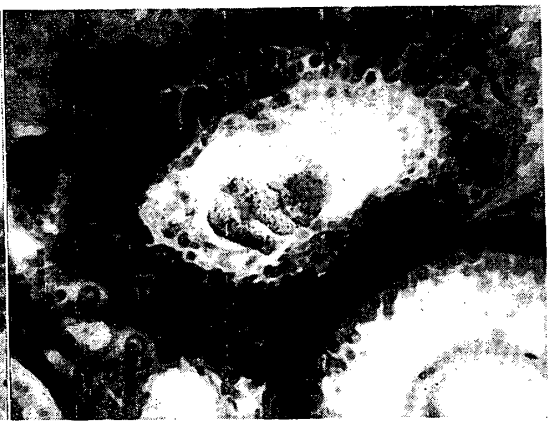

Fig. 2.

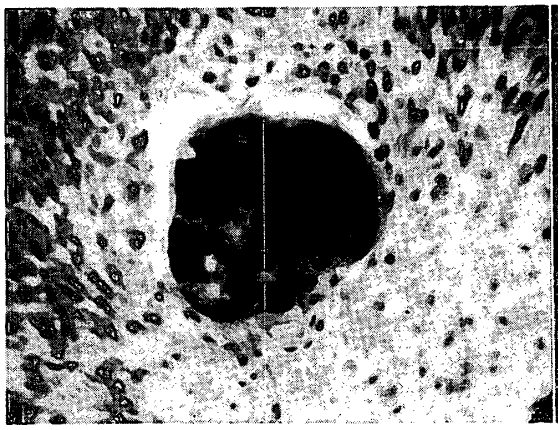

Fig. 3.

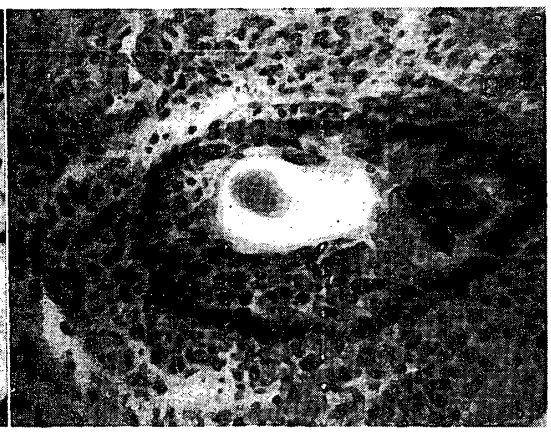

Fig. 4.

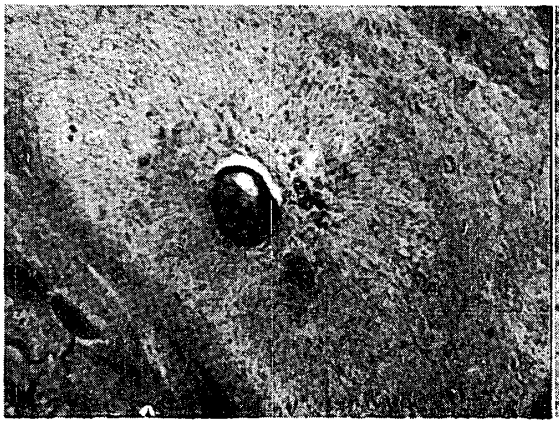

Fig. 5.

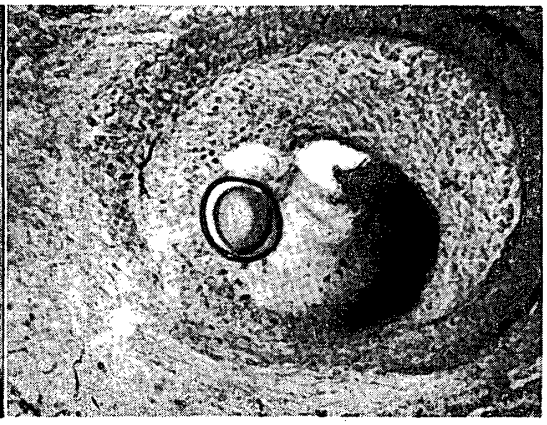

Fig. 6.