Histological and Histochemical Observations on the Atretic Follicles Induced by Adenohypophysectomy in the Hen

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Introduction

The ovarian follicles of the hen ovulate or become atretic in the last stage of maturation. Atresia of the follicles occurs in response to the stimuli which interrupt maturation. Investigations regarding the effect of hormones on atresia have been carried out by various researchers. Histological studies on the characteristics of the atretic follicles have disclosed two main types of atresia, i.e., “bursting atresia” and “invading atresia.” Bursting atresia occurs in follicles containing appreciable amounts of yolk and is characterized by the rupture of the theca. Invading atresia occurs in follicles containing smaller amounts of yolk and is characterized by invasion of cells from the granulosa and thecal layer. However, since these histological findings cannot explain adequately the mechanism of the onset of atresia, more detailed examinations should be performed in taking account of the physiological changes that occur during atretic processes. In the present study, histological and histochemical observations on the atretic follicles (bursting atresia) induced by adenohypophysectomy were carried out, and the mechanism of the bursting of the theca was discussed.

Materials and Methods

White Leghorn hens laying three or more eggs in a clutch (more than 550 days old) were used in this study. Adenohypophysectomy was performed about 11-14 hrs after Cs oviposition, using a stereotaxic instrument as described by Tanaka and Nobukuni. The time interval from the operation to the excision of the follicles and the number of hens used in each experimental group were as follows: 0 hr (8 hens), 12 hrs (4), 18 hrs (6), 24 hrs (8) and 48 hrs (4). In 10 of the larger follicles in each ovary (the largest follicle: F1-the 10th largest follicle: F10), the presence of atretic follicles was recorded. The follicular tissues of the F1 group were fixed in 10% formalin or in cold acetone (4°C). The wall of the non-atretic follicles was cut over a length of about 5 mm before the fixation, to remove approximately half of the yolk material from the follicle. The tissues fixed in formalin were embedded in paraffin by routine procedures, and sections were stained with hematoxylin and eosin. The tissues fixed in acetone for 12 hrs were cleared through acetone-benzene (20 min) and chloroform (40 min), and immersed into paraffin (53°C, 90 min) prior to embedding. The sections were stained to demonstrate the acid phosphatase activity according to the method of Barka and Anderson, while the nucleus was stained with hematoxylin.

Received March 6, 1985
Control sections were stained without the substrate.

Results

None of the follicles developed atretic changes until 12 hrs after adenohypophysectomy except for one follicle of the F9 group. By 18 hrs after the operation, atresia was induced in all of the follicles of F1. The rate of atresia among the follicles of the F2-F5 groups was lower, while that of the follicles in the F6-F10 groups exceeded 50%. At 24 hrs after the operation, all of the follicles of the F1 group and more than 75% of the follicles of the F2-F10 groups except for F4 (62.5%) showed atretic changes. By 48 hrs after the operation, atresia was induced in all of the follicles examined (Fig. 1).

The wall of a normal follicle is composed of the stratum granulosum, theca interna, theca externa, superficial tunic and superficial epithelium (Figs. 2, 3). Until 12 hrs after the operation, structural changes could not be observed under light microscope. At 18 and 24 hrs after the operation, various stages of atresia were observed in the different follicles. The most remarkable change in the structure of the follicular wall was the bursting of the thecal layer. The first sign of the bursting was manifested by the destruction of the inner portion of the thecal layer in which a hollow was formed. The stratum granulosum fell into the hollow (Figs. 4, 5). Finally, the thecal layer burst out, and the stratum granulosum eventually ruptured at the burst portion of the theca. The yolk material was released from the ovum into the superficial tunic through the burst portion (Figs. 6, 7).

Near the burst portion, the tissue of the theca externa became extremely loose, and many

Fig. 1 Rate of atresia induced after adenohypophysectomy in the hen

* Ten of the largest follicles in the respective ovaries were grouped according to their size.
** Numbers in the parentheses indicate the number of hens used.
of the fibroblasts shrank and degenerated showing an intensely stained eosinophilic cytoplasm and pycnotic nucleus (Figs. 5, 7). Around the region distant from the burst portion, the yolk material was aggregated in the superficial tunic, and the stratum granulosum with a basal lamina became markedly folded to form an interspace between the theca interna and these structures. Most of the thecal fibroblasts did not undergo degenerative changes (Figs. 8, 9). At 48 hrs after the operation, the stratum granulosum became thicker with several layers of cells, and many of the granulosa cells were extremely vacuolated. The thecal fibroblasts disintegrated and their nucleus became pycnotic (Figs. 10, 11).

The granulosa cells and thecal gland cells showed an intense acid phosphatase activity, whereas thecal fibroblasts displayed an extremely weak activity in the normal follicles (Fig. 12). In the follicles which became atretic at 18 and 24 hrs after the operation, no change of activity was found in the granulosa cells and thecal gland cells, but a marked increase of the activity was found in the thecal fibroblasts near the burst portion (Fig. 13). The thecal fibroblasts distant from the burst portion did not show such an increase of activity. At 48 hrs after the operation, the acid phosphatase activity of the thecal fibroblasts over the whole follicle had increased (Fig. 14).

Discussion

Previous workers have described that the microscopic signs of bursting atresia in the hen consist of progressive changes of follicular structure including the formation of spaces between the theca interna and basal lamina, and the rupture of the thecal layer allowing the released yolk to aggregate beneath the follicular epithelium. The present study confirmed these descriptions.

The factors leading the thecal layer to rupture, which is the most characteristic change of bursting atresia, have not been fully understood. This paper will discuss primarily the factors. Around the burst portion of the follicle, the thecal tissue became extremely loose, and many of the fibroblasts degenerated with an intensely eosinophilic cytoplasm and pycnotic nucleus. The activity of acid phosphatase, one of the lysosomal enzymes, in the thecal fibroblasts near the burst portion was remarkably increased, as compared with the activity in the normal fibroblasts. These findings suggest that the disintegration of the thecal tissues and the degeneration of the thecal fibroblasts were induced by lysosomal enzymes such as


Fig. 2. The wall of a normal follicle.
Fig. 3. Enlarged view of the follicular wall shown in Fig. 2.
Fig. 4. Site where the bursting of the theca occurs at 18 hrs after operation. Note the destruction of the inner portion of the thecal layer.
Fig. 5. Enlarged view of the theca externa shown in Fig. 4. Note the loosened tissue and degenerated fibroblasts.
Fig. 6. Site where the bursting of the theca has been completed at 18 hrs after operation.
Fig. 7. Enlarged view of the theca externa shown in Fig. 6. Note the degenerated thecal fibroblasts.
Fig. 8. Site distant from the portion where the bursting of the theca occurred at 18 hrs after operation. Arrows show the interspace between the theca interna and basal lamina.

Fig. 9. Enlarged view of Fig. 8. Most of the fibroblasts are normal except for some degenerated ones (arrows).

Fig. 10. Follicular wall 48 hrs after the operation.

Fig. 11. Enlarged view of Fig. 10. Note the vacuolization of the granulosa cells and disintegration of the thecal tissues.

cathepsins present in the fibroblasts. It appears that the bursting of the thecal layer occurs at the area where a large amount of degenerated fibroblasts tends to aggregate. The markedly increased activity of acid phosphatase of the thecal fibroblasts over the whole follicular wall 48 hrs after adenohypophysectomy suggests that the regression of the atretic follicle occurs through the digestion activity of lysosomal enzymes.

On the other hand, the present study indicated that the follicles at middle phase of rapid growth were delayed to become atretic in comparison with the other follicles. This result agrees with that of TANAKA et al. In the follicles at middle phase of rapid growth the amount of estrogen is larger than in the other follicles in the hen. It has been shown that the administration of exogenous estrogen diminishes the rate of atresia in the hypophy-
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Fig. 12-14. Micrographs showing acid phosphatase activity in the thecal fibroblasts. Scale bar: 30 μm.

Fig. 12. Follicular wall of a normal follicle. Note the intense activity in the thecal gland cells (arrow heads) and granulosa cells and weak activity in the thecal fibroblasts.

Fig. 13. Theca externa of the follicle 18 hrs after the operation. Bursting of the theca occurred near this portion. Note the increased activity in the fibroblasts.

Fig. 14. Theca layer of the follicle 48 hrs after the operation. The acid phosphatase activity is markedly increased in the fibroblasts.
sectomized rat\textsuperscript{12}). Autoradiographic investigations have revealed that the specific gonadotropin (GTH)-binding sites are present in the granulosa and thecal gland cells but not in the fibroblasts forming the theca externa in mammals\textsuperscript{13-15}). Therefore, it is estimated that the presence of estrogen delays the occurrence of atresia in the hen, and the degeneration of the thecal fibroblasts in the case of atresia may be induced by the decrease of estrogen contents in the follicular tissue resulting from the deficiency of GTH stimulation.

**Summary**

Histological and histochemical observations on hen's atretic follicles induced by adeno-hypophysectomy were carried out. Atresia was induced at 18 hrs after the operation. In the follicles at middle phase of rapid growth the appearance of atresia was delayed. Thecal fibroblasts in the atretic follicles (bursting atresia) underwent degenerative changes characterized by cytoplasmic eosinophilia, nuclear pycnosis and increased acid phosphatase activity. It is suggested that the rupture of the thecal layer in the case of bursting atresia is induced by the breakdown of the tissue through digestion by lysosomal enzymes from the thecal fibroblasts.

**References**

下垂体除去群における閉鎖卵胞の組織学的ならびに組織化学的観察

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鶏の下垂体除去後に生じる閉鎖卵胞を組織学的ならびに組織化学的に観察した。卵胞の閉鎖は切除後約18時間で誘起された。急速成長期中卵胞では同卵期と後期の卵胞に比べて閉鎖の開始が遅れた。閉鎖卵胞（bursting atresia）の卵胞膜線維芽細胞は、細胞質の顕著なオジョン性を示し、核濃縮および酸ホスファターゼ活性の上昇を伴う変性像を示した。以上の所見から、卵胞閉鎖時に（bursting atresia）における卵胞膜の破裂は線維芽細胞のライソゾーム酵素が組織を破壊する結果とされる。したがって（家禽学会誌、22, 134〜141, 1985）

throughout the estrous cycle: Biol. Reprod. 27, 505-516.