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Microstructure and ultrastructure of atretic follicles in the Chinese giant salamander *Andrias davidianus* *

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Abstract The microstructure and ultrastructure of the atretic follicle and corpora atretica in the ovary of Chinese giant salamander *Andrias davidianus* were observed with light and electron microscopy. The results showed that corpora atretica arise from follicular cells and thecal cells do not invade the oocytes. During the slow growth stage of the ovum, few atretic follicles can be found. In May and June, when most oocytes in the ovary have entered into the previtellogenic stage, atretic follicles were observed, but the steroidogenic features of these cells were not striking. In July and August, when most oocytes were in vitellogenic stage, there were some mitochondria with tubular cristae, plenty of smooth endoplasmic reticulum and lipid droplets, and a well-developed Golgi complex in the corpora atretic cells. These cytological features displayed that the corpora atretica may secrete steroid hormones and regulate normal ovum maturation. We propose that these structures be termed as preovulatory corpora lutea in this species [*Acta Zoologica Sinica* 50 (4): 615 - 621, 2004].
Key words Chinese giant salamander, *Andrias davidianus*, Atretic follicle, Corpora atretica, Microstructure, Ultrastructure

中国大鲵闭锁卵泡的显微和超微结构观察 *

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摘 要 用光镜和电镜观察了中国大鲵卵泡闭锁过程和闭锁小体的显微和超微结构。结果显示, 大鲵闭锁小体是卵泡细胞侵噬卵母细胞并增殖形成细胞团, 膜细胞未参与。在大部分卵泡处于缓慢生长期时, 未发现卵泡闭锁现象; 在 5、6 月份, 卵巢内大部分卵母细胞进入卵黄形成前期, 部分卵泡闭锁, 但闭锁小体细胞的类固醇激素分泌结构特征不明显; 在 7、8 月份, 大多数卵母细胞处于卵黄形成期, 闭锁小体细胞具有管泡状嵴线粒体、丰富的滑面内质网和脂滴、发达的高尔基体等。这些细胞学特征表明闭锁小体可分泌类固醇激素, 以调节正常卵子的成熟。在大鲵中观察到的闭锁小体属于排卵前黄体 [**动物学报** 50 (4): 615 - 621, 2004]。
关键词 中国大鲵 闭锁卵泡 闭锁小体 显微结构 超微结构

Structural variation and histochemistry of atretic follicles before ovulation in fish and amphibians have been reported (Guraya, 1969; Joly and Picherel, 1972; Chieffi et al., 1992; Wang, 1992). The microstructure and histochemistry of atretic follicles after ovulation also have been described in *Rana cyanophlyctis* (Pancharatna and Saidapur, 1983). Yang et al. (1981) observed the structure of atretic follicles in the Chinese giant salamander *Andrias davidianus* using light microscopy, and described them as degraded ova. The atretic follicle of yellow perch *Perca fluviatilis* was considered not to form a corpus luteum with endocrine function, but merely represented a degenerative structure by histochemical, light and electron microscopic methods (Lang, 1981). However, Chieffi et al. (1992) suggested corpora atretica in the electric ray *Torpedo marmorata* perform an endocrine steroidogenic role as evidenced by electron microscopy. In the hamster, immunoreactivity for aromatase was localized in the granulosa cells of healthy developing follicles and Graafian follicles, as well as in newly formed granulosa lutein cells (Yoshinaga and Osawa, 1994). It was apparent from the above review that this is a subject of much inter-

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est and controversy. In investigating normal oocyte development of the Chinese giant salamander and the stream salamander *Batrachuperus pinchonii* (Zhang et al., 1999; Zhang and Jia, 2002), we have seen atretic follicles and corpora atretica in the ovary, so it is important to clarify both structural and functional aspects of atretic follicles to reveal their steroidogenic potential in salamanders during the reproductive cycle. In the present paper, microstructural and ultrastructural observations on the atretic follicle in Chinese giant salamander during the reproductive cycle is investigated.

1 Materials and methods

1.1 Animals

Adult female Chinese giant salamanders were collected between 1994 and 1999 (March 2, May 2, June 1, July 1, August 2) from streams on the south slope of Qinling Mountain, China (109°32′E, 31°53′N), at an altitude between 1 400 to 1 600 m above sea level. Body weight of captured animals ranged from 1 100 to 1 300 g.

1.2 Light microscopy

The animals were anaesthetized with ether and the ovaries were removed. Ovaries were cut into pieces and fixed in Bouin's solution, embed in paraffin and sections of 8 - 10 μm were obtained by an AO-820H rotary microtome and stained with hematoxylin and eosin. The sections were examined and photographed by an Olympus BH-2 light microscope.

1.3 Electron microscopy

Oocytes from 0.35 to 4 - 5 mm in diameter were removed from the ovary and fixed in 2.5% glutaraldehyde and 0.05 mol phosphate buffer (pH 7.2) for 2 - 3 h. In some cases, the oocytes were bisected or punctured to achieve optimum fixation. Then oocytes were fixed in 1% solution of osmium tetroxide (pH 7.2). After graded ethanolic dehydration and passage through propylene oxide, the specimens were embedded in Epon812. Thin sections obtained by LK13 ultramicrotome were doubly stained with aqueous uranyl acetate and lead citrate; then they were examined and photographed by a Hitachi-600 electron microscope.

2 Results

2.1 Microstructure observation

The ovary in Chinese giant salamander was a saclike, as observed in other amphibians. In March, few atretic follicles were present. Meanwhile, most ova in this period were surrounded by one layer of follicular cells and, were still in the slow growth stage. In May and June, most ova were in the previtelligenous or marked growth stag. The biggest ovum was about 3 - 4 mm in diameter. Follicular cells around each oocyte formed two layers. Few atretic follicles could be seen. In atretic follicles, the oocyte's nucleus disappears and the cytoplasm was slightly basophilic while the follicular cells around oocytes proliferated. Some follicular cells engulfed the cytoplasm of oocytes (Plate Ⅰ: 1). A mass of follicular cells sometimes could be observed. In these cells, the boundary was obscure and the nucleus was 15 - 20 μm in diameter. The mass of follicular cell became the primary corpora atretica at the preovulatory stage. Thecal cells were not involved in the cell mass that consisted only of follicular cells. Capillaries were seen in the periphery of the cell mass (Plate Ⅰ: 2).

In July and August, most ova were in the vitellogenic stage or fast growth stage. The largest ova surrounded by only one layer of follicular cells, and had reached 6 mm in diameter. More atretic follicles could be seen in the ovary in July and August. Atresia was observed in medium follicles where the follicular cells and endothelium of capillaries intruded into oocytes, and the follicular cells engulfed the cytoplasm and yolk platelets. Meanwhile, the follicular cells became columnar in shape (Plate Ⅰ: 3). At the same time, many more atretic follicles at different developmental stages were observed. The atretic follicular cell mass contained many capillaries. The atretic follicular cells were large and irregular in shape, their nuclei were 15 - 20 μm in diameter, and the boundaries of the cells could not be discerned. Follicular cells have completed corpora atretica formation before ovulation (Plate Ⅰ: 4).

2.2 Ultrastructure observation

2.2.1 Normal structure of the follicular wall In the ovary of the Chinese giant salamander, the normal developing follicular wall was composed of a vitelline envelope, 1 - 2 layers of follicular cells, capillaries, thecal cells and epithelium. The prominent cytoplasm stretched out from the basic surface of the follicular cells and intruded through the vitelline envelope to contact with the microvilli of the oocyte. The length and density of microvilli were variable during different developmental stages of oocytes. The oocyte has less microvilli in the previtellogenic stage but longer thick microvilli were very numerous in the vitellogenic stage (Plate Ⅰ: 5, 6). Microvilli became short and rare as the time for ovulation approached. In the cortex of ooplasm, just near the base of microvillus, there were some endocytic vesicles, by which the developing oocytes could get nutritious materials from the vitelline envelope. The nucleus of follicular cell was elliptical in shape, with an irregular and wavy edge. The distinction of organelles in the cytoplasm of follicular cells was conspicuous at different developmental stages. In the previtellogenic stage of oocytes, few organelles could be seen in the cytoplasm of follicular cell (Plate Ⅰ: 5). At the vitellogenic stage, the cytoplasm of follicular cells increased. The thecal cells and capillaries changed less in the follicular wall during the different developmental stages.

2.2.2 Follicular cell in the course of atresia During follicular atresia, the most striking feature showed that the ellipse-like nucleus changes into an irregular shape with many fold-like bulges emerging along the nuclear border of the follicular cells (Plate Ⅰ: 7; Plate Ⅱ: 9). When previtellogenic oocytes undergo atresia, their cytoplasm is engulfed as pinocytotic vesicles in the cytoplasm short and rare protuberances seen at the basal surface of the follicular cells (Plate Ⅰ: 8). When atresia occured at the vitellogenic stage, the follicular cells encircled the microvilli of the oocyte and devoured them mainly by stretching its cytoplasmic protuberances forming multivesicular bodies within the follicular cells (Plate Ⅱ: 9, 10). The yolk platelets, mitochondria, lipid droplets and other organelles of the ooplasm were also consumed by the follicular cells. Thus, the follicular cells gradually enlarged and corpora atretica develops.

2.2.3 Cells of corpora atretica The cells of the corpora atretica of the Chinese giant salamander are derived from follicular cells and not from thecal cells. In May and June, there was little cytoplasm and few organelles in the cells of the corpora atretica, and there were no striking cytological features to suggest an endocrine function for these cells (Plate Ⅰ: 7). However, all features of the corpora atretica were different from those of normal follicular cells. In August, most of the normal oocytes were at the vitellogenic stage, and the endocrine features of corpora atretica cell were as follows: (1) most mitochondria with tubular crista were in a circular or elliptical shape (Plate Ⅱ: 11); (2) well-developed Golgi complex (Plate Ⅱ: 12); (3) smooth endoplasmic reticulum increased in mushrooms shape, and some of them surround the mitochondria (Plate Ⅱ: 13); (4) Some of globular or elliptical lipid droplets appeared, which contain high electron-dense substances (Plate Ⅱ: 13, 14); (5) numerous cytoplasmic glycogen granules arranged in a rose-like pattern (Plate Ⅱ: 13, 14).

3 Discussion

3.1 Origin of corpora atretica

The origin of corpora atretica has been the subject of great interest and controversy. The corpora atretica formed from atretic follicles before ovulation might be called preovulatory corpora lutea. Blanchette (1966) has observed that the preovulatory luteal cells are derived from the granulosa cells (follicular cell) in the rabbit. Corpora atretica cells were reported to arise from atretic follicles during the final disintegration of the follicles in birds by Guraya (1976), whereas Marshall and Coombs (1957) thought that they arose from the fibroblast cells of the theca. However, no evidence of corpora atretica formation from atretic follicles was found in white-sparrow (Gupta and Maiti, 1986).

The ovary of fishes is sac-like as found in amphibians, and the corpora atretica cells in fishes are derived from follicular cells (Wang, 1992; Chieffi et al., 1992). Similar to the present observation, in the ovary of the Chinese giant salamander and other amphibians, the corpora atretica also arise from follicular cells, while the thecal cells are not involved the formation of corpora atretica. It has been reported that thecal cells are involved in the formation of the interstitial glands in amphibian (Saidapur, 1978), therefore we suggest that corpora atretica be called preovulatory corpora lutea and they are derived from the follicular cells in the sac-like ovaries of anamniotes.

3.2 Endocrine function of preovulatory corpora lutea

Chieffi et al. (1992) reported the ultrastructure of atretic follicle in the electric ray *T. marmorata* and divided the process of follicular atresia into four stages. The first two comprise the dissolution of the oocyte and its phagocytosis by the smaller cells of the granular epithelium. The third stage consisted of the transformation of the granular epithelium into an active glandular structure and was accompanied by the development of a smooth endoplasmic reticulum. The fourth stage was marked by sclerosis and pigmentary degeneration of the atretic follicle. Taken together, these observations suggested endocrine steroidogenic role of the corpora atretica in the electric ray. The process of follicular atresia in the Chinese giant salamander was similar to these of corpora atretica formation described in the electric ray by Chieffi et al. (1992). Furthermore, Gupta and Maiti (1986) reported that in the Indian pied myna, atretic follicles showed weak 3β-hydroxysteroid dehydrogenase (3β-HSD) activity, a key enzyme involved with steroidogenesis in early stages. Yoshinaga and Osawa (1994) also found that immunoreactivity for aromatase was localized in newly formed granulosa lutein cells in the hamster. It could be concluded that the atretic follicles can secrete steroid hormones in the early stage of atresia and the steroidogenic potential of atretic follicles is gradually lost as the atretic process runs its course. Furthermore, transient steroidogenic potentiality of the atretic follicles at any time is possible. According to the present observations, there are some mitochondria with tubular cristae, plenty of smooth endoplasmic reticulum and lipid droplets, and a well-developed Golgi complex in the corpora atretic cells of the Chinese giant salamander, whose character of secreting steroid hormone was similar to that of atretic follicles in the electric ray (Chieffi et al. , 1992) and in *Salamandra salamandra* (Joly and Picheral , 1972) . In view of the known relationships of structure adapted to endocrine function (Kown et al. , 1991 , 1994) , when the ovum of the Chinese giant salamander was at the vitellogenic stage , the prevulatory corpora lutea may secrete steroid hormones.

The present findings showed that few atretic follicles appeared during the slow growth stage of the ova in the ovary. In May and June , when the oocytes were at the previtellogenic stage , some follicles began to form preovulatory corpora lutea , which initially did not exhibit endocrine function. In July and August , when the oocytes were at the vitellogenic stage , more preovulatory corpora lutea appeared and these cells bear the striking features of secreting steroid hormone. Although the follicular cells have the endocrine structural characteristics at this stage , they were arrayed in one layer. Enhancing the endocrine function of preovulatory corpora luteal cells seemed to make up for the quantity of follicle cells (Dumont and Brummett , 1978) .

Yang et al. (1981) also have observed the structure of atretic follicles in the Chinese giant salamander using the light microscope , and described it as degradation of the ovum. In the present study , it could be observed by electron microscope that the striking proliferation of follicular cells and then engulfing of oocytes were similar to that of Yang et al. (1981) . For yet unknown reasons , the majority of follicles will die through the process of follicular atresia. Apoptosis in steroidogenic cells such as the granulosa cells has a unique feature : steroidogenesis cell can take place in the apoptotic cells as long as the component of the steroidogenetic machinery are not destroyed (Amsterdam et al. , 1998) . It appears that the follicular atresia is not just a degenerative or pathological phenomena occuring during the developmental of oocytes , but these preovulatory corpora lutea may secrete steroid hormones to maintain normal levels required for normal oocyte maturation.

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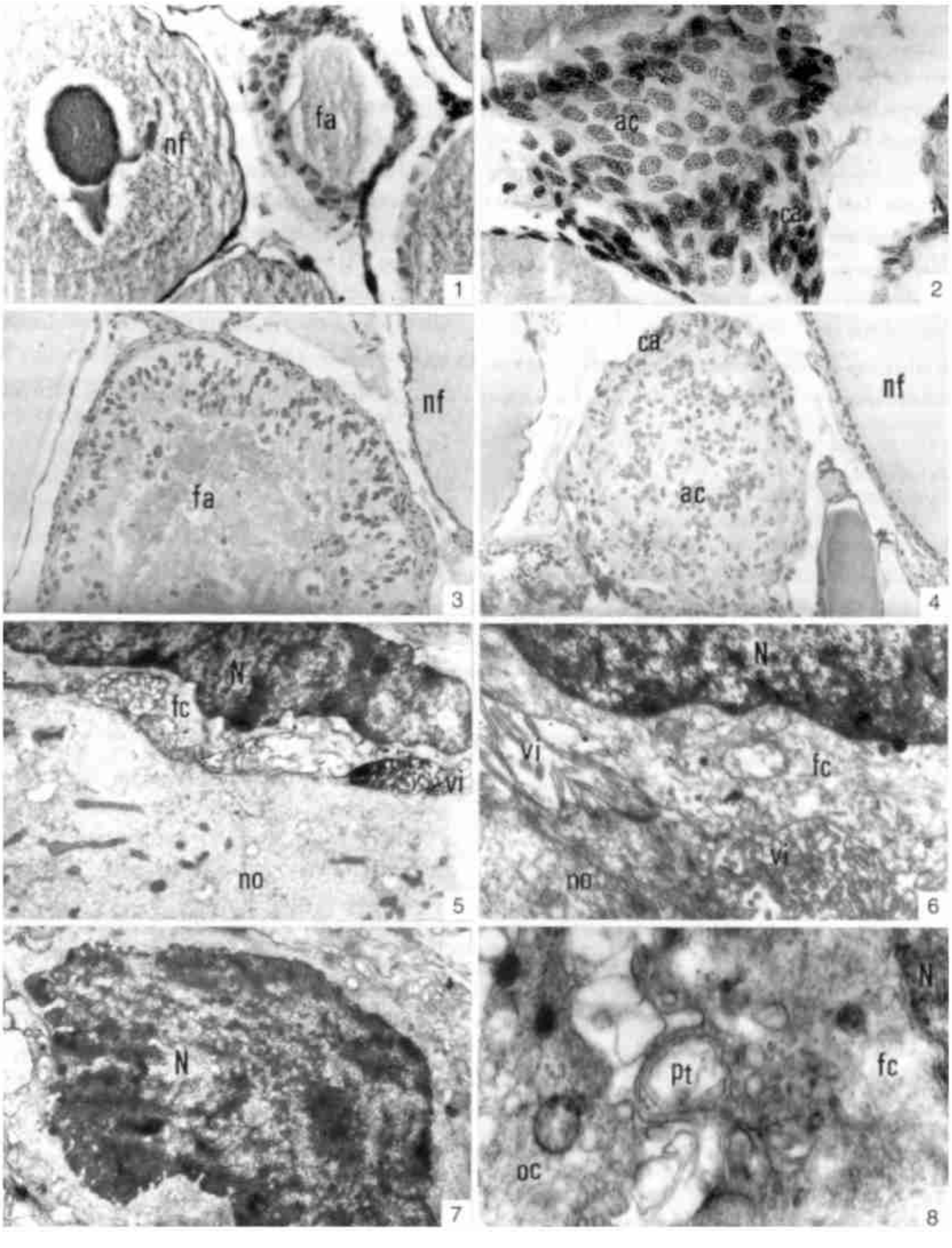
Explanation of Plates

Plate Ⅰ

1. In May, the atretic follicle (fa) appearing in the ovary, the normal follicles (nf) are at previtellogenic stage ×132.
2. The corpora atretica (ac) is found in the ovary in May. (ca) capillary ×198.
3. In Aug. the atretic follicle (fa) is found in the ovary and the normal follicles (nf) are at vitellogenic stage ×132.
4. The corpora atretica (ac) is found in the ovary of Aug. (nf) normal follicles, (ca) capillary ×132.
5. In May, the ultrastructure of normal developmental follicle (no) wall showing less cytoplasm of follicular cell (fc) and oocyte microvilli (vi) are short at the previtellogenic stage of oocyte. (N) follicular cell nucleus ×5 000.
6. In Aug. more cytoplasm of follicle cell and longer oocyte microvilli (vi) appearing in normal developmental follicle (no) wall at the vitellogenetic stage of oocyte ×8 000.
7. Showing less cytoplasma of corpora atretica cell in May. (N) nucleus ×5 000.
8. In May, the follicular cell (fc) engulfing the oocyte (oc) forms pinocytotic vesicles (pt) during the follicle atretic process ×20 000.

Plate Ⅱ

9. In Aug, the multivesicular body (mvb) appearing in the follicular cell cytoplasm. (N) nucleus ×20 000.
10. The follicular cell prominence (pr) engulfs oocyte microvilli (vi) forming multivesicular body (mvb) during follicular atresia in Aug ×20 000.
11. The mitochondria (mi) with tubular cristae and the lipid droplet (li) are striking in the corpora atretica cell ×30 000.
12. The well-developed Golgi complex (go) appearing in the corpora atretica cell. (va) vacuoles, (ve) vesicle ×15 000.
13. The lipid droplet (li) and mitochondria (mi) are surrounded by well-developed smooth endoplasmic reticulum (ser) in corpora atretica cytoplasm. (g) glycogen granules ×15 000.
14. In corpora atretica cell, the cytoplasm showing the abundant lipid droplet (li), glycogen granules (g) and mitochondria (mi) ×10 000.



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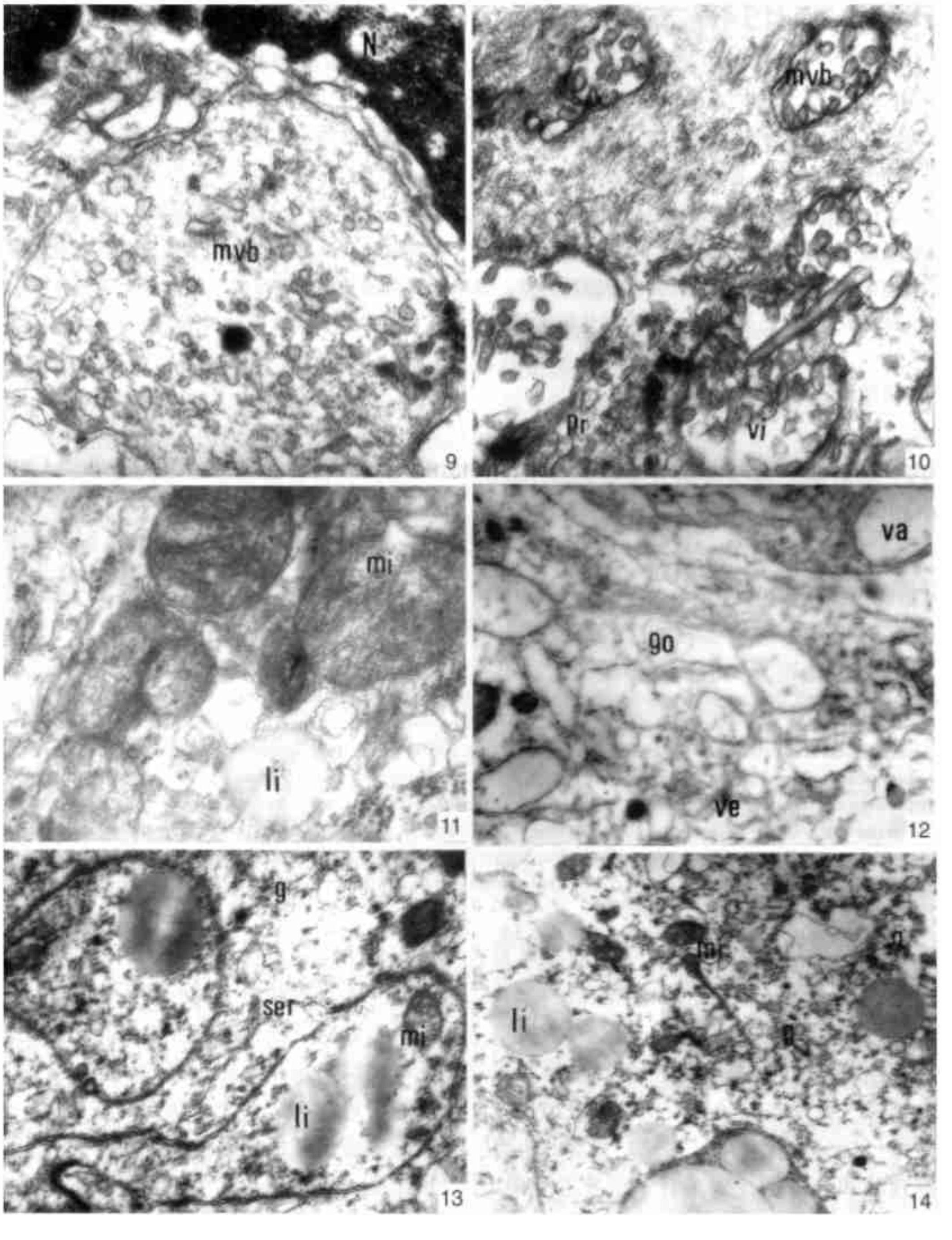

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