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A Study on Sexual Maturation of Hen Clam *Mactra chinensis* Philippi*

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Gonadal development, the annual reproductive cycle and the first sexual maturity of hen clam, *Mactra chinensis* were studied histologically.

Sexuality of the species was dioecious. The gonads were irregularly arranged from the subregion of mid-intestinal gland in visceral cavity to the reticular connective tissue of the foot. The ripe eggs were about 50-60 μm in diameter, and they were surrounded by gelatinous membrane.

The spawning period was from May to September when the water temperature ranged 18.5-27.0 °C, with the peak in June and July. The annual reproductive cycle of *Mactra chinensis* could be classified into five successive stages; multiplicative, growing, mature, spent, and degenerative and resting. The monthly changes of the fatness coefficient closely correlated with the annual reproductive cycle. Percentages of the first sexual maturity of female and male clams were over 50% among those individuals ranging from 3.5 to 3.9 *cm*, and 100% in those over 5.0 *cm* in shell length.

Introduction

Hen clam, *Mactra chinensis* which is commonly found on Korean coast, and it is one of the important commercial bivalves. Some works have been done on the ecological aspect of the clam including the morphometry and growth rate (Hanaoka and Shimadzu, 1949), the development of eggs (Miyasaki, 1933; Lee and Son, 1978), spawning and growing (Kim *et al.*, 1985), and the propagation (Sakai, 1976). However, reproductive biology of this clam is sparse. The main purpose of the present study is to know sexual maturation of the clam based on histological examinations and some morphometric data.

Materials and Methods

Specimens of *Mactra chinensis* were collected by the dredge every two weeks in the vicinity of Piungdo, located in the mouth of the Kumgang estuary from January to December 1984 (Fig. 1).

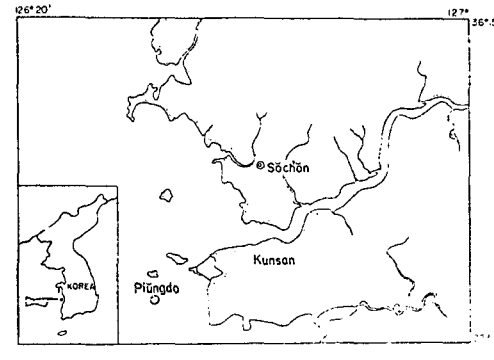

Fig. 1. A map showing sampling station in the study area.

A total of 752 individuals of hen clams were used for the study. The length and weight of each part of specimens were measured, and the fatness coefficient (meat weight x 100/meat weight + shell weight) was calculated by the method of Momoyama and Iwamoto(1979).

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A piece of gonad of each specimen was prepared for histological examination by fixation in Bouin's solution and dehydrated by ethanol. The tissues of each gonad embedded with paraffin were cut into 5-6 μm in thickness, and stained with Hansen's haematoxylin -0.5% eosin, Mallory's triple stain and PAS stain.

Results

1. Position and structure of the gonads

The gonads were irregularly arranged from the subregions of mid-intestial glands in visceral cavity to reticular connective tissues of the foot. The clam was dioecious. The ovary was composed of a number of ovarian sacs, and the testis comprised several testicular tubules.

As the gonads developed, they extended their volume to reticular connective tissue of the foot, and external feature of the mature ovary showed red, and that of the mature testis, lemon-yellow in colour. At this time, if they are slightly scratched, ripe eggs and milky white sperms flow out readily.

2. Annual reproductive cycle

Based on histological observation of the germ cells and tissue cells around them, the gonadal phases could be classified into five stages; multiplicative, growing, mature, spent and degenerative and resting stage as shown in Fig. 2. The criteria used in defining the category of each stage as follows:

1) Multiplicative stage

In this stage, oogenesis occured in the germinal epithelia of the ovarian sacs (Pl. I-Fig. 1). A large number of oogonia appeared along the germinal epithelium. Every oogonium measuring about 10 μm in diameter had a distinct nucleus, while the cytoplasm was very poor(Pl. I-Fig. 2). At this time, a great number of eosinophilic cells and undifferentiated mesenchymal tissues were also

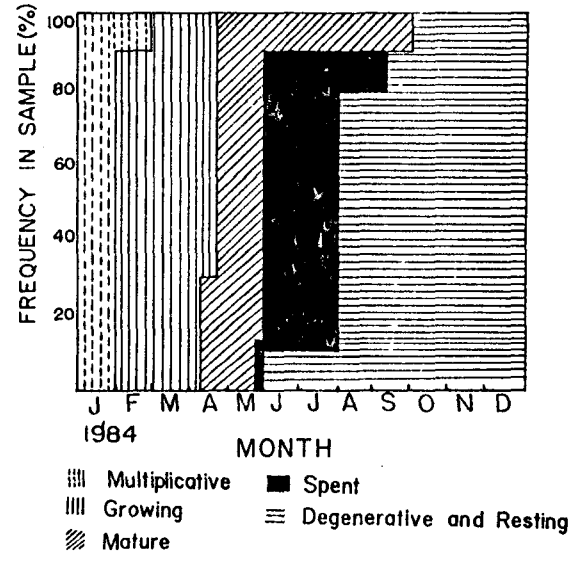

Fig. 2. Gonadal phase of *Mactra chinensis* from January to December 1984.

seen along the germinal epithelium of ovarian sac.

Spermatogenesis occured in the germinal epithelia of the testicular tubules. A large number of spermatogonia were actively proliferating among the eosinophilic cells and the mesenchymal tissues on the germinal epithelia of the testicular tubules in the early development stage(Pl. II-Figs. 10, 11) Spermatogonia were recognized by their nuclei and thin clear cytoplasmic envelops. Spermatogonium was about 5-6 μm in diameter and attached to the germinal epithelium.

The individuals of the multiplicative stage appeared from January to February.

2) Growing stage

In female, the early growing oocyte also had a round nucleus containing a nucleolus and its cytoplasm began to grow in volume(Pl. I-Fig. 3).

When the oocytes grew to 18-30 μm in diameter, each of them made a egg-stalk connected the germinal epithelium, and its nucleus enlarged to be a germinal vesicle having a small nucleolus(Pl. I-Figs. 4, 5).

In male, spermatogonia grew to spermatocytes, spermatocytes moved toward the lumen of the tubule(Pl. II-Figs. 12, 13). These spermatocytes measuring 3-4 μm in diameter showed a flowing structure.

As the further development of the gonad advanced, each of the testicular tubules composed of spermatogonia, spermatocytes, spermatids in groups on the germinal epithelium, and they are arranged in stratified layers(Pl. Ⅱ-Fig. 14).

Growing gonads were found from February to mid-April.

3) Mature stage

In female, the oocyte grew to 30-50 μm in its size, each ovarian sac was filled with the mature oocytes in the center of the lumen. At this time, however the mesenchymal tissues were very few and the germinal epithelium become very thin(Pl. Ⅰ-Fig. 6). Each ripe oocyte measuring 50-60 μm in diameter was surrounded by gelatinous membrane and its cytoplasm contained a large number of yolk granules and a few lipid granules. It had a germinal vesicle and a basophilic clear nucleolus (Pl. Ⅰ-Fig. 7).

In male, spermatids underwent transformation into differentiated spermatozoa. The spermatozoa occupy the center of the lumen of the testicular tubule, and their heads oriented to the basement membrane and tails to the center of the lumen. Ripe gonads are characterized by the formation of stream of spermatozoa in their testicular tubules (Pl. Ⅱ-Fig. 15).

Individuals in the mature stage appeared from the begining of April to September.

4) Spent stage

In the spawning season of female, the ripe oocytes in the ovarian sac were spawned and there remained a few ripe oocytes undischarged as well as young oocytes(Pl. Ⅰ-Fig. 8).

In male, spermatozoa in the lumen were discharged into the surrounding water (Pl. Ⅱ-Fig. 16). The spawning began at the end of May and lasted till mid-September. The main spawnings occured between June and July.

5) Degenerative and resting stage

After the spawning, each ovarian sac was contracted and degenerated for a long time (Pl. Ⅰ-Fig. 9). After degeneration and resting, the rearrangement of the newly formed ovarian sacs followed.

After spawning, a few of remaining spermatozoa were scattered in the lumen of testis, but they began to degenerate(Pl. Ⅱ-Fig. 17). Thereafter, there remained a few degenerating spermatozoa undischarged in the lumen for a long time(Pl. Ⅱ-Fig. 18). After degeneration and resting, the rearrangement of the newly formed testicular tubules followed.

The individuals in this stage were found from June to December.

3. The first sexual maturity

The first sexual maturities of a total of 170 individuals of *Mactra chinensis* were investigated histologically in order to certify these shell length participated in reproduction during the main spawning season between June and July as shown in table 1.

Table 1. The shell length of the first sexual maturity of *Mactra chinensis* during the spawning period between June and July

Shell length (*cm*)	Female		Male	
	Number	Maturity (%)	Number	Maturity (%)
2.6-2.9	13	0	15	0
3.0-3.4	15	20.0	14	28.6
3.5-3.9	18	55.5	14	57.1
4.0-4.4	10	80.0	11	81.8
4.5-4.9	12	91.7	10	90.0
5.0-5.4	8	100	7	100
5.5-5.9	6	100	5	100
6.0-6.3	7	100	6	100
Total	89		81	

Percentage of the first sexual maturity of female and male clams were over 50% among those individuals ranging from 3.5 to 3.9 *cm*, and 100% in those over 5.0 *cm* in the shell length.

4. Seasonal changes of the fatness coefficient and sea-water temperature

Monthly changes of the fatness coefficient are shown in Fig. 3.

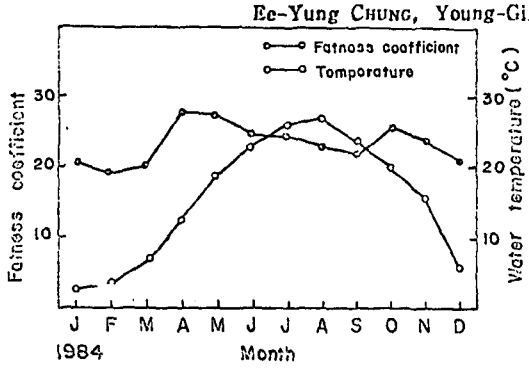

Fig. 3. Monthly changes of the fatness coefficient in *Mactra chinensis* and the mean seawater temperature, from January to December 1984.

In February when the water temperature was very low, the mean value of the fatness coefficient was 19.08, the lowest value during the entire period of experiment, but thereafter it rapidly increased during the spring season, reaching to 27.43 in April. From June to August when the water temperature increased up to 27.0°C, the fatness coefficients were remarkably decreased in the spawning period, which demonstrate the close correlation between the fatness coefficient and gonadal phases.

Discussion

The nutritive material concerning the gonadal development have been reported in several papers, i.e., phagocytic-nutritve cells in *Venus mercenaria* by Loosanoff(1937 a, b), undifferentiated mesenchymal tissue and eosinophilic cell in *Pinctada martensii* by Lee(1972), in *Corbicula fluminea* by Lee and Chung(1980), and in *Solen strictus* and *Solen gordonis* by Chung *et al.* (1986). The undifferentiated mesenchymal tissues and eosinophilic cells which were abundant on the germinal epithelium in the multiplicative stage and gradually disappered with the gonadal development according to the report by Lee and Chung(1980), they could be considered as a kind of nutritve materials.

The observations in the present study showed the same results as Lee and Chung(1980). The fatness coefficients of *Mactra chinensis* were very low when spawning was completed in September, while rapidly increased in the spring season, and reached the peak in April. The fatness coefficients of *Fulvia mutica* were also low after spawning(Inoue, 1955; Matsuoka *et al.*, 1968), and were increased along the growth and the maturity of the gonad(Chang and Lee, 1982). Therefore, monthly changes of the fatness coefficient is well correlated with the reproductive cycle showed by the histoglogical observation of the gonad. Miyasaki(1933) reported that the optimum water temperature for the development of eggs in hen clam was 22-28°C. In the present study, the water temperature ranged from 20.3 to 27°C during the spawning period, and it seems that the spawning period is closely related to the water temperature.

The spawning period of hen clam, *M. sulcataria* was from May to June in Tokyo Bay (Hanaoka and Shimadzu, 1949), but in this study, the spawning season of hen clam, *M. chinensis* was from the end of May to mid-September. Some local variations of the spawning period of hen clams might be related to the geographical differences in the water temperature, time of the food production, and some other environmental factors.

Kim *et al.* (1985) reported that percentages of the first sexual maturity of female and male clams of *M. sulcataria* were 50% among those individuals of 3.64 *cm* corresponding to one year old, and 100 % in those over 4.75 *cm* in the shell length.

In the present study, percentages of the first sexual maturity of both sexes of *M. chinensis* over 50% among those individuals ranging from 3.5 to 3.9 *cm*, and 100% in those over 5.0 *cm* in the shell length. These results were well in accordance with the report by Kim *et al.* (1985).

Explanation of abbreviations

Ct : Connective tissue
Doc: Degenerating oocyte
Dsz: Degenerating spermatozoa
Es : Egg-stalk
Ge : Germinal epithelium
Gv : Germinal vesicle
Ig : Intestinal gland
Lu : lumen
Ml : Muscle layer
Mt : Mesenchymal tissue
N : Nucleus
No : Nucleolus
Oc : Oocyte
Og : Oogonia
Os : Ovarian sac
Rs : Residual substance
Sc : Spermatocyte
Sg : Spermatogonia
St : Spermatid
Sz : Spermatozoa
Yg : Yolk granule

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개량조개, *Mactra chinensis* Philippi의 性成熟에 關한 硏究

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群山大學 海洋開發學科, 群山水産專門大學 養殖科, 釜山水産大學 資源生物學科

개량조개, *Mactra chinesis*의 資源管理 및 增養殖을 爲한 基礎生物學的 硏究로서, 西海岸 옥구군 비응도 근해에서 1984年 1月부터 12月까지 月別로 採集한 材料를 對象으로 生殖巢發達, 生殖年周期, 그리고 群成熟度를 組織學的 方法에 依해 調査하였다.

本 種은 雌雄異體型 二枚貝로서, 生殖巢는 內臟囊의 肝中腸線 下方으로부터 足部의 纖維性網狀結締組織까지 不規則하게 分布되어 있었다.

完熟卵의 卵徑의 크기는 50~60 *μm* 이었으며 젤라틴狀의 皮膜으로 둘러 싸여 있었다.

産卵은 水溫이 18.5~27.0°C 인 5月부터 9月에 걸쳐 일어나며 主産卵期는 6月과 7月이었다.

개량조개의 生殖年周期는 分烈增殖期, 成長期, 放出期, 退化 및 休止期의 連續的인 5段階로 나눌수 있었다.

肥滿度指數의 月別變化는 生殖年周期와 밀접하게 상호관련되어 있었다.

암·수 個體들의 群成熟度는 殼長 3.6~3.9 *cm* 인 個體들은 50% 이상이었고, 殼長이 5.0 *cm* 以上인 個體들은 全 個體가 再生産에 참가하였다.

Explanation of plates

Plate Ⅰ.

Fig. 1. The ovary was composed of a number of ovarian sacs which is located between the mid-intestinal glands and reticular connective tissues. X 100. Fig. 2. Transverse section of an ovary of the multiplicative stage. X 400. Fig. 3. An ovarian sac of the ovary of the same stage above mentioned. X 600. Note proliferation of samll oogonia along the germinal epithelium and undifferentiated mesenchymal tissue and eosinophilic cells. Fig. 4. Section of the growing stage. X 100. Note the early growing oocytes along the germinal epithelium. Fig. 5. The growing oocytes in the ovarian sac. X 400. Note the egg-stalk of the growing oocyte attached to the ovarian sac wall. Fig. 6. Section of a mature ovary. X 100. The lumen was filled with the mature oocyte. Fig. 7. Fully ripe oocytes in the ovarian sac. X 400. Basophilic nucleolus are seen in the large germinal vesicle and yolk materials were seen in the cytoplasm. Fig. 8. Section of the spent ovary. X 100. Note the presence of a few undischarged eggs and residual substances in the ovarian sac after spawning. Fig. 9. Section of ovarian sacs of the degenerative and resting stage. X 100. Note degenerating oocytes in the ovarian sacs.

Plate Ⅱ.

Fig. 10. Transverse section of the testis of the multiplicative stage. X 300. Fig. 11. Testicular tubules of the same stage above mentioned. X 100. Note many small spermatogonia and undifferentiated mesenchymal tissue appeared along the germinal epithelium. Fig. 12. Transverse section of the early growing testis. X 100. Fig. 13. Section of the testis of the growing stage. X 400. Note a number of spermatogonia and spermatocytes on the germinal epithelium. Fig. 14. Transverse section of the late growing testis. X 100. Note the layers composed of spermatocytes and spermatids. Fig. 15. Section of the testis of the mature stage. X 100. Note a large number of spermatozoa with the tails toward the center of the testicular tubule. Fig. 16. Section of the spent testis. X 100. Testicular tubules became withering and a few number of undischarged spermatdzoa and residual substances remained in the tubule. Fig. 17. Stection of the testis of the degenerative and resting stage. X 100. Note degenerating spermatozoa in the testicular tubules. Fig. 18. Section of the degenerating testicular tubule with shrunken appearance. X 100.

Plate I

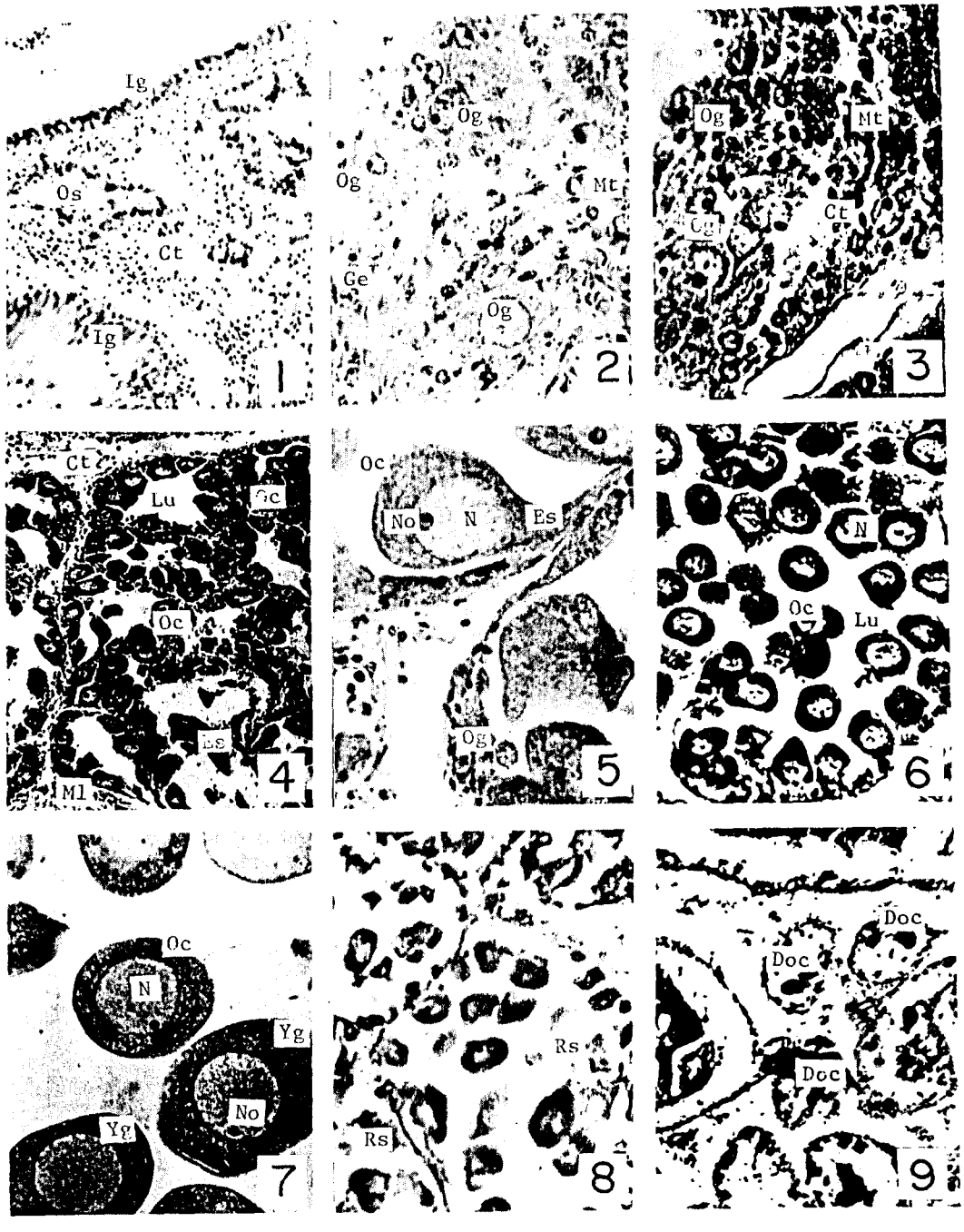
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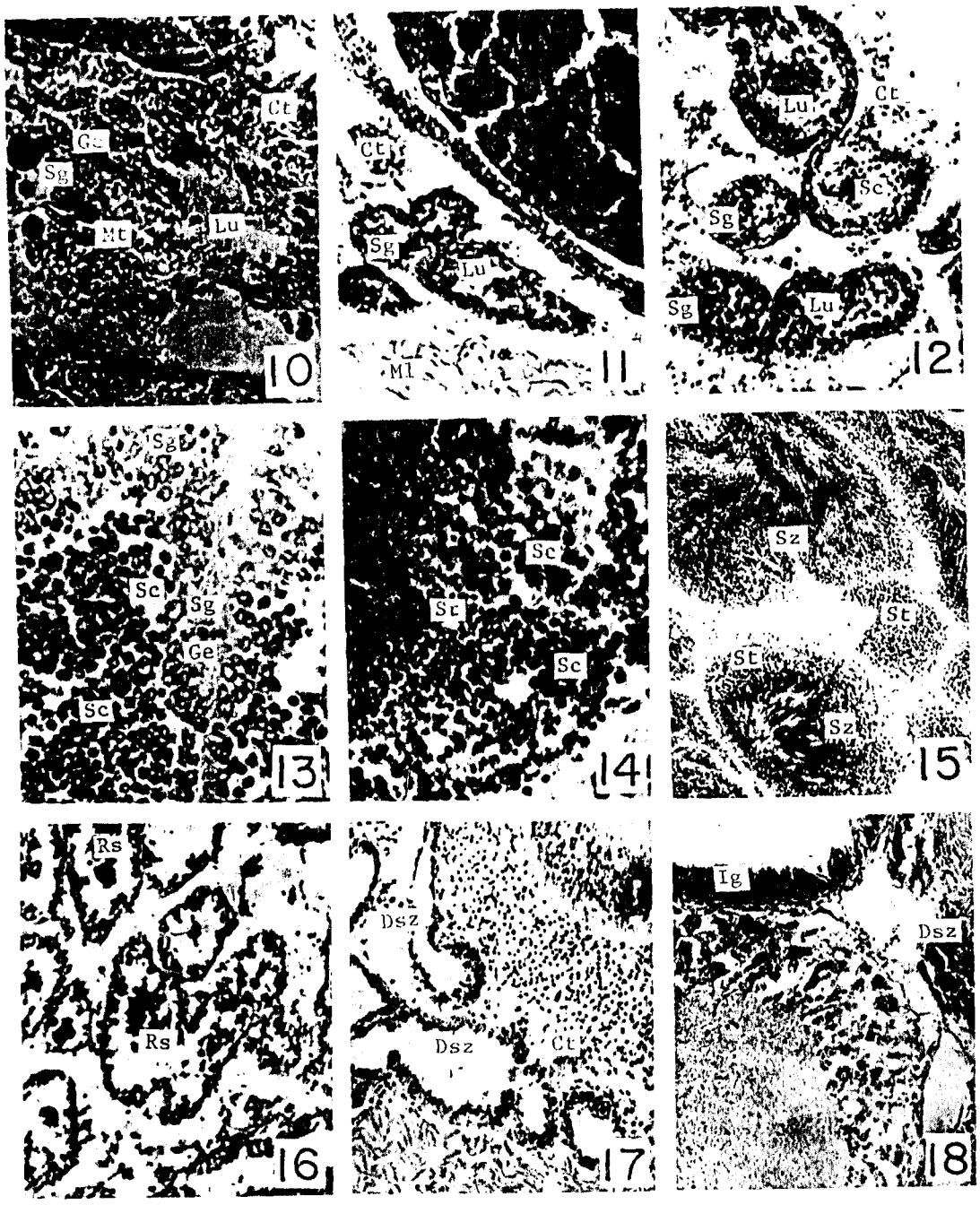
Plate II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