RESEARCH ARTICLES

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DEVELOPMENT OF, AND SEASONAL CHANGES IN, THE REPRODUCTIVE SYSTEM OF *ACHATINA FULICA*

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ABSTRACT

The development of, and seasonal changes in, the reproductive system of the giant African snail, Achatina fulica *were studied by light microscopy. The ovotestis first appeared in 3-month-old snails. It was composed of many acini which contained various stages of developing germ cells; namely, spermatogonia, primary spermatocytes, spermatids and spermatozoa. Groups of sperm heads were found to be adhered to Sertoli cells. The large oocytes were first observed in snails aged 5 months old. They were often located at the periphery of acini and progressively increased in number as the snails reached the age of 7-8 months. Beyond this age, the number of oocytes decreased. During the year, the number of oocytes per acinus was highest in May and September, and lowest in March. Spermatozoa were produced continuously throughout the year.*

INTRODUCTION

Numerous studies have been undertaken on many aspects of the pulmonate gonad. However, as emphasized by recent review,[1] we are still far from understanding gametogenesis and other functions of the organ. The fundamental processes that are involved in gametogenesis and the orientation of gametogenesis to either spermatogenesis or oogenesis have been studied. Runham and Hogg[2] have reported on the development of gonad of *Deroceras reticulatum* (pulmonate). The gonad consisted of small units called acini. During the undifferentiated stage of the gonad, the number of acini increased to 70-180. In later stages, the acini increased in size and often became lobed.[2]

In contrast to other species, there are few reports on the reproductive activity of *Achatina fulica.* Pawson and Chase[3] studied the reproductive activity of this snail and found that sexual maturity was reached at 5 months of age, with a peak in egg production between 210-270 days old. Egg clusters, containing about 100-200 eggs, were hatched synchronously within a 24-h period. Apart from these reports, information on the development of the reproductive system of *A. fulica* has not yet been fully obtained. Hence one of the objectives of this investigation is to study the development of the reproductive system of *A. fulica.*

Furthermore, details of the morphological changes in the reproductive system associated with the breeding cycle are also not available. Runham and Laryea[4] studied the reproductive system of *Agriolimax reticulatus* (pulmonate) and reported that breeding was at maximum in the spring and autumn seasons. These results appear to indicate that the stages in maturation of the reproductive system are related to the growth phases of the animal, and that physiological and environmental (seasonal) factors may control this maturation of the reproductive system. Hence, another objective of this investigation is to monitor the development of the reproductive system of *A. fulica* through various seasons of the year.

MATERIALS AND METHODS

Procedure for studying the development of the reproductive system

Young snails aged 1, 2 and 3 weeks and 1-12 months old were obtained from the snail culture laboratory of the Center for Applied Malacology and Entomology, Department of Biology, Faculty of Science, Mahidol University. At least 4 snails from each age group were used. Snails aged 1-4 weeks were fixed without cutting off the reproductive organs. In older snails, the ovotestis, prostate gland, uterus and albumen gland were cut off and processed for light microscopy. These tissues were fixed overnight in Bouin's fluid, then dehydrate in increasing concentrations of alcohol, and infiltrated with dioxane. Finally, they were embedded in paraffin. Sections were cut on a rotary microtome at 4-5 microns thickness and stained with hematoxylin and eosin. Examination and photography were performed with Olympus Vanox and Leizt Orthoplan microscopes.

Procedure for studying seasonal changes in morphology of reproductive system

Adult snails were collected from the snail culture laboratory in the greenhouse of the Center for Applied Malacology and Entomology and from the field in the area around Ramathibodi Hospital. Every month from April 1987 to March 1988, the ovotestis, prostate gland, uterus and albumen gland were separated and processed for light microscopy as previously described. Oocytes from all ovotestis were counted per acinus, and the t-test was used to compare the number of oocytes from the greenhouse snails with that from snails collected from the area around Ramathibodi Hospital.

RESULTS AND DISCUSSION

Development of the reproductive system

Ovotestis

It was found that there was no ovotestis development in *A. fulica* from hatching up to the age of 2 months (Fig.1). The ovotestis first appeared, in the region of the digestive gland (Fig.2), when the snail reached the age of 3 months. Numerous acini of the ovotestis were observed. Each acinus was completely lined with a basement membrane. The germinal epithelial cells consisted of two types, namely the Sertoli cells and the spermatogenic cells. The latter consisted of the spermatogonia, spermatocytes, spermatids and spermatozoa (Figs.3,4).

Sertoli cells were in contact with the basement membrane. They had large prominent nuclei and very pale cytoplasm (Fig.5). Numerous spermatozoa were attached to the periphery of cytoplasm of the Sertoli cell.

Spermatogonia and spermatocytes were usually found at the periphery of the acinus. The spermatogonia were smaller than the spermatocytes (Figs. 3, 4). The spermatids were characterized by smaller nuclei and more eosinophilic cytoplasm. Numerous spermatozoa were observed. They were usually aggregated in clumps of densely stained heads and long tails. The clusters of spermatozoa heads were found in close association with the Sertoli cells (Figs.4,5).

The histology of the ovotestis in 4-month-old snails was similar to that in snails of 3 months old. However, a striking morphological change was observed in the ovotestis of snails at the age of 5 months, when the huge cells, the oocytes, were first observed (Fig.6). This cell had a prominent nucleolus and clear cytoplasm. It was located at the periphery of the acinus and surrounded by thin processes of follicular cells. The number of oocytes in the ovotestis increased and reached maximum in snails aged 7 months old (Figs.7,8). It was noticed that, at this age, only a small number of spermatozoa was present in the acini containing the large number of oocytes (Fig.8). In snails aged 8-12 months old, the number of oocytes dropped slightly and became stable (Figs.9,10). In contrast to oogenesis, spermatogenesis occurred in the ovotestis in snails aged 3-12 months old (Figs.3-10).

Uterus

The uterus appeared concurrently with the ovotestis. The uterine wall was rather thin in the early stage of development and consisted of mucosa, submucosa and muscular layers (Figs.11-13). Adipose tissue filled up the submucosal layer in snails from the age of 5 months old and onwards. The uterine wall could be divided into three layers based on the organization and the types of tissues; namely, inner, middle and outer layers. The inner layer was narrow and composed of simple ciliated columnar epithelium around the lumen (Figs.12-14). The middle layer was wide and consisted of numerous adipose cells surrounded by lamina propria. Most of the adipose cells were large and had an oval shape. Their nuclei

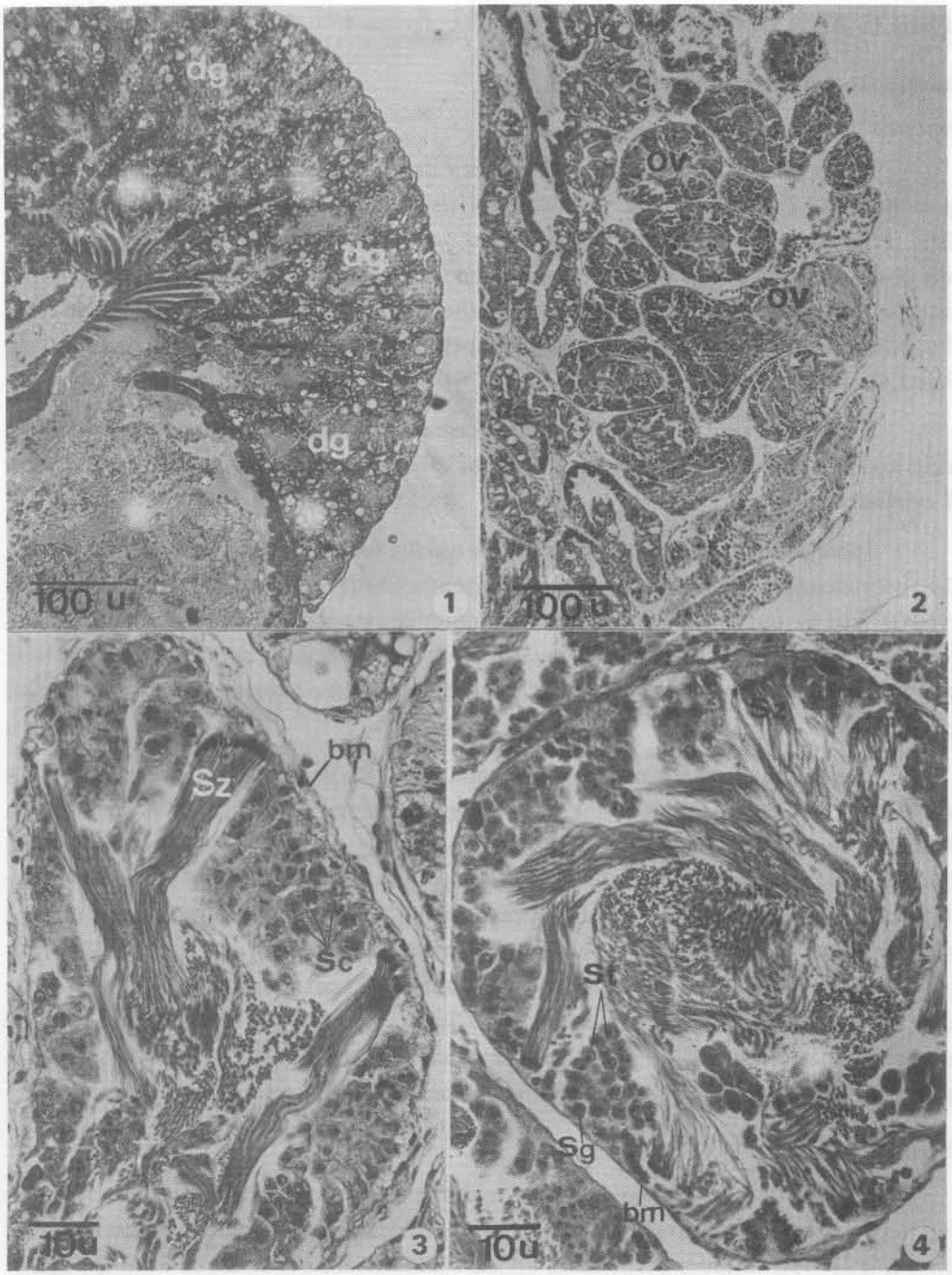


Fig. 1 Paraffin section of reproductive system of *Achatina fulica* at 2 weeks, only digestive gland (dg) is present.

Fig. 2 Three-month-old snail; ovotestis is first observed. ov = ovotestis, dg = digestive gland.

Figs. 3,4 High magnification of ovotestis in 3-month-old snail, showing basement membrane (bm) surrounding acinus. Spermatogonia (Sg), spermatocytes (Sc), spermatids (St) and spermatozoa (Sz) are present.

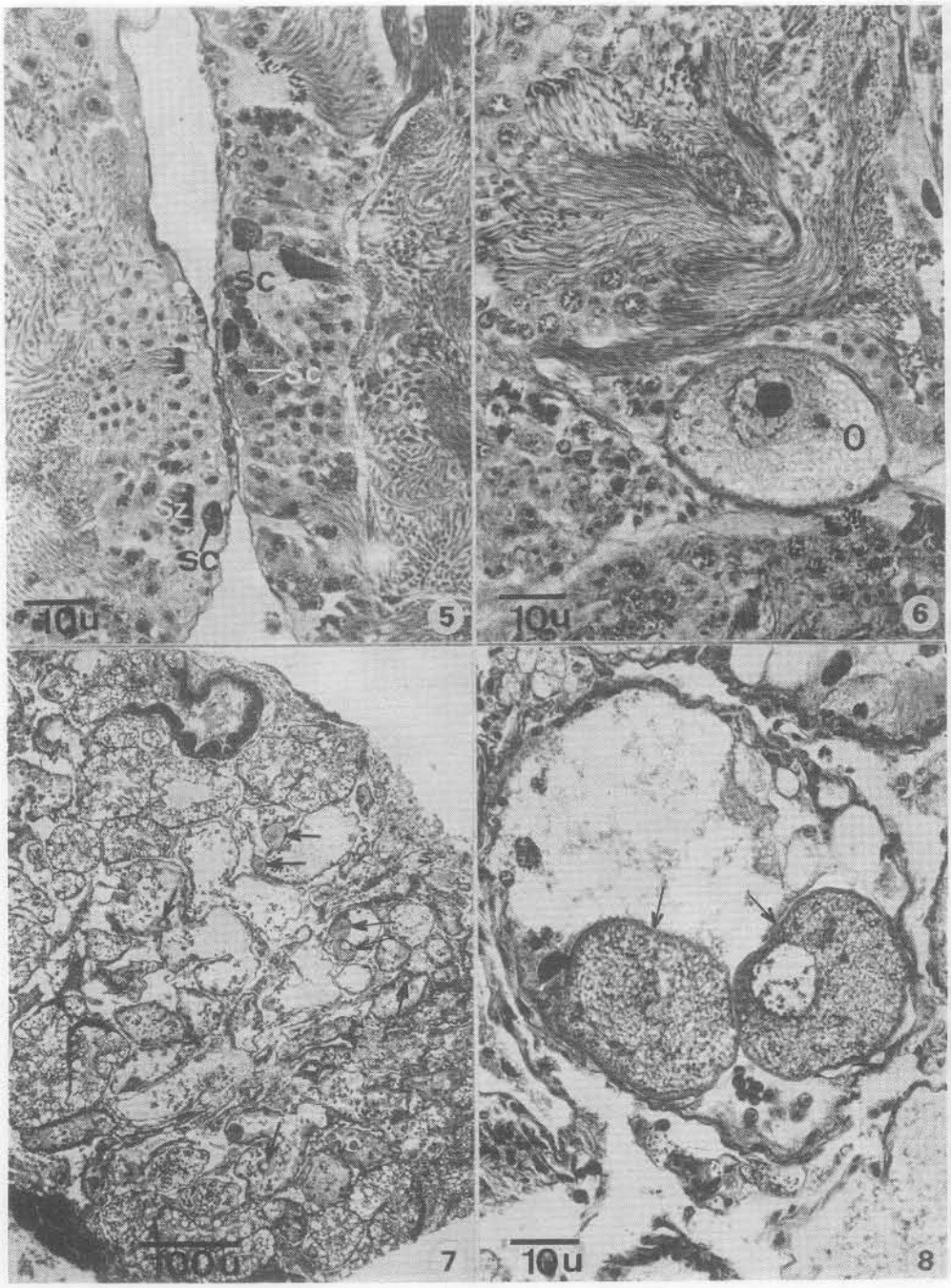
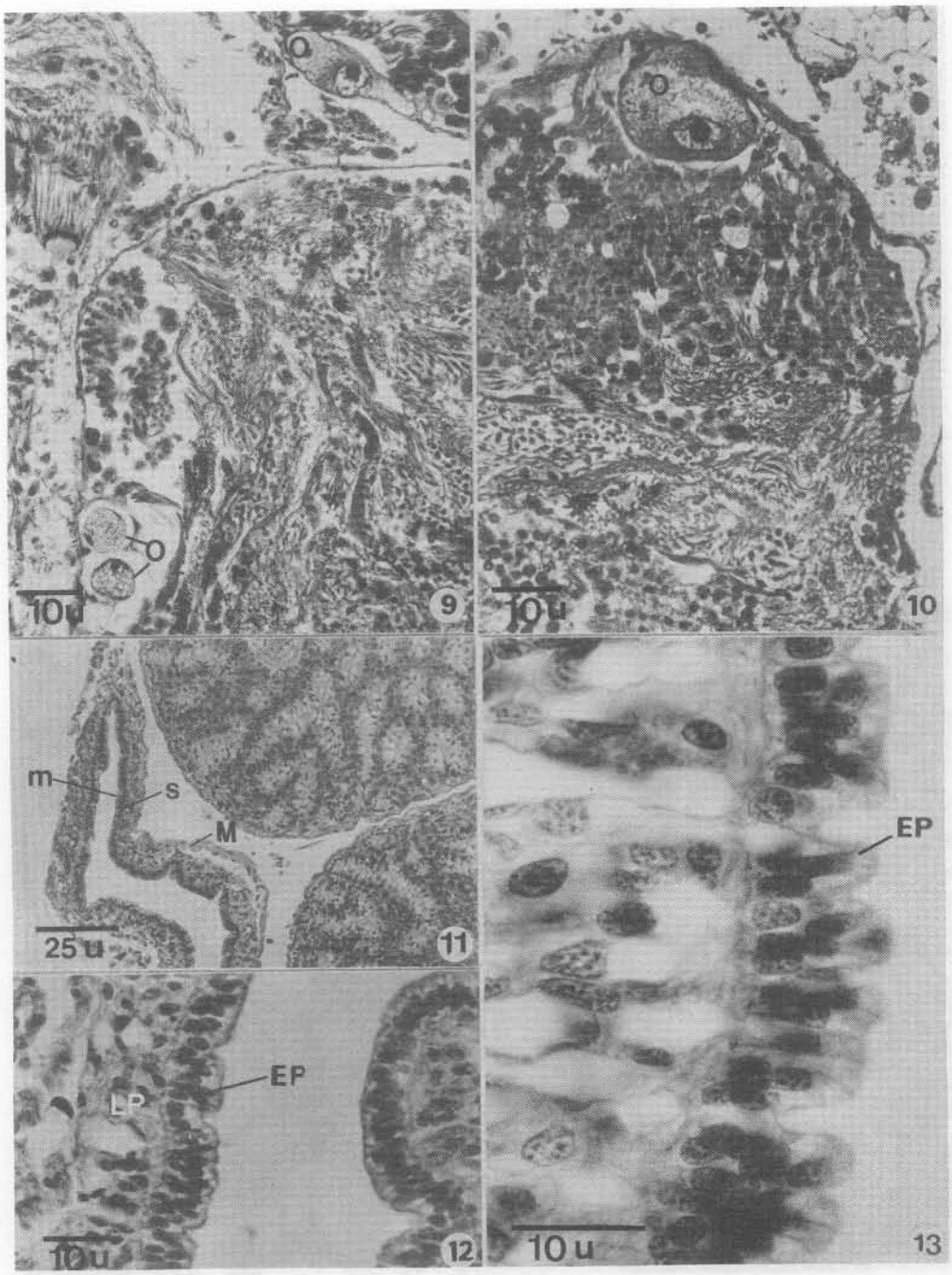


Fig. 5 Medium magnification of ovotestis in 5-month-old snail, showing Sertoli cells (SC) at the base of acinus. Clusters of sperm heads are observed close to Sertoli cell. Sc = spermatocyte

Fig. 6 Cortex of acinus, showing an oocyte (O) with prominent nucleus and highly dense nucleolus, and pale cytoplasm.

Figs. 7,8 Low and high magnification of ovotestis in 7-month-old snail showing a large number of oocytes (arrows).



Figs. 9,10 Ovotestis from snails aged 8 and 9 months, respectively, showing the decrease in number of oocytes (O)

Fig. 11 Low magnification of uterus in 3-month-old snail showing thin uterine wall. The mucosa (m), submucosa (s) and muscular layer (m) are observed.

Figs. 12,13 Medium and high magnification of uterine wall in snails aged 3 and 5 months, showing epithelium (EP) and laminar propria (LP).

were located at the periphery of the cells (Figs.14,15). The presence of the adipose tissue in the uterus could be to function in supporting a large number of eggs that are retained in the uterus before oviposition. The outer layer of the uterine wall contained smooth muscle that was organized in both circular and longitudinal directions. This layer was rather thick and covered with connective tissue (Figs.14,15).

Prostate gland

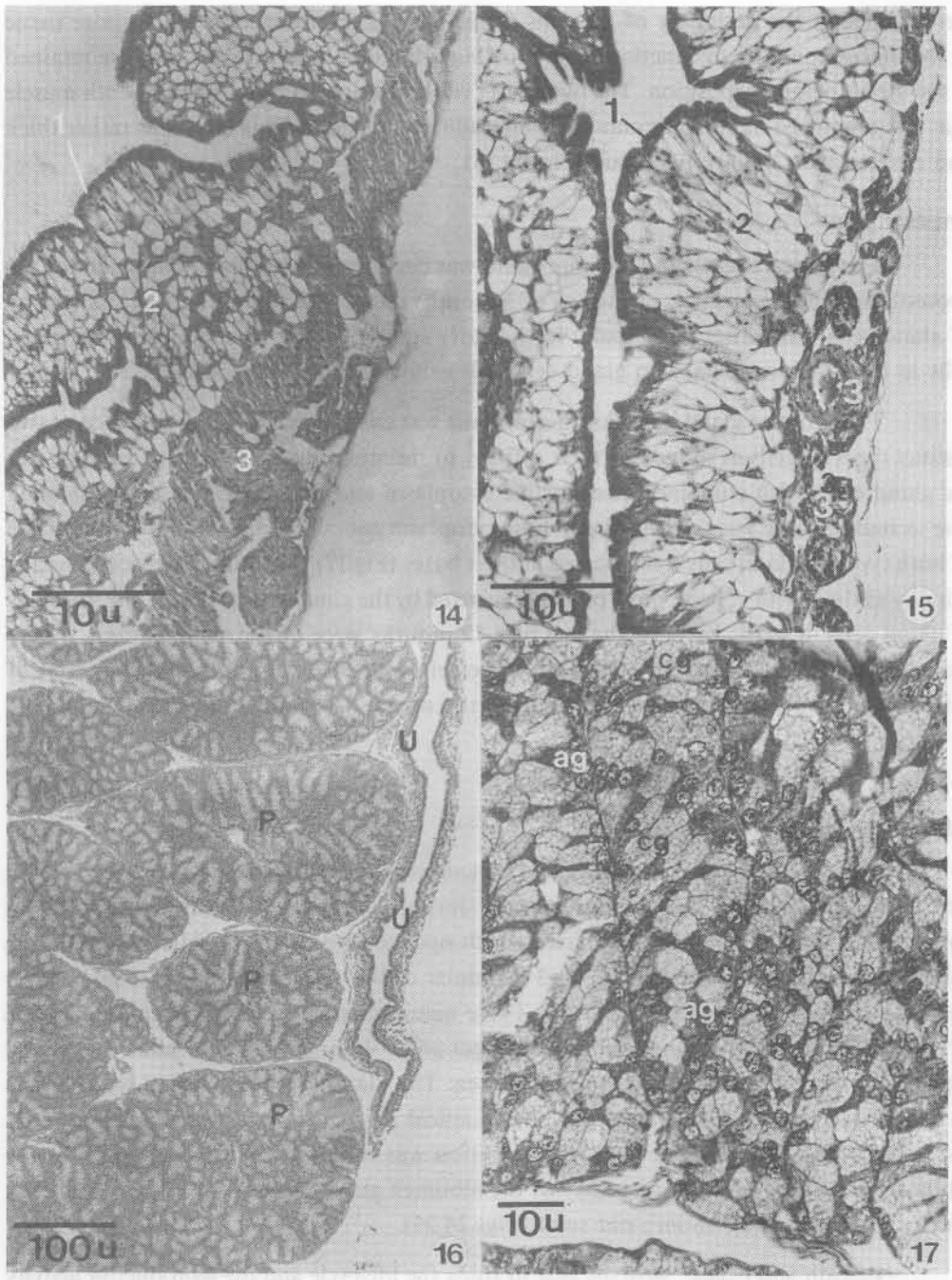
The appearance of the prostate gland was concurrent with the development of the ovotestis and uterus in snails at the age of 3 months old. It was found that the stainability of gland cells was rather poor (pale) in the early stage (Fig.16). The cytoplasm of gland cells in the acini was clear. No gland cell with acidic granules was observed.

The prostate gland in 4-month-old snails was characterized by the presence of two distinct types of cells as judged by their affinity to staining (Fig.17). The first cell type was the gland cell which contained eosinophilic cytoplasm and was not extracted by alcohol. The second cell type was the gland cell whose cytoplasm contained clear granules. The nuclei of both types of gland cells were located at their bases (Fig.17). The lumen in each prostatic acinus was filled with colloid, most probably secreted by the gland cells (Fig.18). The histology of gland cells in snails between the age of 4-12 months were of the same pattern, except that the quantity of colloid being stored in the acini was different, that is, the amount of colloid increased significantly in snails from the age of 10 months upwards (Fig.19).

Albumen gland

The albumen gland also appeared concurrently with the ovotestis, prostate gland, and uterus. The size of this gland increased during development. Histologically, it was composed of a large number of follicles which opened into the main duct (Fig.20). Each follicle was round and consisted of 10-13 glandular cells arranged radially in a single layer around a small central lumen (Fig.21,22). There were two types of cells in each follicle. The first cell type was the gland cell which was larger and columnar in shape, while the second type was smaller and located close to the lumen. The gland cell had a broad basal region in which a large nucleus was located and the apical part of the cell was tapered towards the lumen (Fig.22). The main duct with secretion was found at the central region of the albumen gland (Fig.23). The histology of the albumen gland in the 7-month-old snail was similar to that in the 5-month-old snail (Figs.24,25).

Pawson and Chase[3] were the first to study the life-cycle and the reproductive activity of *A. fulica*. They reported that growth of *A. fulica* in their first month of life was rapid, followed by a less rapid growth in the next two months. Between 4-6 months, the growth rate slowed as the snails became sexually mature. By 5 months of age, mature sexual organs were formed. The peak in egg production occurred between 7-9 months.



Figs. 14,15 The uterine wall in snails aged 7 and 9 months, respectively, showing inner ciliated columnar epithelium (1), middle adipose tissue (2) and outer muscular layer (3).

Fig. 16 Prostate gland in 3-month-old snail, showing the characteristic of prostatic (P) acini; uterus (U) is present close to prostate gland.

Fig. 17 Prostate gland in 4-month-old snail showing two distinct cell types; one contains acidic granules (ag) and the other has clear granules (cg).

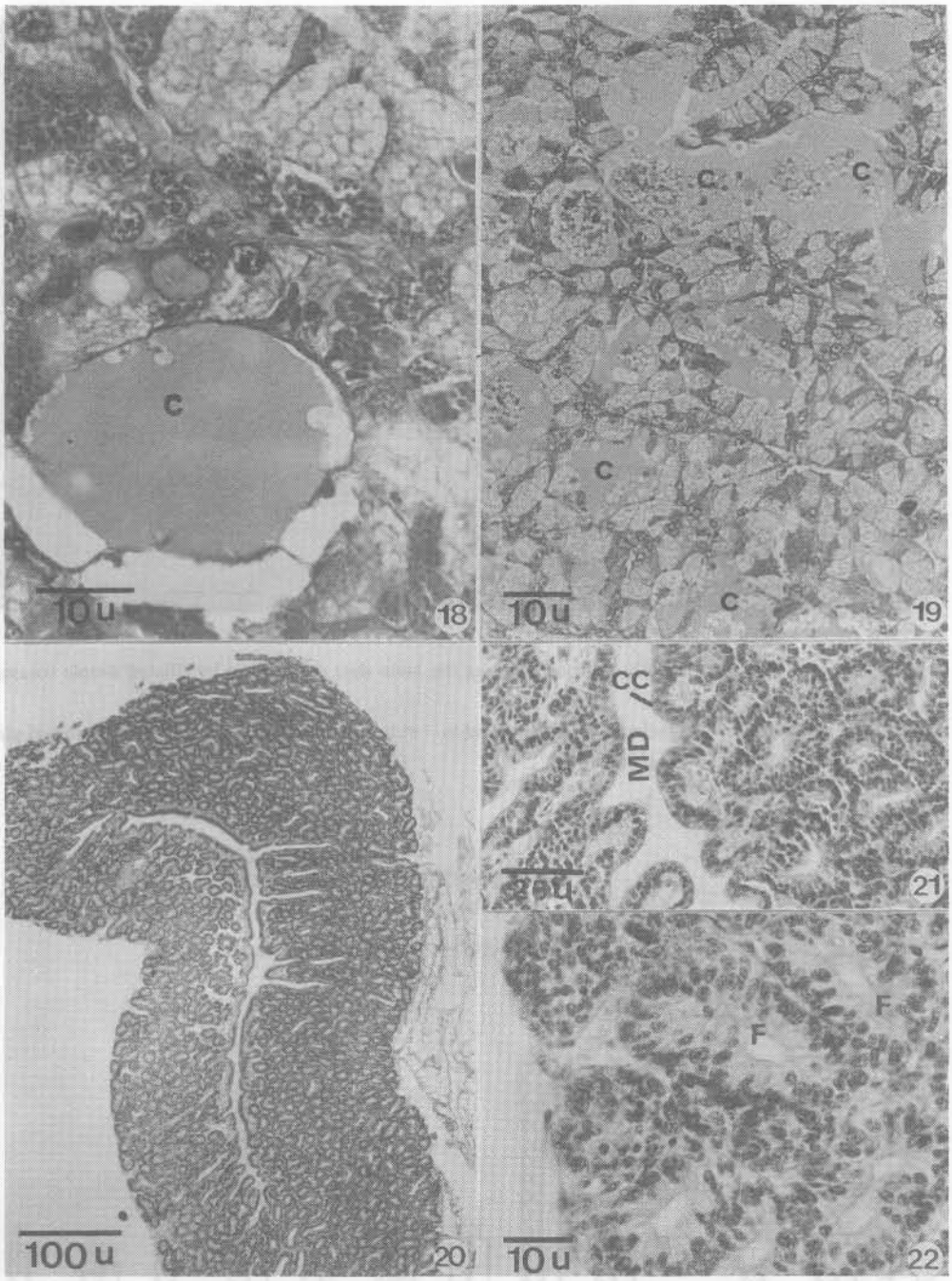


Fig. 18 High magnification of prostate gland in 5-month-old snail showing colloid (C) in the lumen of prostatic lobe.

Fig. 19 Prostate gland in 10-month-old snail showing a large quantity of colloid (C).

Fig. 20 The overall view of albumen gland in 3-month-old snail.

Figs. 21,22 High magnification of albumen gland in 3-month-old snail, showing the main duct (MD) which is lined by ciliated simple columnar cell (cc). The follicles (F) are clearly observed.

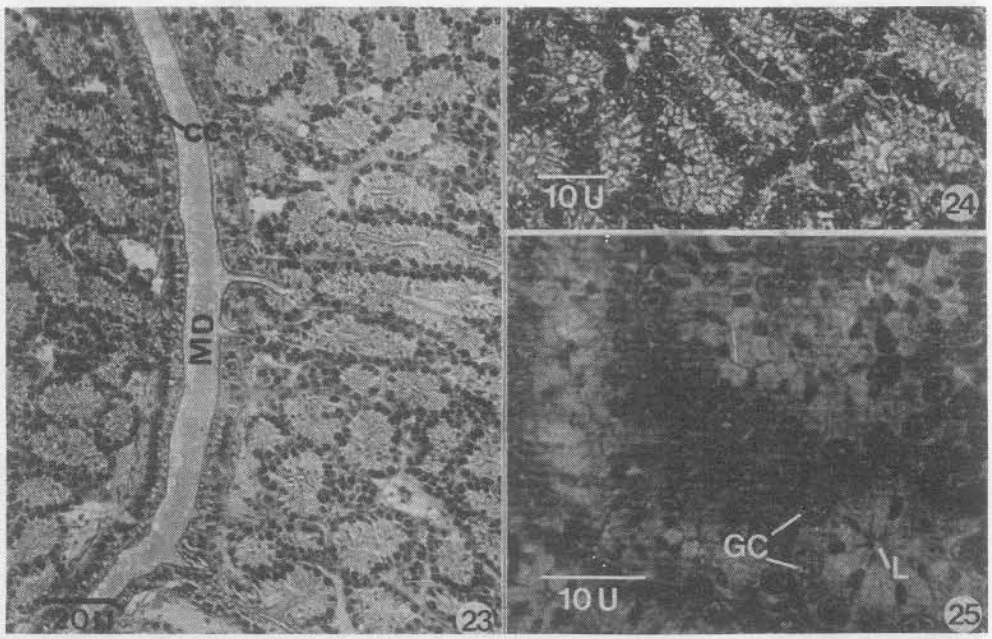


Fig. 23 Albumen gland in 5-month-old snail showing the main duct (MD) lined by ciliated simple columnar epithelium (cc).

Figs.24,25 Cross sections of follicles of albumen gland in 7-month-old snail showing the organization of gland cells (GC) surrounding lumen (L).

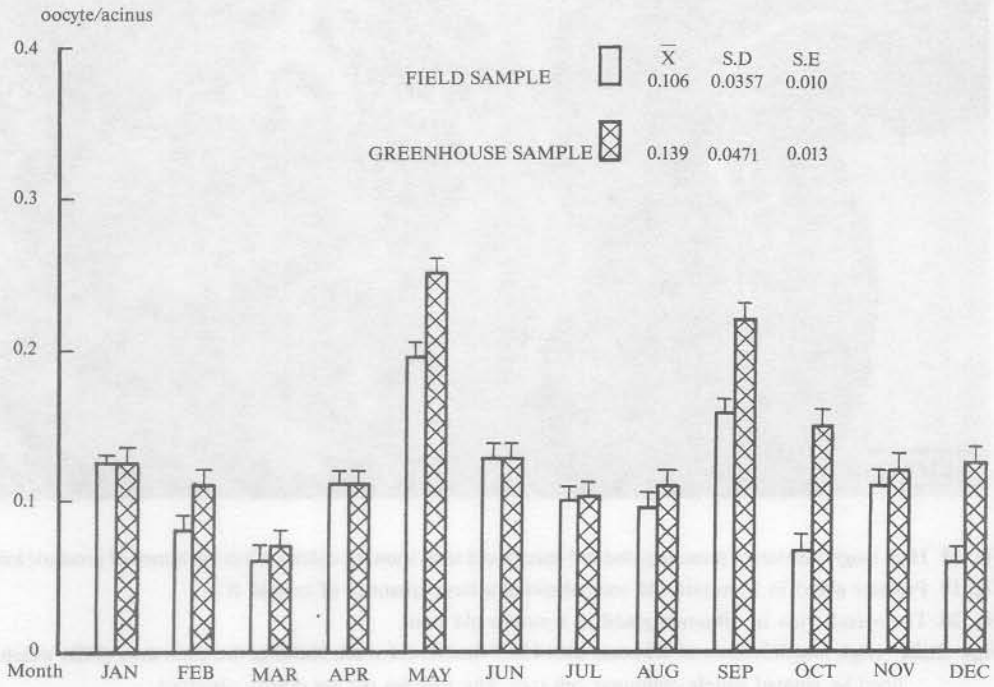


Fig. 26 Histogram showing variation in numbers of oocytes observed from January to December.

Runham and Hogg[2] studied the gonad and its development in *Deroceras reticulatum* and noted that at hatching, the gonad of *D. reticulatum* consisted of 1-3 acini; during maturation the number increased to 70-180, and each acinus also increased in size. Mature oocytes first appeared towards the end of late spermatozoon stage and oogenesis then occurred throughout the life span of the snails.[2] A similar pattern was also observed in *A. fulica.*

Growth of *D. reticulatum* after hatching was at first slow but then increased considerably in parallel with the growth and maturation of the reproductive system. At the end of this phase, the growth rate decreased, and copulation and egg laying took place, quickly followed by the death of the animals, which had a life span only about 6-12 months.[5]

In *A. fulica* of the present study, the ovotestis first appeared when the snails were 3 months old, and the oocyte first appeared when they were 5 months old. The maximum number of oocytes per acinus was highest when the snails were 7-9 months old, later than which this number decreased. In these snails, the male phase also occurred before the female phase, and when the female phase became most active, the male phase decreased in function. The ovotestis and accessory sex organs appeared concurrently in *A. fulica,* whereas in *D. reticulatum,* the development of the male accessory sex organs was completed before the female phase.[2]

Seasonal changes in morphology of the reproductive organs

Ovotestis

It was found that the histology of each acinus had the same pattern throughout the year, and all acini contained both spermatogenic cells and oocytes. All stages of spermatogenic cells including the clusters of spermatozoa were observed all year round, both in snails taken from the greenhouse and from the field. However, the number of oocytes per acinus was different from month to month, and these variations are shown in Fig.26. It was found that the number of oocytes per acinus in the snails obtained from the field was highest in May (0.183) and September (0.145) respectively, while in March the number of oocytes per acinus was lowest (0.064). Similarly, the number of oocytes per acinus in the snails obtained from the greenhouse was highest in May (0.249) and lowest in March (0.064). The numbers of oocytes per acinus from field snails and greenhouse snails were only significantly different ($p<0.05$) in May, September, October and December.

Prostate gland

Throughout the year, the histology of the prostate gland obtained from the field snails was not different from that of the greenhouse snails. Furthermore, the histology of the gland did not vary throughout the year. Two distinct cell types and colloid were always observed in the glands as previously described in adult snails.

Uterus

The histology of the uterus obtained from snails collected from the field and the greenhouse were similar in pattern all year round. The uterine wall consisted of the inner ciliated columnar epithelium, the middle layer which was filled up with adipose tissue, and the outer muscular layer as previously described in adult snails.

Albumen gland

The histology of the albumen gland obtained from the snails collected from the field and the greenhouse did not differ throughout the entire year. The morphology was generally the same as that of adult snails described previously.

From this study, the numbers of oocytes per acinus from greenhouse snails and field snails were highest between May and September, while the lowest were observed in March, Therefore, environmental conditions play an important role in the egg laying and in the hatching of embryos. During May and September, humidity, availability of food and temperature are optimal for the laying of eggs and for survival upon hatching. In the drier season in March, the number of oocytes was lowest probably due to the summer conditions of low humidity, high temperature and limited availability of vegetation for the snails. The number of oocytes per acinus from the greenhouse snails was higher than that from the field snails because in the former case food was more plentiful and the environment was not subjected to as severe variations as prevailed in the natural habitat.

ACKNOWLEDGEMENT

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บทคัดย่อ

การศึกษาการพัฒนาของ โอโวเทสทิส ตั้งแต่ระยะฟักตัวจนถึง 12 เดือน โดยกรรมวิธีของกล้องจุลทรรศน์ธรรมดา พบว่า โอโวเทสทิสปรากฏรูปร่างชัดเจนเมื่อหอยอายุได้ 3 เดือน มีลักษณะเป็นอะซีนัสจำนวนมาก แต่ละอะซีนัสถูกแบ่งด้วยเนื้อเยื่อเกี่ยวพัน ภายในอะซีนัสประกอบด้วยเซลล์สืบพันธุ์เพศผู้ในระยะต่าง ๆ ได้แก่ สเปอร์มาโตโกเนีย สเปอร์มาโตไซต์ขั้นต้น สเปอร์มาติด และสเปอร์มาโตซัว ในบางอะซีนัส พบว่ากลุ่มของหัวสเปอร์มาโตซัวเกาะอยู่กับเซลล์เซอโทไล ส่วนการสร้างโอโอไซต์เริ่มพบตั้งแต่ 5 เดือน ส่วนมากอยู่ตามขอบของอะซินัส จำนวนของโอโอไซต์เพิ่มขึ้นเมื่อหอยอายุ 7-8 เดือน หลังจากนั้นจำนวนจะลดลง เป็นที่น่าสังเกตว่าการเป็นเพศผู้ในหอยทากยักษ์ มีการพัฒนาก่อนระยะการเป็นเพศเมีย

จากการศึกษาอิทธิพลของฤดูกาลที่มีต่อจำนวนของโอโอไซต์ต่ออะซีนัสของหอยที่เลี้ยงและจากธรรมชาติด้วยกรรมวิธีของกล้องจุลทรรศน์ธรรมดา พบว่าจำนวนโอโอไซต์ต่ออะซีนัสของหอยเหล่านี้จะสูงที่สุดในเดือนพฤษภาคม และ กันยายนและต่ำที่สุดในเดือนมีนาคม ในทางตรงข้ามสเปอร์มาโตซัวมีการสร้างตลอดทั้งปี