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Observations on Spermatogenesis in *Gerris paludum* (Heteroptera)

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소금쟁이의 精子形成에 對한 硏究

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적 요

소금쟁이의 精子形成에 對한 顯微鏡的 觀察을 통해 다음과 같은 結果를 얻었다. 1. Germarium은 合胞體로 된 多核의 apical complex를 포함한다. 2. Trophocyte는 不規則的인 모양을 하며 精原細胞에서 sperm bundle까지 分布해 있으며 核內 有系分裂에 의해서 크기가 증가한다. 3. 대부분의 昆虫에 있어서는 成虫이 되기 전 마지막 脫皮시에 減數分裂이 完成되는데, 本種은 成虫에서도 減數分裂이 일어나서 精子形成이 계속되며 染色體數는 $2n=24$로 나타났다. 4. 精子完成을 染色質濃縮, 細胞質內 顆粒, 核의 크기와 모양의 차이에 따라 7단계로 나누었으며, 이 단계들은 核의 모양에 따라 球形, 伸張形, 막대形의 3群으로 類別된다.

INTRODUCTION

There have been some morphological and cytological studies on the spermatogenesis in various insects (Alexander and Chippendale, 1972; Anwar *et al.*, 1970; Ashrafi and Roppel, 1972; Retnakaran, 1971; Kaye and McMaster-Kaye, 1966; Carminda and Beig, 1976). Also, many phenomena of the spermatogenesis in the hemipteran sperm have been reported by light and electron microscopic studies (Montgomery, 1911; Bowen, 1922; Bonhag and Wick, 1953; Payne, 1966; Pratt, 1968). However, in *G. paludum*, the spermatogenesis has been so far little revealed. This species was found to possess unusual morphological characteristics, such as "H" shape of each testis and the outer mid-peripheral position of germarium in a follicle (Lee and Lee, 1980).

Descriptions of different spermiogenic stages including the observation of trophocytes are presented here. Further elucidation of the morphological details of these cytoplasmic changes will be presented in the near future.

MATERIALS AND METHODS

The last instar larvae and adults of *G. paludum* were sampled from two ponds in our University and were reared in the laboratory. Testes were dissected in 0.8 percent physiological saline, and immediately fixed both in buffered neutralized formalin solution for 3-4 hours and in Carnoy's solution for 3 hours. After fixation, the samples were dehydrated in a graded series of ethanols, cleaned in chloroform, embedded in paraffin of 53°-56° C MP and sectioned at a thickness of 3-4 microns. The sections were stained with Harris', Ehrlich's hematoxylin and eosin, aceto-orcein and acetocarmine.

For phase contrast and Nomarski microscopes, some testes were carefully isolated, fixed in the above solutions, washed in running tap water, and stained for 15 minutes with acetocarmine. Then these were lightly squashed in a drop of 45% acetic acid on the slide under rubber press for 10-15 seconds to be examined under phase contrast and Nomarski lenses of Olympus photomicroscope.

RESULTS

The interior of each testicular follicle consists of germarium, a series of cysts and sperm bundles. The germarium contains a multinucleated apical complex and young spermatogonia in small cysts (Fig. 3). A series of cysts in each successive stages are classified as spermatogonia, spermatocytes and spermatids. The cells of each cyst are all in the same stage of development except those in processing first and/or second spermatocytes division.

The germarium appears at the outer mid-peripheral part of a long follicle. The apical complex is surrounded by the small cysts which are composed of many young pyramidal spermatogoia. The grown spermatogonia are smaller in size but more in number than the young ones. They are located in the outer peripheral side around the young, pyramidal spermatogonia (Fig.1). The number of chromosomes in spermatogonia are counted to be twenty four as presented in figures 7A and 7B.

At the outer mid-peripheral part of the testicular follicle, the germ cells are differentiated into a number of spermatogonia and trophocytes or nurse cells, during differentiation the former undergo the mitotic division and the latter the endopolyploidization (Fig. 5). The spaces among cysts, between membranous wall and cysts

(Fig. 4B), and around the sperm bundle (Fig. 4A), are filled with trophocytes of irregular shapes.

The primary spermatocytes grow in volume and are located in the outer marginal region of a follicle. The secondary spermatocytes lie proximally adjacent to the primary ones, which are followed by cysts of spermatids with various stages of development. The spermiogenesis occurs in the remainders of the testis follicle.

The differentiated spermatozoa which lie in the vicinity of the vas efferens are grouped in bundles and the sperm heads, which are very tightly packed, seem to move toward the vas efferens (Fig. 3). In the seminal vesicle there are many single free sperms separated from bundles (Fig. 6).

Meiotic divisions are observed in spermatocytes. In the first and the second meiotic divisions, metaphase and late metaphase are distinctly observed.

The spermiogenesis, the subsequent growth and development of the spermatids into mature spermatozoa is divided into seven stages depending on chromatin, cytoplasmic granules, and nuclear size and shape.

The formation of spermatid after meiotic division is referred to as the first stage.

At the second stage the nucleus is spherical, and the chromatins are evenly scattered as small spherical bodies within the nucleus (Fig. 8: N). And the cytoplasm contains eosinophil granules (Fig. 8: Ne), which disappear gradually during the next stage.

At the third stage (Fig. 9) the chromatin is identical with that of the second stage. The diameter of the nucleus seems to be decreased as the spermatid proceeds toward the third stage. The cells are irregularly shaped, but the nuclei are less spherical than in previous stage (Figs. 8, 9).

At the fourth stage (Figs. 10A, 10B) the nucleus becomes cuboidal, of which meaning we restricted to the cuboids twice as high as its maximum diameter. The chromatin at this stage is more homogeneous than at the previous stage, but appears to be more concentrated at the adjacent part of the tail. With the advancement of differentiation, acrosome grows larger than the nucleus (Figs. 10A, 10B, 11).

At the fifth stage (Fig. 11) the nucleus gradually elongates and decreases in diameter, with the result of the condensation of chromatin, and the columnar shape of the nucleus. The nuclear material showing clear spaces in the middle is not entirely homogeneous (Fig. 10B). Through the five stages, the nucleus gradually changes from spherical via cuboidal to columnar shape (Figs. 8, 9, 10, 11).

At the sixth stage (Fig. 12) the nucleus gradually becomes smaller and, in the end, a elongated and narrowed rodform, and the density of the nucleus increases.

At the last seventh stage (Fig. 13) many sperms are found to be packed together in bundles.

Therefore, spermatids of such seven successive spermiogenic stages could be in turn subdivided into three main groups in terms of their various shapes: the spherical spermatid (stages 1-3), the elongating spermatid (stages 4-5), and the rodform spermatid (stages 6-7).

The larvae of the fifth instar show more embryonic tissues and less sperm bundles than the adults.

DISCUSSION

The embryonic tissues are generally found in the apical region of the testicular follicle in other species. But in this species, the germarium is in the outer median peripheral side of a follicle. The fifth instar larvae contain much more young germ cells and their wider distribution in the follicle than adults. So, it is assumed that the embryonic tissues of the young instar larvae may take much more part than those of the old ones, and at the early stages the germ cells may remain immature without further differentiation.

The apical cell is of wide occurence in the various orders of insects. Henking and Gross reported the presence of an apical cell in ***Pyrrhocoris apterus*** (Heteroptera), and Kornhauser studied its structure in Membracidae (Homoptera) (*In* Bonhag and Wick, 1953). Bonhag and Wick (1953) reported that the apical complex in the milkweed bug is a trophic tissue, which is analogous to the apical trophocytes of the ovariole in its cytological appearance and position, on the ground that the apical complex supplies nutrient to spermatogonia. Also in the European glowworm, the complex was reported to play a role of nutritive cells for the developing spermatogonial cysts (Counce and Waddington, 1973). The apical complex of *G. paludum* consists of a multinucleated syncytium, which seems to play a nutritive role for the early spermatogonia.

Connections between the apical complex and the spermatogonia are soon lost, and among the cysts trophocytes are scattered in Heteroptera (Chapman, 1974). In this study, the trophocytes which are irregularly large cells, are on the whole scattered throughout the space of spermatogonial cysts to sperm bundles. And they seem to be similar to Sertoli cells in the seminiferous tubules of the vertebrates.

Trophocytes increase in size by endomitosis and endopolyploidization (Vanderberg, 1963; Cave, 1975). On the basis of the fact that endopolyploidization are very frequently found within follicles, it is thought that trophocytes of *G. paludum* also increase with the mechanism.

The primary spermatogonia might have undergone several mitotic divisions before they finally became spermatocytes. Secondary and subsequent spermatognia are divided synchronously, forming cysts ensheathed with mesodermal interstitial tissues. Bonhag and Wick (1953) reported that trianglar or flask-shaped spermatogonia, closely associated with the germarium, form an irregular rosette, and are larger than the secondary spermatogonia. The structure of the spermatogonia in *G. paludum* was identical with that of milkweed bug.

The earliest stage of sperm development takes place in the distal region of the germarium, and the latest one does proximally adjacent to the vas deferens. In *G. paludum*, the youngest germ cells lie around the apical complex in the outer lateral side of the follicle while the oldest, sperm bundles, adjacent to the vas efferens connecting the inner lateral sides (Lee and Lee, 1980).

Kaye and McMaster-Kaye (1966) reported that nuclear differentiation of the house cricket is divided into three stages, i.e., the early spermatid stage, the speckled stage and shell stage according to the chromatin distribution, nuclear size and shape among the stages. Kierszenbaum and Tres (1975) identified 16 successive spermiogenic steps in the mouse testis and this classification was based on the shapes and condensation of the nucleus.

In the spermiogenesis of the present species, seven stages have been classified according to chromatin concentration, cytoplasmic granules, and nuclear size and shape. The eosinophil granules of the second stage gradually disappear during the third stage and do not appear at all in the subsequent stages. It is assumed that this granules form nebenkern at the second stage and begin to form slender sheath of mitochondria from the head at the third stage.

Carminda and Beig (1976) observed that one of the most obvious structural changes in the nucleus is a progressive condensation of the chromatin during spermatid differentiation, and young spermatids possess irregular nuclei with less condensed chromatin. In *G. paludum* the spherical spermatid (1-3 stages) has spherical nucleus with chromatin scattering in small spherical bodies.

A slight elongation of the nucleus was seen in the late shell stage of the house cricket (Kaye and McMaster-Kaye, 1966). In *G. paludum* the nucleus attains to a cuboid form during the fourth stage and elongates into a columnar form from the fifth stage.

Chapman (1974), and Dass and Ris (1958) suggested that as the nucleus is elongated and narrowed, the nucleoplasm begins to condense finally consisting of a uniformly dense material. In the species employed in our studies, the nucleus appears to be filled with dense material at the sixth stage after nuclear reduction.

The mature sperms of Parlatoria (Homoptera), Rhodnius (Heteroptera) and Orgyia (Lepidoptera) are filamentous in form, about 300 microns long and less than a micron in diameter. The head and the tail of the sperm are approximately of the same diameter.

During the sixth stage of *G. paludum*, the sperm consists of a rodform nucleus together with a long acrosome and a tail ultimately giving a very long filamentous shape, while the diameters of the head and the tail remain approximately the same. Afzelius *et al.* (1976) reported that the backswimmer sperm is one of the largest in the animal kingdom.

According to Johnson *et al.* (1970), spermatogenesis continues during the imaginal period since male weevil and male house fly frequently mate during their adult stage. But in some insects which eat nothing during adulthood, spermatogenesis may be completed before the adult emerges. In *G. paludum,* spermatogenesis is maintained during the adult stage because of mating and eating in its adult life.

In a number of insects, sperms are at least grouped together in bundles for some period of their existence and the bundles sometimes persist even after transference to the female. In *G. paludum,* movement of a bundle seems to result from the combined activities of the sperms within it.

Spermiogenesis is completed by the time the adult emerges, and the germ cells complete their tranformation in the seminal vesicles. In *G. paludum,* there are a number of motile sperm bundles in the follicle, but separated free sperms in the seminal vesicles.

SUMMARY

The germarium contains the apical complex consisting of a multinucleated syncytium. Apical complex has a nutritive role for the early spermatogonia. Trophocytes are irregularly shaped and are distributed throughout the space from spermatogonial cysts to sperm bundles, and they increase in size by endopolyploidization. As meiotic divisions are not complete until the final moult, spermatogenesis continues even in the adult. Chromosome number is $2n=24$. Spermiogenesis is divided into seven stages in terms of chromatin concentration, cytoplasmic granules, and the nuclear size and shape. These stages could be grouped into the spherical, the elongating and the rodform spermatids in shape.

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EXPLANATION OF FIGURES

Fig. 1. L.S. through the testis follicle showing young pyramidal spermatogonia (PSG) and grown spermatogonia (SG) along the outer periphery. ×400.

Fig. 2. L.S. through the germarium (G) showing developments of apical complex (APC) and small cysts. SPC, the zone of spermatocytes. ×400.

Fig. 3. Sperm bundles (SB) heading towards the vas efferens (VE) in L.S. ×100.

Figs. 4A. 4B. Distribution of trophocytes (TC) within a follicle in L.S. ×400.

Fig. 5. Trophocyte undergoing endopolyploidization. (under phase contrast microscope) ×1,000.

Fig. 6. L.S. of the seminal vesicle showing the free sperm heads (SH). ×400.

Fig. 7A. The spermatogonia showing 24 chromosomes ($2n=24$). (under Nomarski lense) ×1,000.

Fig. 7B. A spermatocyte showing 12 chromosomes ($n=12$). ×1,000.

Fig. 8. The 2nd stage: spermatids showing the spherical nuclei (N) and nebenkern (Ne), the eosinophil granular material of the cytoplasm. ×1,000.

Fig. 9. The 3rd stage: irregular cells with the spherical nuclei (N) without cytoplasmic granules. ×1,000.

Fig. 10A. The 4th stage: cuboidal nuclei (CN) and acrosome (A) undergoing elongation. ×1,000.

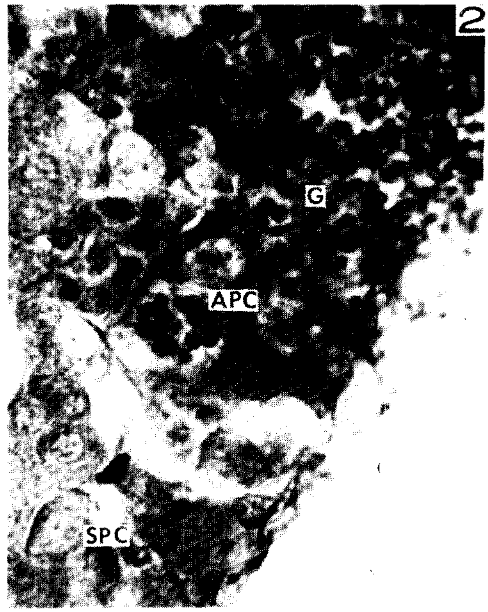
Fig. 10B. The nuclei enclosing vacuole (i.e., colourless area in the figure). ×1,000.

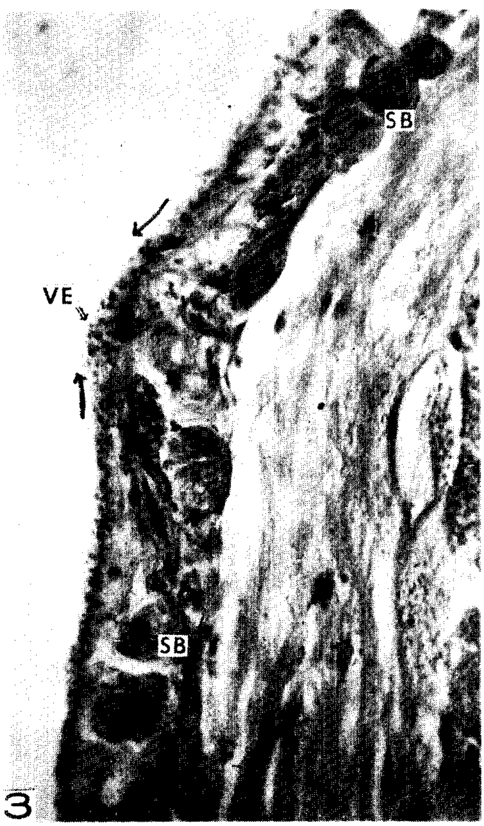
Fig. 11. The 5th stage: columnar nuclei (CoN) in various lengths. ×1,000.

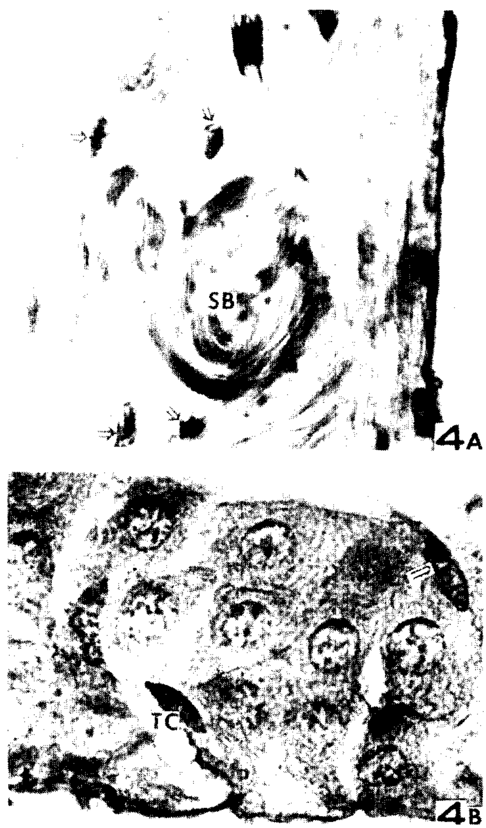
Fig. 12. The 6th stage: fully elongated rodform nuclei (RN). ×1,000.

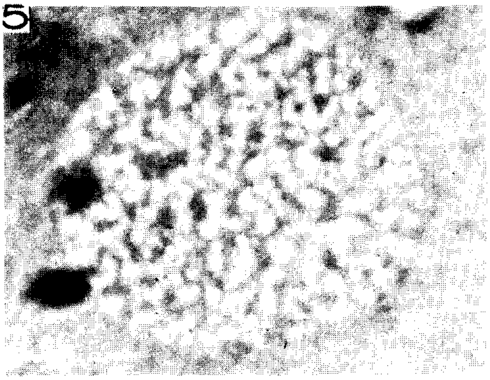
Fig. 13. The 7th stage: morphologically matured sperms in a bundle (B). ×1,000.


1
PSG
LSG


2
G
APC
SPC


SB
VE
SB
3


SB
4A
TC
4B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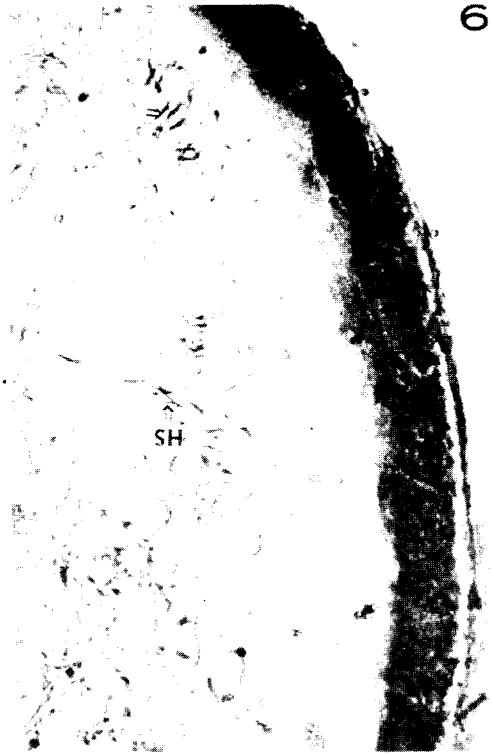
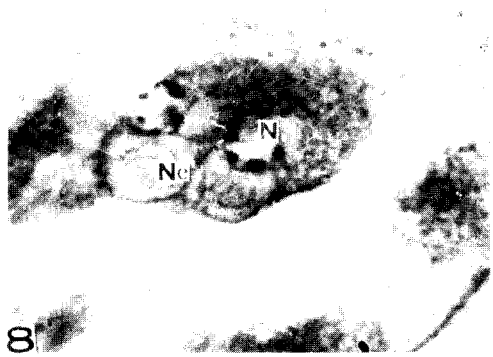

6
SH


7A


7B


8
N
Ne


N
9


10A
A
CN
A
10B
11
CN
12
RN
13
B