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Histological study on the regeneration of the sexual organs in planarian, *Dugesia gonocephala*

Tetsuji KIDO and Makoto HASEBE

Biological Institute, Faculty of Science, University of Kanazawa

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Introduction

It is well known that in planarian reproduction there are two different ways; one is sexual and other is asexual. The sexual form contains both male and female reproductive systems, while the asexual form does not contain any reproductive organs. These worms reproduce themselves by fission alone.

Okugawa (1957) observed that the sexual forms of Japanese fresh water Planarian, *Dugesia gonocephala*, make their cocoons during the winter months and when the season was over, many of the sexual individuals died away, only leaving a few survivals. In such survivals the sexual organs degenerate and a capacity of fission is restored in the summer months. Thus he suggested that some of the worms would repeat the cycle of degeneration and regeneration of the sexual organs.

It is interesting that the sexual organs can regenerate, if they are experimentally removed in the sexual period. In this case the regeneration is surmised to occur by virtue of the neobrasts buried amidst the mesenchymal cells, because both ovaries and testes can arise even in a piece without any trace of the sexual organs.

Vandel (1920) stated, however, that, when a sexually mature planarian was cut into two pieces between the pharynx and the copulatory apparatus, the latter organ in the rear piece degenerated to become an asexual worm, whereas in the anterior piece a new copulatory apparatus regenerated so far as the active testes were present in it. This result seems to indicate that formation and maintenance of the copulatory apparatus depend on the presence of the active testes.

Kenk (1941) and Okugawa (1957) pointed out that if an anterior piece of a sexual form and a posterior piece of an asexual form were united to together,

the gonads and copulatory apparatus developed in the asexual piese. There are two possibilities in this case, one is that the regenerated sexual organs originated from the tissue of the asexual component under the influence of the sexual graft and the other is that the organs develop from the cells which has migrated from the sexual component. Their conclusion is that the former seems to be right. But their conclusion was not based on the histological observation.

The present paper is an extension of their works and particularly concerned with the histological investigation of the formation of the sexual organs in the transected pieces.

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Material and Method

The material used was the mature sexual form of *Dugesia gonocephala* collected in a stream running out from a spring at Yasuhara, suburb of Kanazawa City.

The Yasuhara strain is apparently different from Nishioji strain of Okugawa's concerning the duration of the sexual season, namely we can find a number of cocoons both in nature and also in laboratory during a comparable long period, from April to November. In addition, even after the breeding is over, a majority of the individuals survivied and maintained the sexual organs.

Prior to operation the worms which were 16-18mm. long, were starved for over a week, and transected into two pieces through different levels of the body; 1) piece A is an anterior half of a worm cut through the level immediately posterior to the pharyns, 2) piece B is a caudal half of the worm cut through the level in the middle between the pharynx and the copulatory apparatus and 3) piece C is a posterior half of the worm cut through slightly posterior to the copulatory apparatus. Regenerating process of the sexual organs was examined from time to time under a low power microscope.

Histological preparation was made by the ordinary way of paraffin sectioning, and stained with Heidenhain's or Delafield's haematoxylin with counter staining of eosin. Mallory's triple staining was occationally applied.

The completely mature copulatory apparatus is indicated in Fig. 1.

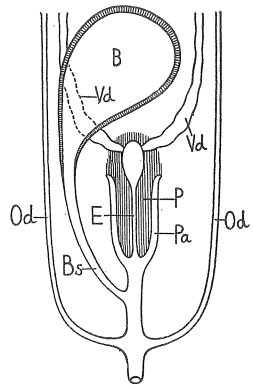

Fig. 1. Scheme, showing the copulatory apparatus of *Dugesia gonocephala*. B : bursa, Bs: bursa stalk, E: ejaculatory duct, Od : oviduct, P : penis, Pa : penis atrium, Vd : vas deferens.

Observations

Regeneration of the sexual organs in piece A: This piece includes the overies, testes and the pharynx but excludes the copulatory apparatus. By the fifth day after cutting, the wall of numerous follicular testes showed a sign of disintegration and the boundary between the follicle and the mesenchyme became obscure. Masses of sperms disappeared completely from every follicle and the shape of other germ cells became irregular and tended to disperse into the mesenchyme (Fig. 2). The nuclei of these cells were deeply stainable with haematoxylin. The dispersed cells in mesenchyme became hardly distinguishable from the mesenchynal cells and eventually were completely unrecognizable in ten days after cutting. Simultaneously, vasa deferentia and oviducts disappeared also. It was quite difficult to ascertain whether the germ cells transform into the neoblasts indistingushable from the mesenchymal cells or they undergo complete cytolysis. By contrast, a pair of overies lying in postcephalic area were intact and did not show any morphological change from the original feature.

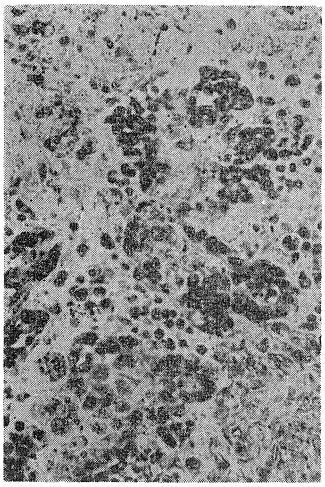

Fig. 2. Photomicrograph, showing the degenerating testes. The follicular testes disintegrate and germ cells disperse into the mesenchymal tissue, on the fifth day after cutting.

The regeneration blastema from cut-surface reached to the normal tail size in ten days after cutting, and the lateral intestinal tracts were completed up to the tail end.

On the fourteenth day after cutting in autumn, the neoblasts with a large nucleus deeply stainable with haematoxylin and with a scanty cytoplasm were seen to aggregate, forming a strand which ran backwards from a part immediately posterior to the pharyngeal atrium, between two lateral intestinal tracts newly formed (Fig. 3, A), like the pharyngeal formation (Kido, 1961). Besides, it was found that the cells of the distal wall the pharyngeal atrium dissociated and migrated caudad. The significance of these cells to the neoblasts remains uncertain (Fig. 3, B). The assembly of the neoblasts was delayed about four to five days in spring. Any way, numbers of the gathered cells became increased, especially at a distal portion of the strand and eventually made a knob of these cells (Fig. 4, A). Thereafter, accumulation of the neoblasts occurred slowly and a subsequent develoment of the knob was retarded.

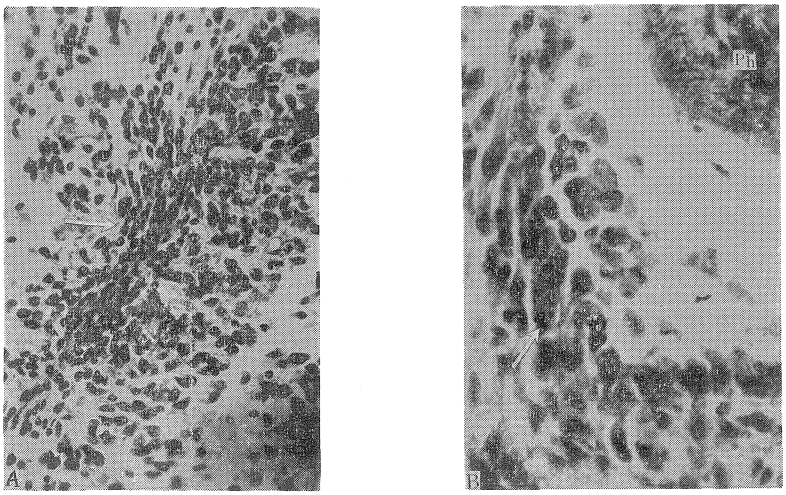

Fig. 3. Photomicrographs, showing the cell migration of piece A in fourteen days after cutting in autumn. A, showing the aggregation of the neoblasts, indicated by arrow, which migrate from a part immediately pcsterior to the pharyngeal atrium (upper end of the photograph). B, showing that cells of the posterior wall of the pharyngeal atrium, indicated by arrow, are loosely arranged, and migrate posteriorly. Ph : pharynx.

Accordingly, the time required for formation of a cavity in the center of the knob was about twenty-six days after cutting in autumn and about eighty days in spring, although even in the same season it varied considerably among the individuals. (Fig. 4, B). The cavity extended antero-posteriorly as a lumen of the bursa stalk. Thus the primordium of bursa stalk developed first in the accumulation of the neoblasts.

When the cells of the primordial bursa stalk began to take an epithelial arrangement, an anterior part of the lumen enlarged and became a cavity of the bursa (Fig. 4, C). As to the further development of the organs, the discription is given with the autumn specimens.

At forty days after cutting, the posterior end of the lumen of the bursa stalk bent anteriorly and expanded into a cup-shape. The cup-shaped cavity will represent finally a penis atrium (Fig. 4, D). Some of the neoblasts invaded into this cup-shaped cavity and constituted a penis primordium. After a while, a cavity developed in the penis primordium as a bulbar lumen of the penis. The bulbar lumen was later connected with the penis atrium with the intercalation of the ejaculatory duct. The development of the penis itself was, however, slowly and its muscular and glandular tissues were morphologically poor (Fig. 5). Later the genital pore opened at the point where the penis atrium attached to the ventral side of the body. At this time, numerous small round

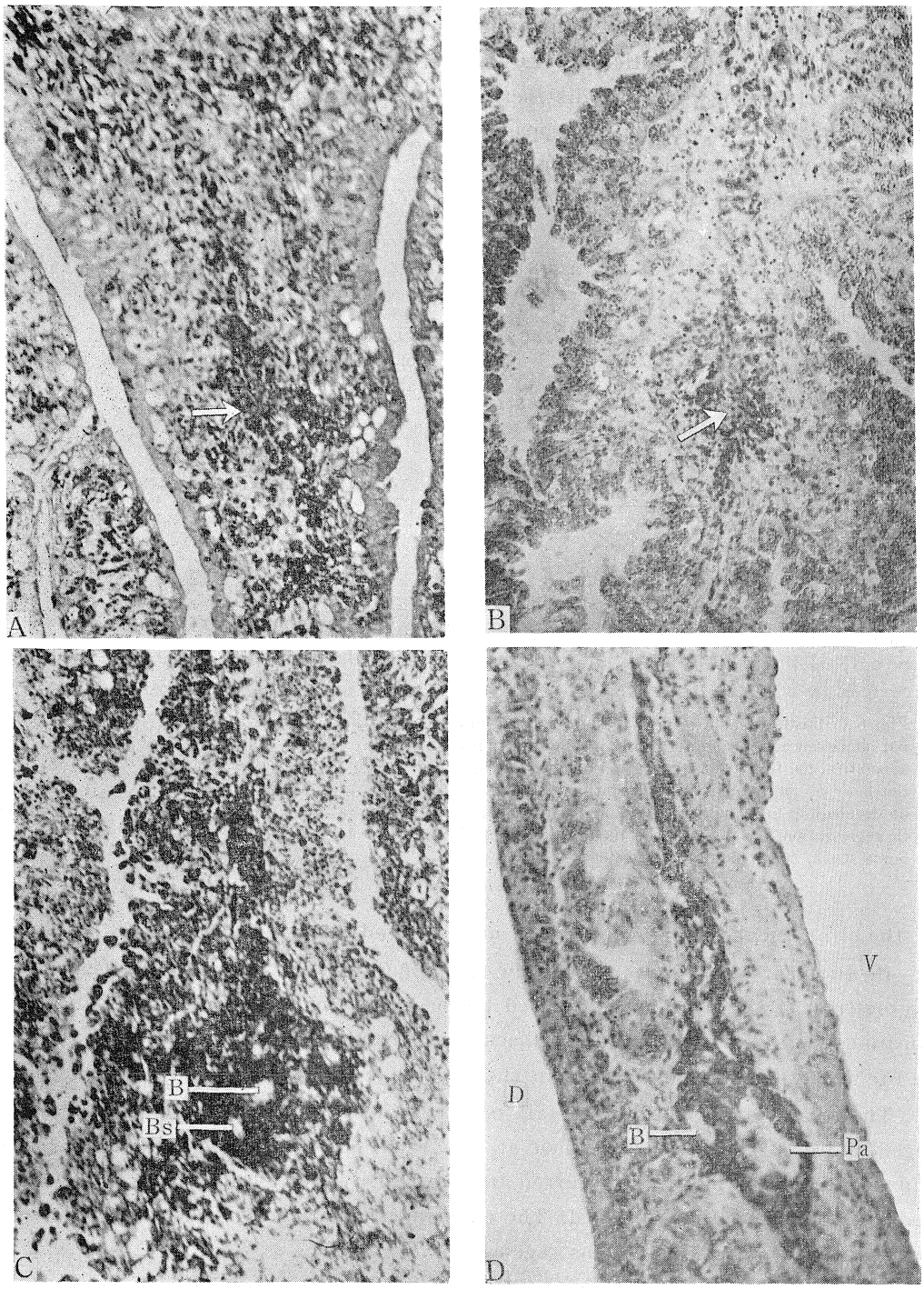

Fig. 4. Photomicrographs, showing regeneration of the copulatory apparatus in piece A, cut off in autumn. A, showing the aggregation of the neoblasts, indicated by arrow, forming a knob in the germinal region, in sixteen days after cuting. B, showing a cavity, indicated by arrow, occuring in the knob in twenty six days after cutting. The cavity develops to the bursa stalk. C, showing that cavities of the bursa(B) and of the bursa stalk (Bs) are formed in the knob in thirty two days after cutting. D, showing a cavity of the penis atrium, occuring in the knob in forty two days after cutting. B: the cavity of the bursa, D: dorsal side of the worm, Pa: penis atrium, V: ventral side of the worm.

bodies reappeared in the mesenchyme and soon became the follicular testes. The oviducts reappeared also distinctly and opned into the copulatory apparatus. The vasa deferentia were not yet regenerated at this time.

The experiment indicated that there was difference in the time required for regenerating the sexual organs according to the seasons tested, as is shown in Fig. 6.

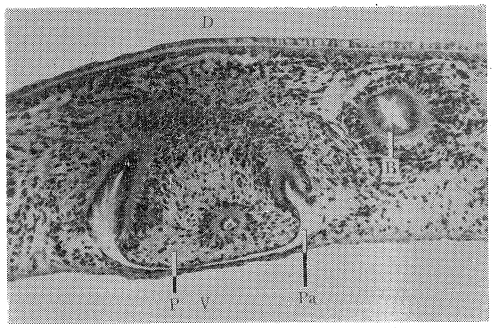

Fig. 5. Photomicrograph, showing formation of the penis. B : bursa, P : penis, Pa : penis atrium.

Regeneration of the sexual organs in piece B: This piece includes the copulatory apparatus and testes but excludes the overies and the pharynx.

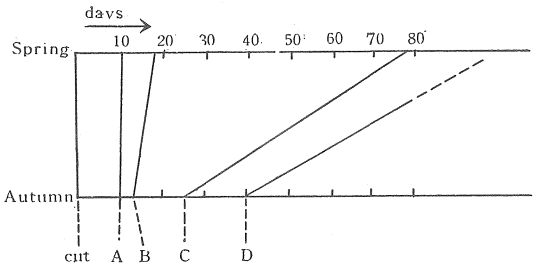

Fig. 6. illustration, showing that there is the seasonal difference on regeneration of the copulatory apparatus. the time of degeneration of the testes, B : the time of the neoblastic aggregation. C : the time of developing to a cavity in a knob as the neoblastic aggregation, D : the time of the formation of the bursa stalk, bursa, penis and testes.

Soon after cutting disintegration occurred first in the tissue of bursa and then in the tissue of the bursa stalk and the penis. The entire copulatory apparatus disappeared in ten days after cutting. A new pharynx developed in the place where the copulatory apparatus had disappeared. The testes also disappeared as in piece A. The sequence of the pharyngeal formation was already reported elsewhere (Kido, 1961).

In autumn, in a few days after the pharynx was completely established, aggregation of the neoblasts appeared to form the primorduim of the copulatory apparatus as the strand extending backwards from a part immediately posterior to the newly formed pharynx as in the case of piece A.

The formation of the bursa stalk occurred in forty days after cutting, but the regeneration of the testes was poor at this time. The ovaries could not be found. The copulatory primordium occurred in every case after the pharyngeal formation had been completely established. The experimental worms could not be reared so long as the entire sexual organs regenerated.

In spring, aggregation of the neoblasts for the copulatory apparatus did not occur even in eighty days after cutting.

Regeneration of the sexual organs in piece C: This piece includes a little quantity of the testes but excludes the ovaries, the copulatory apparatus and the pharynx.

In around ten days after cutting, the head and pharynx were completely established, and the testes almost disintegrated and disappeared.

In autumn specimens, marked aggregation of the neoblasts were seen to appear behind the pharyngeal atrium in forty to sixty days after cutting, but the subsequent development was less marked during the experimental period. Both genital organs did not regenerate.

In spring specimens, very slight aggregation of the neoblasts was found only in a few cases in about eighty days after cutting, but any morphological development of the sexual organs was not encountered in about three months after cutting.

Discussion

The early phase of regeneration of the copulatory apparatus in Japanese fresh water planarian, *Dugesia gonocephala* was aggregation of the neoblasts as in the case of the pharyngeal formation (Kido, 1961). The site of the pharyngeal primordium depends on the presence of a new tissue regenerated from a cut-surface of the intestinal tracts. But it is unknown by what factor(s) does control the location of the germinal primordium. According to Vandel (1921), the pharynx may be formed by cells derived from the copulatory apparatus. An occurrence of the inverse phenomenon from the pharynx to the copulatory apparatus may be considered on the basis of the facts that the neoblastic migration for the formation of the copulatory apparatus was first found at the site posterior to the pharyngeal atrium, and that the posterior wall of the pharyngeal atrium seemed to contribute cells to the aggregation of the neoblasts. In addition, the present experiment indicates clearly that, in the piece in which the pharynx and the copulatory apparatus were discarded aftter cutting, no neoblastic migration for the formation of the copulatory apparatus occurred unless the pharygeal formation had been completely established. From these facts, it may be said that the neoblasts can regenerate the sexual organs, only in the presence of the pharynx.

Using *Polycelis tenuis*, Lender and Le Moigne (1960) pointed out that an aggregation of the neoblasts is first visible in the genital region, and that in the mass of these cells two cavities appear as the male genital atrium and the common atrium. But in the present species a cavity of the future bursa stalk arises first in the knob of the neoblasts and then a cavity appears as the penis atrium. The formation of the penis of the present species is good accordance with that of *Policelis tenuis*.

Kenk (1941) grafted the anterior region of a sexual *Dugesia tigrina* including ovaries and testes into the posterior region of the asexual form without genital organs. Several months later, the genital glands and the copulatory apparatus

were seen to have appeared in the asexual component. Okugawa (1957) carried out a similar and more complete experiment and obtained the same result. Two possibilities were considered for these formations. One was that they were produced by the neoblasts derived from the sexual graft, and the other was that they were established only by the neoblasts locating in the mesenchyme of the asexual component. If the latter is the case, Okugawa claims, differentiation of the sexual organs would be induced in the asexual component by a hormonal substance(s) from the testes.

The present experiment has revealed that regeneration of the sexual organs in autumn is quicker than in spring. It seems probable that such a time difference of regeneration between autumn and spring is due to the sexual conditions in the worms. In addition, the regenerating power of the sexual organs is the lowest in piece C which contains the least quantity of the testes among the three pieces tested. Under these situation, it is likely that hormonal substance(s) from the testes on their remnants is responsible for the activation of the neoblasts.

Summary

1. Regeneration of the sexual organs in planarian, *Dugesia gonocephala* was histologically examined in the pieces coming from three different levels of the body ; i. e., 1) piece A is an anterior half of an animal cut through the level immediately posterior to the pharynx, 2) piece B is caudal half of the animal cut through the level of the middle between the pharynx and the copulatory apparatus and 3) piece C is a posterior half of the animal cut through the level slightly posterior to the copulatory apparatus.

2. Soon after cutting, the testes degenerates in all pieces but the overies remain in the original state in spite of degeneration of the oviducts.

3. The regenerating sequence of the sexual organs is slightly different depending on the three kinds of pieces tested.

Generally speaking, cell migration occurs from a part immediately posterior to the pharyngeal atrium in the posterior direction between two lateral intestinal tracts and in the germinal region the migrated cells aggregate as a strand with a knob at its terminal. This feature is the first stage of regeneration of the sexual organs. In the second stage, a cavity appears in the knob as a cavity of the bursa stalk and then extention of the cavity produces the penis atrium. In the third stage, the penis is formed from the invaded neoblasts in the penis atrium.

4. Although there is a individual difference in the time required for the regeneration of the sexual organs, it is a rule that it is longer in spring than in autumn.

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