Morphological and Histochemical Studies on Regeneration in a Freshwater Planarian, *Dendrocoelopsis lacteus*[1)]

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(With 1 Table and 1 Plate)

Recent advances in the histochemical and cytochemical techniques during the last two decades have stimulated an analytical study of the planarian regeneration from a metabolic angle. The literature dealing with this research field is reviewed in Brøndsted ('55). Up to this time, the histochemical studies using the planarian tissues have been reported by many workers (Freisling & Reisinger '58; Lender & Gabriel '60; Lindh '56, '57 a, b, c, d; Pedersen '59a, b). There still remain many questions to be solved.

For years fundamental studies on planarian regeneration have been in progress in our laboratory. Namely, the two articles, the histological observation of the blastema formation and the origin of cells for larval regeneration in *Dendrocoelopsis ezensis* (Ichikawa '50) and the histochemistry of the distribution of DNA, polysaccharides, SH groups and alkaline phosphatase in mature worms of *Dendrocoelopsis lacteus* (Yamamoto '57), were published. The present work is the third report of a study on these topics. The authors have investigated the process of regeneration and the movement of neoblasts in morphological aspect and compared their results with those reported by earlier workers. Further, metabolic changes through regeneration process are studied histochemically.

Material and method

In the present study, the unpigmented freshwater planarian, *Dendrocoelopsis lacteus* Ichikawa et Okugawa, was employed. This triclad flatworm is a very suitable material for the histochemical study, since it has no pigment in any part of the body except for some pairs of the eye spots (Ichikawa & Okugawa '58). Mature and immature specimens of this species collected from the brook at Kotoni, Sapporo City, were prepared as material. They ranged from 15 to 20 mm in length.

The wroms are starved at least for a week prior to the operation. They are placed on paraffin-board and then cut transversely with a sharp scalpel under the binocular microscope just anterior to the pharynx (prepharyngeal level). Most observations are carried out using the anterior pieces, i.e. on the posterior regeneration of the head pieces.

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They are transferred into a glass container of 20 cm in diameter and 10 cm in depth containing about 2000 cc of well water which is renewed every day. During the observation of regeneration process, the container is kept in an electric refrigerator at about 10°C, and no food is given to the worms.

For the morphological studies, in most cases, the worms are fixed with Zenker's or Allen-Bouin's fluid. The materials are then washed, dehydrated, cleared and embedded in paraffin (m. point 50°C), and serial sections are prepared by the ordinary method (7-10μ). The sections are stained by Heidenhain's azan stain.

In the histochemical studies, iron, nucleic acids, polysaccharides, lipoid, lipase and alkaline phosphatase are detected by the following methods.[3] :

1. *Iron.* After fixation with absolute alcohol for 20 minutes, the materials are sectioned and then stained with prussian blue according to the revised method of Perls-Stieda. The sections are incubated for 2 hours at 80°C, during which the staining medium is renewed 3 times. In control slides, iron is dissolved by a pretreatment with 1N HCl for 16 to 20 hours at 37°C.

2. *Nucleic acids.* The materials fixed with Zenker's fluid for 45 minutes are sectioned. For stainings of nucleic acids, Feulgen's technique and Unna's methylgreen-pyronin method (cf. Pearse '53) are employed.

3. *Polysaccharides.* The materials are fixed with Allen-Bouin's fluid for an hour at 37°C. Staining of the sections with Schiff's method (PAS-reaction) is performed, modified after Hotchkiss (cf. Pearse '53). For differentiation of glycogen from other polysaccharides, the slides are subjected to diastase treatment for 40 minutes at 37°C.

4. *Lipoid.* The sections are prepared from the materials fixed and treated by Ciaccio's method. Staining with Sudan black B is performed at room temperature.

5. *Lipase.* The materials are fixed with absolute aceton for 24 hours at 5°C and embedded rapidly in paraffin. The sections are incubated in a substrate medium containing Tween 40. Incubation is performed for 8 hours at 37°C. Control slides are incubated in the medium lacking the substrate.

6. *Alkaline phosphatase.* The sections prepared as in the case of detection of lipase are treated by the revised method of Gomori (cf. '52). As the substrate, sodium β-glycerophosphate buffered to pH 9.4 is used. In general, the sections are incubated in the substrate medium kept at 37°C for 3 hours and colored by the cobalt method (cf. Lillie '54). Control slides are incubated in a buffered solution lacking the substrate.

Observations

I. Morphological

When the head pieces obtained by the transverse cut in the prepharyngeal level are cultured in well water, they regenerate the new pharynx within about 10–14 days. The regeneration process of the posterior wound surface is as follows.

Even just after the time when the worm is cut, the head piece creeps actively emitting some tissue cells near the cut surface through the wound openings. From the body surface the animal excretes a large amount of mucus and at the same time it is observed that the wound opening begins a remarkable contraction.

In 6 hours after the operation, the epidermal cells in the wound border

3) For details, see Lison ('53) and Romeis ('48).

become particularly columnar. In such pieces, remarkable changes are recognized not only in the wound but also in the tissues far from the wound. The whole nerve cords become loose and increase in their thickness. The subepidermal muscles have a loose appearance also in every part of the body. The cells of the rhabdite-forming glands near the wound become irregular form and appear as if they are putting off their rhabdites out of the gland cells and undergo dedifferentiation. These figures suggesting emission of the cell inclusions are found not only in the rhabdite-forming glands but aslo in the intestinal and the cells of the yolk glands near the wound.

The epidermal cells of the wound begin to close the wound surface on 1st-2nd day of the regeneration. The columnar epidermal cells fall down over the wound and begin to creep toward the wound surface from the cut end of the epidermis. These creeping epidermal cells, transforming to thin discoidal ones, spread on the wound surface. The wound tissues under the epidermal covering consisted mainly of the compact parenchymal cells, but a few neoblasts and a little amount of the parenchymal free materials such as food granules, mucoid granules, and damaged yolk cells are also observed. The loosening and waving of the nerve cords become more remarkable. Furthermore, the connection between each intestinal cell become loose. Each of the intestinal cells assumes nearly bipolar shape with condensation of the cytoplasm at two poles of the nucleus having a distinct nucleolus. The parenchymal cells move from place to place and begin to assume nearly bipolar shape. In the present paper, such morphological changes are called in a term of "transfiguration". Besides, the parenchymal mucoid increases gradually in its amount.

In 3rd day's pieces, the wound covering of the epidermal cells is completed. The transfiguration becomes more marked in every tissue. The parenchymal cells approach morphologically to the neoblasts, while the neoblasts increase in number. Then, a few neoblasts begin to arrange under the covering of the wound. The parenchymal cells near the wound generally direct their long axes toward the wound. The neoblasts in this region also direct their long axes to the wound. The yolk granules, food granules and mucoid granules are scattered in this region as if they are drifted to the wound.

A mass of regenerative cells, called regeneration bud or blastema by earlier workers, was formed in the wound on 4–6th day after the operation. The neoblasts are found especially near the nerve cords. They appear to increase rapidly in number, though the mitotic figures are very few. The cut ends of the nerve cords are broken up into fibres and extend into the blastema and intermingle with the regenerative cells. The parenchymal free materials, especially the mucoid granules, are found to be increasing near the blastema, but are scarcely found in the blastema.

A rudiment of the pharyngeal cavity occurs in the ventral side of the blastema on 7–8th day after the operation. A narrow slit appears in the ventral

side near the blastema. This is a rudiment of the pharyngeal cavity. The slit extends toward the dorsal side being lined by somewhat large bipolar cells. Anteriorly, near the region of the pharyngeal cavity the accumulation of the regenerative cells are found. This is the rudiment of the pharynx. As differentiation of the pharynx proceeds, another parenchymal slit occurs between the base of the pharyngeal rudiment and the cut end of the intestine and free cells derived from the epithelial cells of the cut end of the intestine enter into it. These free cells are nearly bipolar and contain no food granules. The staining property of such cells quite resembles to the intestinal cells, but the former cells are somewhat transparent than the latter. Further, each nucleus of these free cells has a distinct nucleolus. Slightly anterior region from here is occupied with the transitional cells. Namely, the transfiguration of the intestinal cells are observed in that region and cell inclusions released from them are found in the intestinal tract. These transforming cells are also found in the blastema lateral to the pharyngeal cavity and they seem to give rise to the posterior trunks of the intestine in the regenerated worm.

The parenchymal slit of the pharyngeal base grows into a tube piercing through the core of the new pharynx, establishing the rudiment of the pharynx lumen. This change is found on 8–9th day of the regeneration. The lumen has already lined with the epithelium. On the other hand, some regenerative cells begin to transform into the mesenchymal cells of the pharynx. In the postpharyngeal region of the blastema, the regenerative cells begin to differentiate into the parenchymal cells and the muscles. Also in this region, nucleus-like bodies are often found. It is thought that they may be degenerating regenerative cells. In the periphery of the regenerated parenchyma, the rhabdite-forming glands containing several small rhabdites are apparently recognized. The regenerated epidermis is restored to the state found in the intact worm.

The mouth is formed by disappearance of a part of the ventral wall of the pharyngeal cavity at the level of the tip of the pharynx. This is observed usually in 9–10th day of the regeneration. At the same time, the nuclei of the outer epithelial cells of the regenerated pharynx assume the insunk type as in the intact worm. In the postpharyngeal region, the parenchymal muscles become more distinct and the mucoid is accumulating immediately beneath the epidermis. It is likely that these accumulated mucoid has a certain relation with regeneration of the basement membrane.

Characteristic changes are observed in the regenerated pharynx on 11–12th day of the regeneration. Namely, the muscle fibres are well recognized in the pharynx. At first, the longitudinal muscle fibres appear beneath the inner epithelium of the pharynx and then radial muscle fibres also occur. The outer epithelium of the pharynx is composed of the cells with completely insunk type nuclei, but without cilia. In the regenerated part posterior to the pharynx, the subepidermal muscles are divided into two layers. The epidermis of the regenerated

part has a very thin basement membrane beneath it. The neoblasts in all the parenchyma of the regenerated part seem to decrease in number, while the parenchymal cells increase. In the old part of the body, the intestinal cells are firmly connected to each other and form again the continuous epithelial layer. The parenchymal cells have stopped their transfiguration and have restored the nearly normal feature. The nerve cords, however, remain still loose in this time.

The head pieces complete their regeneration in about 14 days after the operation. The regenerated animal has all the elements found in the intact worm, though the nerve cords remain loose. In the pharynx, the outer epithelium becomes to posses cilia and the outer muscle layers are also well differentiated. The inner muscle fibres increase in number and show an intermingled structure as in the intact worm ; it is structurally typical of the family Dendrocoelidae. In the base of the newly regenerated part, i.e. in the pharyngeal region, the differentiated nerve cords are found. But the authors have failed to find the nerve cords in more posterior part. The regeneration of the nerve cords seems to complete, at least, in more than 30 days after the operation.

II. Histochemical

Iron : In the intact worm, the most intense reaction for iron is found in the basement membrane, but the moderate reaction appears in the intestine, the marginal glands and a part of a glandular layer of the pharynx.

During regeneration, the old tissues of the head pieces always react in a similar manner as in the intact worm. In the wound and blastema, iron is not detected except in the mucoid granules which show the positive reaction. Since the basement membrane does not appear entirely in the regenerating part until 9–10th day of the regeneration, iron was not detected in the corresponding part, but when the basement membrane appears, the positive reaction is recognized in this part. In the regenerating pharynx, iron is also detected on 8–9th day of the regeneration. However the inner epithelial cells of the regenerated pharynx show no positive reaction for this test, though they are derived from the transfigurated intestinal cells (Fig. 1). On the other hand, all of the intestinal cells, including the free cells for the regeneration of the posterior trunk, always show the positive reaction even during their transfiguration.

Nucleic acid : DNA is found only in the nuclei of all the tissue cells and in the spermatozoa. As the blastema is a compact mass of the regenerative cells, DNA appears to be rich in this region than in other parts. By the use of Feulgen's reaction, the mitotic figures are easily recognized in the regenerating worms. Some mitotic figures are found in the blastema, particularly near the regenerating part of the intestine, but not in the wound covering epidermis. As the mitotic figures are scarcely found in the old tissues, it may be said that the regenerating part possesses a comparatively high mitotic activity.

In the methylgreen-pyronin preparation, the nuclei of all the tissue cells and

the spermatozoa are stained with methylgreen, indicating the presence of DNA, and the cytoplasm of the tissue cells is stained uniformly with pyronin indicating the presence of RNA. The coloration of the neoblasts and the blastema cells is particularly intense. Probably, these results suggest that the cytoplasm of the tissue cells contains a fairly amount of RNA and that the neoblasts and the blastema cells are particularly rich in the amount of RNA. The authors also have observed the intense coloration with pyronin in the cytoplasm of primary yolk cells of the intact worm at breeding season.

Polysaccharides: In the intact worm, polysaccharides other than glycogen demonstrate in the basement membrane, the rhabdite-forming glands, the parenchymal mucoid and the other epithelium of the pharynx. The yolk glands also show the positive reaction. The other tissues do not usually contain the polysaccharides other than glycogen. The parenchymal mucoid, which shows intensely positive reaction to PAS-test, indicates an axial gradient in its distribution, found more in the anterior and less in the posterior part of the body. It is especially rich in the parenchyma of the body margin and also in the parenchyma anterior to the pharyngeal region (Fig. 2).

During regeneration, the reaction in the old tissues does not differ from that in the intact worm. In the wound and the blastema, the reaction is found only in the mucoid granules.

Glycogen is usually found in all the tissues of the intact worm. The glandular cells and the muscle layers of the pharynx and food granules appear to be especially rich in glycogen. In the regenerating pieces, the old tissues show the same reaction as those of the intact worm.

During regeneration, the reaction of the regenerating tissues is so weak as in the parenchyma of the intact worm, but it becomes intense in the glandular cells of the pharynx after completion of regeneration.

Lipoid: In the intact worm, by Ciaccio's method, certain food granules in the intestinal cells and in the parenchyma around the intestine show the presence of lipoid (Fig. 3). The other tissues are stained faintly but the same coloration is observed frequently in the materials fixed with alcohol or Bouin's fluid.

No difference is observed in the localization of lipoid during regeneration. The wound, the blastema, and the regenerated tissues are not stained with Sudan black B except food granules. The regenerating intestinal cells and the regenrating inner epitherial cells of the pharynx have not contained Ciaccio-positive granules at all.

Lipase: In the intact worm, high activity of lipase is demonstrated only around the food granules, probably lipoid granules. In the starved worm, the reaction is found only in the peripheral parenchyma of the body and in the epidermis. In the well fed worm, some food granules in the intestinal cells and in the parenchyma around the intestine show the intense reaction. In this case, the weak reaction is also observed over the cytoplasm of the intestinal cells (Fig. 4).

During regeneration, no significant differences in the localization of the enzyme are found in the tissue cells.

Alkaline phosphatase: In the intact worm, alkaline phosphatase is ditected in the brain,the pharynx and in a part of the penis papilla with 3 hours' incubation. The other tissues usually do not react at all in this incubation period, though the nerve cords show a faint reaction in some cases (Fig. 5). With 24 hours' incubation, however, the nerve cords and the parenchyma of the whole body show the moderate reaction and the other tissues show weak reaction except in the intestine where reaction is scarcely observed.

During regeneration, a significant change of the activity of alkaline phosphatase is observed with 3 hours' incubation (Table 1). When the nerve cords become loose in 6 hours after the operation, high activity of alkaline phosphatase

Table 1. Activities of alkaline phosphatase in various tissues.

Day of regeneration	Stump				Newly regnenerated part			
	Brain	Nerve cord	Parenchyma	Wound	Blastema	Rudiment of pharyngeal cavity	Rudiment of pharynx	Pharynx
Intact worm	++	±	−					
0 – 1	++	++	−	−				
2 – 3	++	++	±	±				
4 – 6	++	++	±		−			
7 – 8	++	++	±			+	+	
9 – 10	++	++	−					−
11 – 12	++	++	−					+
14	++	++	−					++

++ : intense; + : moderate; ± : inconstant or doubtful; − : negative.

suddenly appears in them. This high activity of the enzyme persists throughout the regeneration processes and disappears after the completion of regeneration which is indicated by the smoothing of the wavy nerve cords. At first, the high activity of phosphatase in the nerve cords appears to show a gradient from anterior to posterior part of the piece. The bipolar parenchymal cells transfigurated during regeneration often show inconstant phosphatase reaction with 3 hours' incubation. In some cases, the parenchymal cells around the nerve cords show the intense reaction, as if the enzyme oozed out of the nerve cords. This change of the activity of alkaline phosphatase in the parenchyma is found mainly in 2–8 days after the operation. The blastema cells do not show usually the reaction in the incubation for 3 hours. The nerve branches from the cut ends of the nerve cords traverse the base and the ventral side of the blastema, and these regions show the high activity of the enzyme. With 24 hours' incubation, the blastema cells show slightly higher activity of the enzyme than the parenchyma cells. When the pharyngeal cavity and the rudiment of the pharynx appear, the moderate reac-

tion is to be found around them. This reaction in the rudiment of the pharynx is found only in its base until 7–8th day of regeneration, when the rudiment grows to some length. But the reaction in the pharynx generally disappers on 8–10th day. When the regeneration of the pharynx is completed, the intense positive reaction appears again in the pharynx on about 14th day. At first, the reaction appears in the inner muscle layers and then extends over all the tissues of the regenerated pharynx (Figs. 6–12).

Discussion

According to Curtis ('36), Curtis & Schulze ('24. '34), Hein ('28), Lang ('12, '13), Steinmann ('26, '27 and others), and Weigand ('30), etc., bipolar wandering cells play a major rôle in the regeneration. This wandering cell is called "Bildungszellen", "Regenerationszellen", "Stoffträger", "Stammzellen", "formative cells", or "neoblast". Recently, much interesting researches have already been done on the planarian neoblasts and blastema formation by several workers (cf. Brøndsted's review, '55). Namely, it has been proved that the neoblasts may migrate through the entire body, migration being induced by factors in the wound, and the blastema is made of neoblasts (Dubois '49 ; Pedersen '59b ; Török '58). Furthermore, the distribution and the actual number of the resting neoblasts in planarian body were studied by A. & H. V. Brøndsted ('61) in *Planaria torva* and *Dendrocoelum lacteum*, and Lender & Gabriel ('60) in *Dugesia lugubris*.

Needham ('52) described that "the dedifferentiating cells usually enlarge, the nucleus with enlarged nucleolus stains more readily, and the cytoplasm became denser". In the present study, in *Den. lacteus*, we have observed that the cytoplasm of the intestinal cells is condensed and their nucleoli become distinct during the loosening of the tissue. These pictures may quite resemble to that of the dedifferentiating cells reported by Needham. As stated already, the parenchymal cells have been transfigurated and would seem to easily transform into the neoblasts. Though the degree of the dedifferentiation may vary in different tissue cells, it may safely be said that the dedifferentiation occurs more or less in all of the tissue cells of the present material.

The characteristic distribution of the neoblasts in several planarian species has previously been described in a series of papers from Brøndsted's team in Copenhagen (op. cit.). As already mentioned, however, in the early stage of the blastema in our material, the neoblasts were most abundant ventrally, especially around the nerve cords. In this connection the resemblance between the descriptions of the above cited literatures and our observation is very striking.

Concerning the origin of the tissue cells of the regenerated organs, the old intestinal, epidermal and nerve cells seem to build respectively the new tissue with incorporations of little amount of neoblasts. On the contrary, the pharynx, parenchyma, and muscles are probably formed only by the regenerative cells, i.e. transfigurated cells and neoblasts. Kido ('58, '61a, b) studied the regeneration of the intestine and pharynx in Japanese *Dugesia gonocephala* and reached the same conclusion. On the other hand, a discrepancy of the opinions exists on the regeneration of the nerve cords. According to Flexner ('98), the nerve cords in *Pl.*

torva arise from the parenchymal cells. From the experiments of tissue culture in *Dugesia dorotocephala*, Murray ('31) stated that new nerve tissue might be derived from common parenchymal cells. But she does not distinguish between the neoblasts and fixed parenchymal cells. In the present study, however, the new nerve cords appear to be arisen by elongation of the old ones with help of the neoblasts. The minute branches of the old nerve cords penetrate into the blastema and moreover the neoblasts near the nerve cords became to be gradually bipolar and to resemble with the appearance of the nerve cell. Our observation is very similar to that of the description of Török ('58), who studied the regeneration blastema formation in *D. lugubris*.

The localization of the parenchymal mucoid seems to show an axial gradient in the anterior half of the worm. Histochemically, the mucoid is predominantly composed of polysaccharides. As stated already, a large amount of the mucoid appears in the parenchyma of the regenerating worm. Probably this increase of the mucoid may be derived from the metaplasm excreted from the tissues during their dedifferentiation. It is worthy to note that, in Zenker-fixed material, the mucoid often shows positive reaction to the Feulgen's test in the regenerating worms. As it does not stain with methylgreen, the positive reaction to the test may not be due to the presence of DNA. Probably, the change on the staining property of the mucoid indicates that some metabolic changes occur during regeneration. The parenchymal free materials, i.e. the mucoid granules and food granules, gradually enter into the regenerating tissues and appear to be absorbed in later stages of the regeneration process.

As stated already, the food granules are found not only in the intestinal cells but also in the parenchyma. Probably these food granules are absorbed into the parenchyma through the phagocytotic cells of the intestine. Food granules in the parenchyma show the same reaction histochemically as those in the intestinal cells. This fact may suggest that the food granules are digested also in the parenchyma to a certain extent. It may be supported also by the fact that lipase is found only in the parenchyma near the body surface in the starved worm.

Histochemically, the most interesting fact was found in the case of detection of alkaline phosphatase. As reported already by Yamamoto ('57) in our laboratory, the high activity of alkaline phosphatase was not found in the nerve cords of the intact worm of *Den. lacteus*. In the present study, however, we have found that the nerve cords have come to show the positive reaction of this enzyme as the result of the operation of the intact worm. These results indicate that a considerable change occurs chemically in the nerve cords of the regenerating worm. The supposition that the nervous system plays a significant rôle in the regeneration, has long been held by many workers not only in invertebrate animals but also in vertebrates.

In the present study, the nerve cords far from the wound also show the change in the phosphatase reaction. This observation supports the veiw that the regeneration may be affected not only by the nervous cells in the wound but also

by those in the region far from the wound. As to the alkaline phosphatase in the nerve cord of the regenerating worm, it may be highly possible that the enzyme comes from the brain, where the phosphatase reaction is positive also in the intact worm (cf. Yamamoto '57). Probably, if the worm is wounded in the body, alkaline phosphatase of the brain moves along the nerve cords toward the wound. On the other hand, active synthesis of alkaline phosphatase may occur in the regenerating worm, since the brain always shows the high activity of this enzyme in spite of its outflow into the nerve cords. The intense phosphatase reaction of the new pharynx as found in that of the intact worm (cf. Yamamoto '57) has also been observed after the completion of regeneration. And the moderate reaction found in the base of its rudiment in 7–10th day of regeneration seems to be due to the minute nerve element distributed there. Alkaline phosphatase found in the normal pharynx of the worm is supposed to relate with its functional activities. We also have observed that the muscular penis papilla which possesses high contractility shows the positive phosphatase as found in the pharynx. The enzyme in these organs may possibly be related to its muscular movement. Perhaps this consideration may be supported by the fact that the positive phosphatase reaction is observed after the appearance of the muscle fibres in the new pharynx of regenerating worms.

Summary

The posterior regeneration of the head pieces obtained by a transverse cut through the prepharyngeal level of the planarian, *Dendrocoelopsis lacteus*, has been studied morphologically and also histochemically. The results are summarized as follows:

1. The regenerated tissues of epidermis, intestine and nerve cords come mainly from the cells of the old tissues. On the other hand, the regeneration of pharynx is performed by the regenerative cells including the transfigurated cells and neoblasts as an embryonic stock.

2. Parenchymal mucoid appears to have an axial gradient tendency in its distribution, found more in the anterior and less in the posterior part of the body.

3. Digestion takes place not only in the intestine but also in the parenchyma of the worm.

4. Alkaline phosphatase is detected in the brain, the pharynx, and the penis papilla of the intact worm. Regenerating worm, however, shows a high activity of alkaline phosphatase in the nerve cords in addition to the brain.

5. From these results described above, a possible rôle of alkaline phosphatase in the regeneration process is discussed.

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Explanation of Plate XVI

Abbreviations. *b* : brain, i : intestine, nr : newly regenerated part, ph : pharynx, pm : parenchymal mucoid, rb : regenerating blastema, rp : rudiment of the pharynx, rpc : rudiment of the pharyngeal cavity, W : wound.

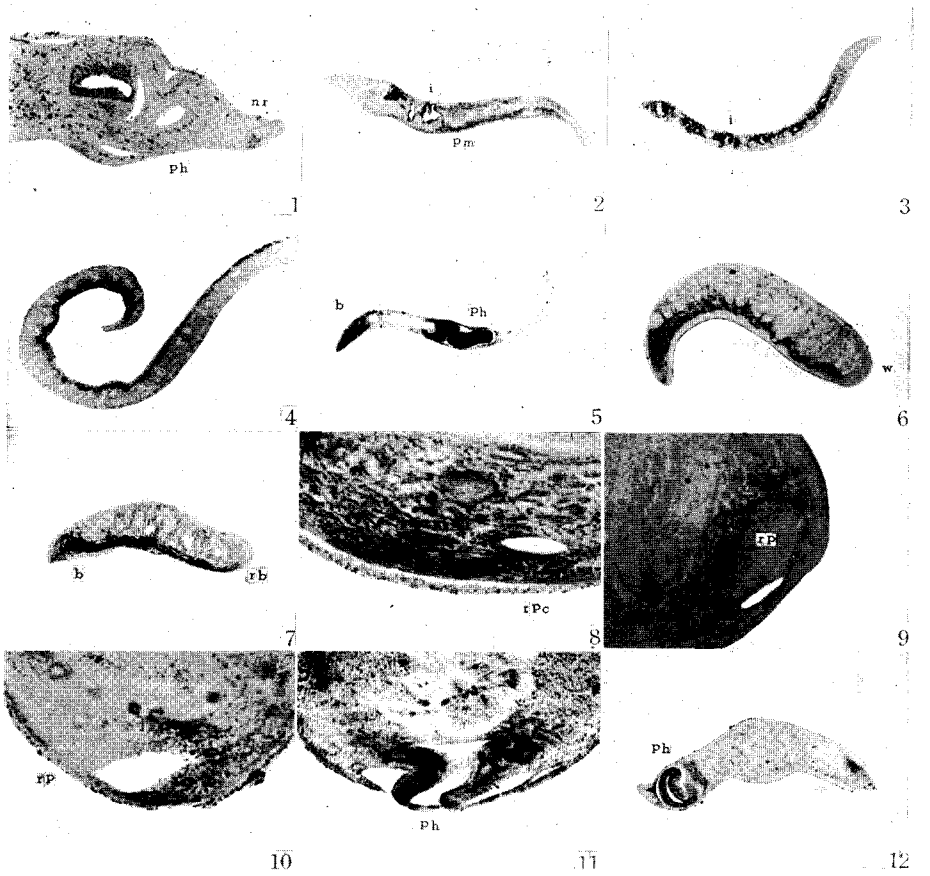
Fig. 1. Sagittal section of the regenerating worm on 14th day. Iron preparation. Notice the positive reaction in the intestinal epithelia. ca. ×40.

Fig. 2. Sagittal section of the regenerating worm on 14th day. Polysaccharide preparation. Glycogen is removed previously by the diastase treatment. Notice the positive reaction in the parenchymal mucoid. The black coloration in the intestinal epithelium is due to the colored food granules. ca. ×10.

Fig. 3. Saggittal section of the regenerating worm on 3rd day. Lipoid preparation. Notice the positive reaction in the intestinal epithelium and the parenchyma around the intestine. ca. ×10.

Fig. 4. Sagittal section of the intact worm. Lipase preparation. Notice the intense reaction in the peripheral parenchyma of the body. ca. ×15.

Figs. 5–12. Sagittal sections of the worms. Alkaline phosphatase preparation. Fig. 5. Intact worm showing the positive reaction in the brain and the pharynx. ca. ×3. Fig. 6. Regenerating piece on 3rd day. Notice the positive reaction in the nerve cord. ca. ×15. Fig. 7. Regenerating piece during blastema formation. Notice the positive reaction in the nerve cord. ca. ×15. Fig. 8. Notice the positive reaction near the rudiment of the pharyngeal cavity. ca. ×90. Figs. 9 and 10. The positive reaction in the base of the rudiment of pharynx. ca. ×90. Figs. 11 and 12. The positive reaction in the regenerated pharynx. Fig. 11. ca. ×90. Fig. 12. ca. ×15.



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