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Studies on the Pharynx Regeneration in Planarian, *Dugesia gonocephala*

I. Histological Observation in the Transected Pieces

By

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Introduction

During about thirty years since the beginning of this century, the histological and cytological investigations on the regenerating process of planarians had been extensively carried out because of the strong power of regeneration in these worms (Bardeen, 1901, 1902, 1903, 1904 ; Stevens, 1907, 1909 ; Ude, 1908, Lang, 1912, 1913 ; Bartsch, 1923 ; Curtis, 1928, 1936 ; Curtis and Schulze, 1924, 1934 ; Steinmann, 1908, 1926, 1927, 1928, 1932, 1933 ; Bandier, 1936). But these authors disputed mainly about the origin and the behavior of the cells participating in regeneration, and so far their works have hardly contributed to the analytical study of the mechanism of morphogenesis. The approach to the causal analysis of morphogenesis has been thereafter tried from an angle of the physiological gradient of the worm by Child and his coworkers. However, an important question is still left unsolved, whether such physiological gradient can be the cause for initiation of the planarian morphogenesis in regeneration. Actually, Child's gradient theory has faced criticisms. For example, Brøndsted (1946) claimed that the differential power of dye-reduction in the regions arranged along the anteroposterior body axis has nothing to do with the general morphogenetic activity in these regions, because, since the organs in a given region take the dye too variable quantities to give a homogenous staining, the result obtained with such dye is not so simple as to indicate some relation to the frequency of head regeneration as Child claimed, but, roughly speaking, it reflects rather the integrals of the metabolic rate of various organs in each region. Teshirogi (1959) stated that in terms of the intensity difference of Nadi-reaction, which represents the head forming frequency, the pieces of *Bdellocephala* treated with LiCl did not indicate the regional difference along the anteroposterior body axis, notwithstanding the fact that they could regenerate a head from the anterior and a tail from the posterior cut-surface respectively. This finding seems to be adverse criticism to the gradient theory. In this

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connection Ikushima's experiment (1953) concerning the differential oxidation of dye in amphibian embryo is also instructive in the fact that the oxidation-reduction potential in a given part of embryo does not necessarily reflect the developmental activity of that region as would be expected from the gradient theory. Based on his finding of the absence of the metabolic gradient in *Nereis*, *Planaria* and some actinians, Parker (1929) argued that the metabolic activity of an organism cannot be a cause for the formative process but it is rather a result of such a process.

In the previous experiments with *Dugesia gonocephala*, the writer (1958, 1959) has pointed out that a new pharynx was always formed at a definite site from the cut-surface, but the site was nothing to do with the length of the piece, in so far as the piece was taken from the pre- or postpharyngeal region. Buchanan (1927) stated that the more anterior a piece is taken from, the more posterior a new pharynx develops of it. This statement cannot agree with the above data of the author.

On the other hand, the writer (1952) has already demonstrated clearly in the transplantation experiment that the nerve plays an important role in the pharyngeal formation, although a number of planarian investigators vaguely assumed a nervous contribution, in some unknown way(s), to regeneration of the worm.

Child and Watanabe (1935) have maintained that a new regenerating part in planarians may be formed through a process of reorganisation of the old parts rather than by the undifferentiated "formative cells". Child and his associate, however, have so far not carried out the histological or cytological investigation to make this comment.

Under these considerations, it seems to be necessary to perform the histological and cytological investigations in terms of the modern knowledge of regeneration.

The present paper deals with the histological investigation of the pharyngeal formation in the transected pieces.

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Material and Method

The material used was the asexual forms of *Dugesia gonocephala* collected in the vicinity of Kanazawa City. Prior to operation the worms had been starved for over a week. Regenerating process was microscopically examined of the pieces coming from three different levels of the body : i. e., 1) the postpharyngeal piece which is a caudal half of a worm cut apart at the middle between the distal end of the pharynx and the tip of the tail, 2) the prepharyngeal piece which is an anterior half of the worm cut

through the level slightly anterior to the pharynx and 3) the pharyngeal piece which is a rear half cut apart through the level slightly posterior to the pharyngeal basis.

Fixation of the regenerated specimens was done either with Lang's solution or with 90 per cent ethanol at various occasions from four hours to five days after cutting. Before fixation, the worm had been placed on a glass plate and immersed in 1% of 1 N HCl solution in order to make it flat. Transversal, frontal and sagittal sections were prepared at 6–8μ thick by ordinary technique, and stained with Delafield's or Heidenhain's iron-haematoxylin coupled with eosin or orange G. Sometimes, Mallory's triple staining was applied. The last staining was effective in differentiating the different kinds of tissues.

Observations

I. *Regeneration of the postpharyngeal piece*

In the previous investigation (Kido, 1959), it was confirmed that the new pharynx is always formed within the old tissue near the anterior cut-surface, and the posterior cut-surface has, at least morphologically, nothing to do with this pharyngeal formation. In the present experiment, therefore, the posterior cut was not inflicted.

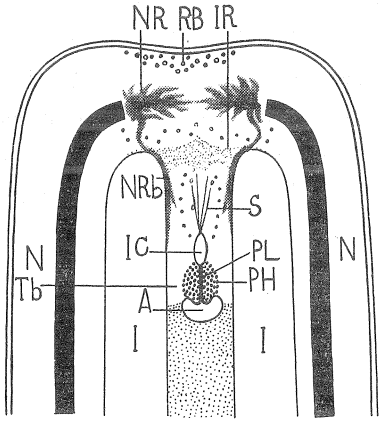
Microscopical observation at four hours after cutting: By contraction, the wound surface exposed becomes narrow soon after operation. This causes the inward bend of the cut end of the nerve cords and their mutual approach. Microscopical observation of the sagittal sections reveals that the cut ends of the nerve cords were also bent in the dorsal direction due to severer contraction of the dorsal tissue than the ventral one.

The old epidermis near the cut begins to spread over the wound as a semitransparent, thin, syncytial tissue, but it does not yet cover the whole cut-surface at this stage (Fig. A on Plate I). This new epidermis is clearly distinguishable from the original one because of it being thinner than the latter.

Soon after cutting, conspicuous histolysis occurs in the tissues not only near the wound, but also in the regions distant from it. The first remarkable phenomenon in the histolysis is the disappearance of fibrous structure in the mesenchymal tissue and the migratory movement of its constituent cells. Among such migrating cells there are found numerous large basophilic cells. These cells take occasionally spindle-shape as is shown in Fig. B on Plate I. The nuclei are larger than in the ordinary mesenchymal cells and hardly stainable with haematoxylin. There is an eosinophilic nucleolus in each of them. These cells seem to be identical with the "Bildungszellen" of Bartsch (1923) and Weigand (1930), the "wandering cells" of Steinmann (1933) and also "neoblasts" of Dubois (1949). At this initial stage of regeneration these cells are not found near the cut-surface, but in an area distant from it.

The second remarkable phenomenon is the clearance of the mesenchymal tissue from the anterior part between two lateral intestinal tracts, and the invasion of the disintegrated

mesenchymal tissue from the cut-surface into this part. Such part is seen as a bottle-shaped area (Tb in Text-Fig. 1).



Text–Fig. 1

Text-Fig. 1. Scheme, showing the pharyngeal formation in the postpharyngeal piece. A : atrial primordium, I : original intestinal tract, IC : primary cavity formed in the distal end of "streaming pathway", IR : bridge of syncytial tissue derived from original intestinal tract, N : original nerve cord, NR : mass of regenerating nervous tissue, NRb : regenerating nerve branch, PH : pharyngeal primordium made of MR-cells, PL : canaliculus which passes through the pharyngeal primordium from the primary cavity to the atrial cavity. It becomes enlarged later to the pharyngeal lumen, RB : accumulation of the MR-cells of a regeneration blastema, S : "streaming pathway" derived from the regenerating intestinal tissue, Tb : bottle-shaped area.

Intestinal tissue is harshly injured by cutting not only at the cut-surface but also at the distant parts. The tissue fabric of the intestinal inner layer is disintegrated and sometimes the constituent cells scatter, and the cells of the outer layer adjacent to the cut-surface are also dispersed. Cytoplasm of these dispersed cells are stainable with haematoxylin and eosin, while their large nuclei are hardly stainable with any dyes, except for the eosinophilic nucleoli which are frequently found. These cells are to make the syncytial tissue.

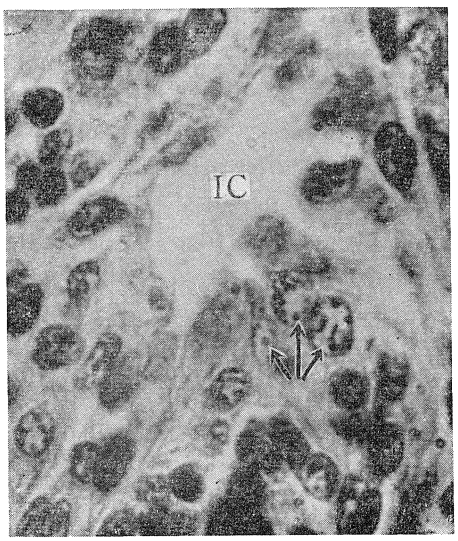
Transverse muscles near the cut-surface disappear, leaving sometimes fragmental remnants, whereas circular muscles and nerve cords are hardly affected by cutting, except for their limited cut ends. In front of each cut end of the nerve cord, a small mass of syncytial tissue is found (Fig. C on Plate I). The nuclei of this tissue are slightly larger, round and unstainable with basic dyes. It is sure that the syncytial tissue is made of regenerative cells derived from the original nerve cord. The designation as NR-cells will be adopted to denote them in the following description.

Microscopical observation at eight hours after cutting : The new epidermis covers the wound surface completely, but its cells are still flat and loosely connected with each other. The disintegrated mesenchymal cells gather together under the new epidermis. Just inside of this gathering and also along the nerve cords, the cells with large, basophilic nucleus and scanty cytoplasm are found to accumulate. In these cells no mitotic figure is found, but somtimes nucleolus is visible. Presumably, these cells have been derived from the mesenchyme directly or via the so-called spindle-shaped cells. It seems very likely that accumulation of these cells is the first step of the formation of regeneration blastema. These cells will be designated as MR-cells in the following description.

There appear fine dendriform processes from the assembly of the NR-cells. Some of those processes extend towards the cut-surface of the piece, and other, towards the end of the main intestinal tract. Now, the wounds at the cut ends of the two lateral intesti-

nal tracts have healed completely, and they are not exposed to the cut-surface. From the medial corner of each healed cut end of intestinal tract, a band of deeply eosino- and slightly basophilic tissue appears. This tissue is syncytial and seems to be originated from the intestine (Fig. D on Plate I). As is seen in Text-Fig. 1, these two bands grow in the medial direction and eventually fuse together with a resultant production of a bridge between the two intestinal cut ends (IR in Text-Fig. 1). These cells will be designated as IR-cells in the following description. Nuclei of the IR-cells are slightly larger in size than those of the MR-cells, but contain less numerous granules ; sometimes they contain a large nucleolus each as is frequently found in the intestinal cells.

Microscopical observation at twelve hours after cutting: Gathering of the disintegrated mesenchymal cells which has happened to block up the wound at the early stage of regeneration is now gradually replaced by the increasing accumulation of MR-cells, among which fine fibrous structures begin to appear (RB in Text-Fig. 1). Thus, the area lying between the intestinal bridge of the syncytial tissue and the cut-surface of the piece becomes occupied almost by MR-cells. This feature indicates the second step of the formation of regeneration blastema. No mitotic figure is found at the distal portion of the blastema, while at the proximal part adjacent to the cut ends of the nerve cords, a few cells are sometimes found dividing. The intestinal bridge of the syncytical tissue becomes gradually distinct, and the original lumen of the intestine begins to invade this bridge. From the middle of the bridge two projections appear in both anterior and posterior directions. The posterior projection extends more quickly than the anterior one, and infiltrates into the bottle-shaped area mentioned above. Such a feature of infiltration is referred to as a "streaming pathway", because it consists of some fine strands streaming out of the syncytial tissue (S in Text-Fig. 1 and Fig. E on Plate I). The distal end of



Text-Fig. 2

Text-Fig. 2. Photomicrograph, showing the primary cavity (IC) formed at the distal end of the "streaming pathway" at twenty-four hours after cutting. Arrow indicates the hardly stainable nuclei and eosinophilic nucleolus in the cells of intestinal origin. MR-cells with deeply stainable nuclei are seen below the nuclei indicated by arrow.

this "streaming pathway" makes a knob of the syncytial tissue at the bottom of the bottle-shaped area.

A transverse, slender nerve cord with fine branches occurs within the blastema and it makes light connection with the cut ends of the two original cords (Fig. F on Plate I). Presumably, the cephalic ganglion will arise from this new cord. Besides, a slender nerve branch runs along the inner side of each lateral intestinal tract (NRb in Text-Fig. 1). It is very difficult, if not impossible, to trace its proximal and distal ends, because the syncytial tissue arising from the intestinal tract conceal them from the closer observation. However, it seems likely that each mass of NR-cells sprouts out this branch.

Microscopical observation at twenty-four hours after cutting : There appears "primary cavity" within a knob of the syncytial tissue which is situated at the end of the "streaming pathway" (IC in Text-Fig. 1, Text-Fig. 2 and Fig. G on Plate II). A heavy accumulation of MR-cells occurs in contact with a posterior part of the primary cavity-wall (PH in Text-Fig. 1 and Fig. G on Plate II). It is sure that the accumulation is a pharyngeal primordium. It can be assumed that migration of the MR-cells formerly accumulated near the wound surface takes place to this area, because there are many histological sections which can demonstrate the continuous distribution of these cells from the blastema to the cavity along the "streaming pathway" as well as along the inner sides of the lateral intestinal tracts. The primary cavity evaginates into the pharyngeal primordium as a slit (PL in Text-Fig. 1). Subsequently, the distal end of the slit bulges into the secondary cavity behind the pharyngeal primordium. Thus, the cavity gives rise to the atrium later (A in Text-Fig. 1 and Fig. G. on Plate II). The figure thus established is essentially similar to that already shown by Bandier (1936).

As was already described, the anterior projection from the syncytial tissue of intestinal origin enters the blastema and will constitute a median intestinal tract in the prepharyngeal region (Fig. H on Plate II). The new nerve cord in the blastema becomes thicker and more ramified, its connection with the original nerve cords being clear. The new nerve branches along the inner sides of the lateral intestinal tracts also show further extention.

Microscopical observation at fourty-eight hours after cutting : The pharyngeal and atrial primordia increase gradually in size, especially, the atrial growth is so predominant that the pharyngeal primordium is enclosed by it. Thus, the scheme of the pharyngeal part is established.

On the other hand, the primary cavity extends in the anterior direction so as to communicate with the narrow lumen, which has been developed in the tissue of "streaming pathway". This lumen crosses another lumen running transversely within the syncytial tissue of the bridge between the lateral intestinal tracts (Fig. H on Plate II). A cross-shaped lumen thus produced is the rudiment of the intestinal system characteristic of *Triclada*.

As the regeneration blastema grows markedly, the fibrous structure within the mesenchyme becomes distinct. The horseshoe-shaped cephalic ganglion becomes enlarged

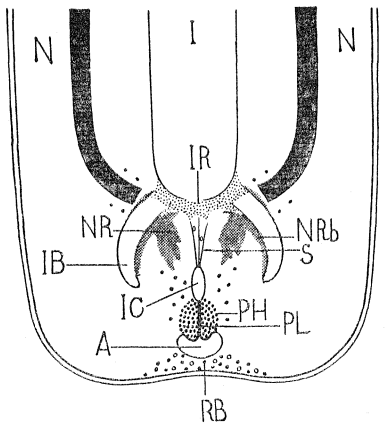
and gives off numerous ramifying fibres into the blastema. At three or four days after cutting, slender muscle fibres are found in the blastema and the new pharyngeal wall. In addition, the secretory cells occur also in the blastema at this stage. Simultaneously, a caudal tip of the atrial wall begins to extend towards a ventral body wall, and finally it will open to the outside as the mouth opening.

When the tail end is cut off from the postpharyngeal piece, a marked contraction of the tissues occurs on either cut-surface of the piece, though it is always less conspicuous at the posterior surface. Motile mesenchymal components near the posterior cut-surface infiltrate into the area laying between the lateral intestinal tracts, though less extensively than in the anterior part. The nerve cords and the intestinal tracts in the posterior part continue to grow separately without respective fusion, while their fusions occur in the anterior part as described above. Accordingly, the syncytial bridge of the intestinal tissue does not appear in the posterior part. It seems likely that this is the reason why the "streaming pathway" and the new pharynx cannot develop in the posterior part. The pharyngeal formation takes place only in relation to the anterior cut-surface in the same way as the case of a piece with the anterior cut-surface alone.

II. *Regeneration of the prepharyngeal piece*

So far as no special mention is made, a cut was applied only to the posterior end of the piece, because it has been previously demonstrated by the writer (1959) that in the prepharyngeal piece with both anterior and posterior cut-surfaces the pharynx is always formed in relation to the posterior cut-surface alone.

The first step of regeneration is nearly the same as was already elucidated in the postpharyngeal piece. Since, however, the intestinal system in this piece is different from that of the postpharyngeal piece, the mode of pharyngeal formation is necessarily different.



Text-Fig. 3. Scheme, showing the pharyngeal formation in the prepharyngeal piece. IB : intestinal branch. Other abbreviations are the same as in Text-Fig. 1.

Microscopical observation at eight hours after cutting : The intestinal tract retracts itself from the cut-surface in considerable degree and a band of syncytial tissue appears behind the end of median intestinal tract (IR in Text-Fig. 3). Lateral extremities of this band run backwards a little and come to contact with the anterior cut ends of the old intestinal branches, which happen to be there. From the middle of the band the "streaming pathway" of the syncytial tissue grows posteriorly towards the cut-surface. Just inside of the cut-surface, numerous components of the disintegrated tissues gather to block up the wound and a small number of MR-cells are found

scattering in the area anterior to this gathering of components. As is shown in Text-Fig. 3, both cut ends of the nerve cords bend in the median direction owing to the shrinkage of the cut-surface of the piece, but the regenerative tissue derived from the injured nerve cords are not so easily seen as in the case of the postpharyngeal piece. MR-cells are found along the nerve cords, especially near the cut ends of them.

Microscopical observation at twelve hours after cutting: The "streaming pathway" of the syncytial tissue of the intestinal origin becomes distinct and extends towards more posterior part, where the MR-cells gather to form the regeneration blastema. But the cells are still small in number at this stage, and the components of disintegrated tissues exist behind it.

Regeneration of the nerves is more extensive from the original cords towards the median direction of the piece, but these nerves do not fuse with each other to form a cephalic ganglion. A branch of regenerating nerve cord extends along the inner side of an old intestinal branch on either side (NRb in Text-Fig. 3).

Microscopical observation at twenty-four hours after cutting: The distal end of the "streaming pathway" bulges out to develop into a primary cavity. A large number of MR-cells gather together around there, especially around the posterior end of the cavity. This accumulation will develop in due course into the pharyngeal primordium, as was already described in the case of postpharyngeal piece (PH in Text-Fig. 3 and Fig. I on Plate II).

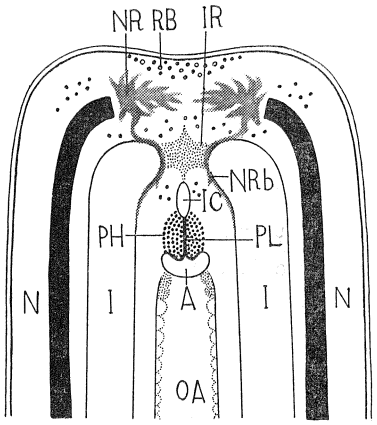
The further development of the pharyngeal formation is almost similar in the case of the postpharyngeal piece. But, the two lateral intestinal tracts newly formed are the remoulding of the old intestinal branches which happen to be near the cut end of the median intestinal tract (IB in Text-Fig. 3 and Fig. I on Plate II).

When another cut was applied to the anterior part of the piece in addition to the posterior one, fusion of two nerve cords for the formation of a cephalic ganglion always occurs only in respect of this anterior cutting, while the "streaming pathway" and the primary cavity are produced in relation to the posterior cutting alone. Consequently, a new pharyngeal formation takes place only at the posterior portion of the piece. The old intestinal branches which happen to be near the anterior cut end of the median intestinal tract are communicated with the latter, and function as before, while the original branches which fuse with the posterior cut end of the median intestinal tract develops into the new lateral intestinal tracts of the regenerating worm.

III. *Regeneation of the pharyngeal piece.*

Concerning the pharyngeal piece the writer (1959) stated already that the new pharynx develops from the old pharyngeal remnant, but at that time he omitted intentionally the histological description of it. The present microscopical observation on this piece is the supplement to the previous information.

The worm was transected into two halves through the level slightly posterior to the base of pharynx, and the microscopical observation was done on a rear half thus made.



Text-Fig. 4. Scheme, showing the pharyngeal formation in the pharyngeal piece. IC : cavity derived from the original atrial wall, and being comparable to the primary cavity, OA : original atrium, other abbreviations are the same as in Text-Figs. 1 and 3.

Immediately after operation, an old pharynx was discarded, leaving only a large part of the atrium.

Microscopical observation at four hours after cutting : As was seen in the post- and prepharyngeal pieces, a contraction takes place at the cut-surface. Sinece this contraction at the middle part of the cut-surface is more marked than that at the lateral sides, the cut end of the atrium situated on the median line of the piece retracts itself remarkably. Accordingly it lies behind the cut ends of the nerve cords and the lateral intestinal tracts (Text-Fig. 4). The regenerating feature of the nerve cords and the lateral intestinal tracts running along either side of the atrium is the same as was alredy described in the case of the postpharyngeal piece. Disintegration of the atrial wall occurs first near the cut end, and the cells liberated are found scattering there. Occasionally, an anterior part of the atrial cavity is constricted off from the original cavity (PC in Fig. J on Plate II). In any case the cells which remained in the anterior part of the atrial wall tend to form a syncytium. The cytoplasm of such cells is stainable with haematoxylin and eosin, while the nucleus is large and unstainable with these dyes, except for a large, eosinophilic nucleolus. Therefore, these cells bear a striking resemblance to the syncytial tissue of the intestinal origin. The atrial wall of the remaining part is also disintegrated in various degrees, and even a necrosis is seen in some of the cells (Fig. J on Plate II).

Microscopical observation at the twelve hours after cutting : The aforesaid syncytial tissue of the atrial origin extends forwards, and soon later a cavity is produced in it. The cavity communicates, through a canaliculus of the same origin, with the original atrial cavity. MR-cells are found scattering near the cavity and the cut-surface. On the other hand, another syncytial tissue of the intestinal origin appears in a form of the bridge between the cut ends of the lateral intestinal tracts, as was already described in the postpharyngeal piece. From the middle of this bridge two projections emerge in the anterior and posterior directions. The posterior projection will unite later with the wall of the cavity in the syncytial tissue of the atrial origin.

Microscopical observation at twenty hours after cutting : A remarkable aspect is the accumulation of MR-cells around the canalicular part of this cavity (Text-Fig. 4 and Fig. K on Plate II). This accumulation is certainly a pharyngeal primordium. Therefore, this atrial cavity can be comparable to the primary cavity of the intestinal origin in the pre- and postpharyngeal piece. Some of the MR-cells are found dispersedly along the lateral

intestinal tracts, and they continue to the heavy accumulation of the same cells near the cut-surface of the piece. In this stage, there occurs a secondary small cavity in the atrial syncytial tissue just behind the pharyngeal primordium. This secondary cavity is surely the atrial primordium. At the same time a new nerve appears in front of the bridge and runs transversely.

At twenty-four hours after cutting, the pharyngeal primordium augments, and the canaliculus passing through it develops into the pharyngeal lumen.

The further sequence of regeneration in the present piece is almost the same as in the postpharyngeal piece, except for a minor point of the rapid growth of the new atrium (Fig. L on Plate II).

When the secondary cut was made at a level of the posterior end of the pharynx, the original pharynx is easily discarded, leaving only the atrial wall. The sequence of regeneration from this cut-surface is identical with that from the posterior cut end of the postpharyngeal piece. However, the disintegrated posterior cut end of the atrial wall is rejuvenated to form a syncytial tissue. This syncytial tissue is supposed to take part in the repairment of the atrium, but no cavity is formed in it. Therefore, formation of the new pharynx does not occur in spite of the accumulation of the MR-cells at the posterior cut end of the piece. By contrast, the new pharynx is always developed in relation to the anterior end of the piece as was described above.

Considerations

The present histological and cytological observations on the transected pieces of *Dugesia gonocephala* reveal clearly that the regenerative changes occur not only at the cut-surface, but also in a portion distant from it.

In the postpharyngeal piece, at four hours after cutting, a bottle-shaped area filled with components of disintegrated mesenchymal tissue appears between the anterior parts of the lateral intestinal tracts. It is a rule that the new pharyngeal formation occurs always at the bottom of this bottle-shaped area. Since the extent of such area is always constant whenever the cutting is made, the new pharynx arises uniformly at a definite site with respect to the cut-surface. The writer's measurement (1959) indicates that the distance from the anterior cut-surface to the new pharynx is approximately 0.5 mm. long in the postpharyngeal piece. This distance certainly corresponds to the extent of the disintegration of the mesenchymal tissue between the lateral intestinal tracts. The fact that this distance is constant was confirmed by our previous experiment (Okada and Kido, 1943). In the regenerating piece of *Dugesia gonocephala*, so far as the length of the postpharyngeal piece was less than 0.5 mm, the piece lost its polarity and was very frequently capable of developing a head in reversed direction. This tells us that the tissue in such small piece is so extensively damaged throughout the whole length that the original polarity is lost.

In the prepharyngeal piece, as was already described, the new pharynx appears always in contact with the regeneration blastema established by the accumulation of the MR-cells at the posterior cut-surface. Since the length of the blastema in every case is almost constant at twenty-four hours after cutting, the new pharynx is to appear at the definite site from the posterior end of the piece.

This histological observation is in line with the previous view of the writer (1959) that in the piece taken from either the pre- or the postpharyngeal region, the new pharyngeal formation always initiates at a definite site from the cut-surface.

Bandier (1936) is of the opinion that the new pharynx is formed by the self-differentiation of a mass of embryonic cells derived from the mesenchymal tissue, but he does not refer to the details of the morphogenesis of the regenerative cells. The present observation discloses, however, that the formation of the primary cavity is prerequisite to the pharyngeal formation from the MR-cells. The primary cavity is formed in the distal end of the "streaming pathway" of the syncytial tissue of intestinal origin both in the pre- and the postpharyngeal pieces, while in the pharyngeal piece the comparable cavity occurs in the syncytial tissue derived from the old atrial wall. But the atrial wall itself is a derivative from the wall of the primary cavity. After all, the primary cavity is derived in every case from the intestinal tissue.

The pharyngeal primordium is made of the MR-cells, which accumulate behind the primary cavity regardless of the origin of the latter. From this feature, it is assumed that the cells of the primary cavity of the intestinal origin may exert an inductive influence upon the MR-cells to develop the pharynx.

In the postpharyngeal piece the new pharynx is always produced at a position where the lateral intestinal tracts fuse each other. This fact was already pointed out by Bardeen (1903). In the postpharyngeal piece with two anterior and posterior cut-surfaces, the new pharynx is always developed in relation to the anterior cut and never to posterior one. This seems to be due to the fact that the intestinal fusion is established only in the anterior region of the piece, and not in the posterior region. Concerning this point, Sugino (1938) reported a good example, with *Planaria gonocephala*, in which the pharngeal formation occurred in the prepharyngeal region, where the fusion of the intestinal tracts had took place after removal of the large median part of the worm. From this we can extend Bardeen's finding to the prepharyngeal piece, and say that the intestinal fusion is one of the factors necessary for the pharyngeal formation regardless of the original region from which the regenerating piece is taken. But we cannot consider it in the backward direction, i. e., the intestinal fusion is not necessarily the causal requisite for the pharyngeal formation. In the prepharyngeal piece, the new pharynx occurs at the position where the original intestinal branches fuse with the posterior cut end of the median intestinal tract, but it never occurs near the anterior cut end, although such a fusion occurs there also. In the pharyngeal piece the fusion of the anterior cut ends of the lateral intestinal tracts occurs as in the postpharyngeal piece, but it cannot induce the accumulation of the MR-cells indispensable for the pharyngeal formation, presumably because

of the lack of the primary cavity derived from the intestinal syncytium. Under these considerations, it is safe to conclude that the intestinal fusion is one of the factors favourable, but not sufficient, to the pharyngeal formation. The necessary and sufficient factor must reside in the primary cavity.

There are some unpublished data of the writer which show that inhibition of the new tissue formation either from the anterior cut-surface of the postpharyngeal piece, or from the posterior cut-surface of the prepharyngeal piece results in the failure of the pharyngeal formation. Similarly, in the transplantation experiment with Planaria Okada and Sugino (1937) and Okada and Kido (1943) pointed out that whenever no tissue is developed between the graft and host tissues, no pharynx appears. Taking the present observation into consideration, it may be surmised that prevention of the MR-cells from migration towards a prospective pharyngeal area will cause the inhibition of the new pharyngeal formation.

Here we arrive the tentative conclusion that the factors indispensable for the pharyngeal formation are dual ; one is the primary cavity formed generally in the fusion of the intestinal tissue, and the other is the migration and accumulation of the MR-cells towards behind this cavity.

What is a factor responsible for the migration of the MR-cells ? Many investigators have commonly assumed that it is mere cutting or a factor released by cutting. In fact, histological and cytological changes occur in a drastic manner immediately after cutting in the tissues not only near the cut-surface, but distant from it as well. Thus, mesenchymal tissue becomes motile everywhere and its cells move towards the cut-surface or towards the place where the pharynx will arise. However, a question how the pathway is settled of the cell-migration is left unsolved. The present observation can contribute nothing to these questions. But it seems probable that the principle liberated from some injured cells is attractive to the MR-cells. If so, the MR-cells can be drived by something like a chemotaxis to the injured parts ; namely, near the cut-surface and the presumptive place of the new pharynx.

Many authors have so far stated the possibility that the nerve plays some role in the regeneration of animals. In Planaria, Beyer and Child (1930) and Wilson (1940) pointed out that the regeneration blastema is primarily formed in relation to the cut ends of the nerve cords. In this connection, Schleip's observation (1934) in Sipunculus, *Phascolion strombi*, is of special interest. In this animal a strand of embryonic cells and a nerve run parallel with each other within so-called "Bauchmark". When the worm is cut out, the embryonic cells migrate out of the "Bauchmark" and accumulate at the cut end to develop into the epidermis and nerve. This fact seems to be identical with the writer's observation (1957) on Planaria that the numerous MR-cells are found along the nerve cords, and they contribute to the formation of a new pharynx and a regeneration blastema.

On the other hand, Coe (1934) stated from the observation on nemerteans that the cut end of the nerve liberates some growth stimulating substance which will exert double influence upon the adjacent dormant cells of parenchyma so as to transform them into

active, regenerative cells and to guide their movement towards the anterior region. Similarly, in the regeneration of earthworm Okada and Kawakami (1943) postulated the possibility of the humoral substance secreted from the cut end of the nerve. In Planaria, Lender (1956) and Dubois and Lender (1956) claimed that ganglionic secretion occurs in association wtih the regeneration of the ganglion and the eye. Thus, detailed informations of the nerve secretion will be expected to elucidate the mechanism of cell-migration in the regeneration process of Planaria. So far any definite statement cannot be made as to the mechanism of the cell-migration, but the present observation may suggest that the direction of the growth of nerve will partly contribute, in addition to the humoral substance, to the determination of the pathway of the migrating MR-cells.

As to the origin of cells participating in the planarian regeneration, there is difference in opinion ; some investigators (Schulz, 1902 ; Stevens, 1909 ; Abeloos, 1930 and Curtis, 1936) insisted that these cells are already reserved in the mesenchymal tissue, as have been known in certain annelids (Hämmerling, 1924), while others argued that mesenchymal cells are transformed into the embryonic totipotent cells due to cutting to form the regeneration blastema (Bartsch, 1923 ; Castle, 1924 ; Weigand, 1930 and Steinmann, 1933). According to Bandier's observation (1936) on the land planarians, both of the new nerve and the new intestine are produced from the cells originated from their respective organs after the cells have been transformed into the regenerative cells. Bartsch (1923) believed that the spindle-shaped cells are transformed into the MR-cells on the way of their migration along the nerve to the cut-surface. In the present observation, it was not able to trace whether or not the so-called spindle-shaped large cells were the origin of the MR-cells although it seemed to be possible. But it is apparent that the epithelial, nervous and intestinal cells can be transformed into regenerative cells in addition to the mesenchymal cells.

In other invertebrate animals such as *Corymorpha*, there are some informations that the rejuvenation of cells occurs in the inner layer and in *Syllidian* even the transformation appears of outer epithelial cells into nerve cells (Okada, 1927, 1929).

In planarians, however, these transformed regenerative cells are considered not to be totipotent, but to retain their original specificity, although Bartsch (1923 a, b) and Dubois (1949) insisted that so-called MR-cells are totipotent. The present microscopical observations indicate that the MR-cells can differentiate into only limited organs. In other words, they are unable to develop into any other tissues than mesodermal ones. Formation of the new pharynx will be a good example in this respect. This organ is exclusively derived from the MR-cells accumulated around the primary cavity.

In the piece having no old pharyngeal remnant, say, in the pre- and the postpharyngeal pieces, tissue of the primary cavity which is the prerequisite to the accumulation of the MR-cells is of the intestinal origin, while in the pharyngeal piece the tissue of the cavity is derived from the atrial wall, although the latter has previously been originated from the intestinal tissue. As these regenerative cells of intestinal origin are again transformed into the new intestinal tissue and its derivative, the retention of the original specificity

can be assumed in these regenerative cells.

To sum up, the regeneration blastema is surmised to be the complex of cells with different specificities, that is, it is an accumulation of different cells stigmatized as the epithelial, nervous, intestinal and mesodermal tissue.

Dubois (1949) demonstrated that planarian neoblasts undergo mitosis at least once before they accomplish the differentiation within the regeneration blastema, and McWhinnie and Gleason (1956) also observed the cell-division of mesenchymal amoebocytes on the way of their migration towards the cut-surface. However, Dresden (1940), Verhof (1946) and many others could not find the mitotic figure in any place. In the present observation, mitosis is very scarce. The cells participating in regeneration must be the cells themselves coming from the old tissues. Consequently, the strong power of movement of the cells is necessary for the material-supply to the regenerating tissues. Recently, Pedersen (1958) stated with *Planaria vitta* that the mitotic activity becomes prominent on the third and the fourth day after decapitation. The fact seems to the present author to indicate that the cell-division can be the way for supplying the lost cells at the later stages of regeneration after the establishment of various primordia, because, although the planarian species used in the present observation is different from Pedersen's material, the time required for the pharyngeal formation is only twenty-four hours. Therefore, it seems likely that mitosis may be a roundabout way to contribute the cells to the initial process of the reconstitution of the organs.

Summary

The microscopical study was carried out for the purpose of analysing histologically the process of pharyngeal formation in the regenerating piece taken from the pre-, post- and pharyngeal regions of Planaria, *Dugesia gonocephala*.

It was found that the formation of the new pharynx occurs in intimate relation to the mode of the intestinal and nervous regeneration. Namely, it is revealed that the factors responsible for the pharyngeal formation are dual ; one is the primary cvaity derived form the intestinal tissue, and the other is the migration and accumulation of the MR-cells derived form the mesenchymal cells towards behind the primary cavity. This migration of the MR-cells seems to be escorted by the regenerating nerve fibers.

In the postpharyngeal piece, the regenerative syncytial tissue derived from the anterior cut-surfaces of both lateral intestinal tracts fuse each other to form a bridge between the tracts. From the middle of this bridge a strand of syncytial tissue emerges posteriorly as "streaming pathway" into a bottle-shaped area lying between the two lateral intestinal tracts. Finally, at the distal end of the "streaming pathway", i. e., at the bottom of the bottle a primary cavity is formed. At the same time, MR-cells coming from the surroundings gather together in this area, and accumulate behind the primary cavity to give rise to the pharyngeal primordium. Nearly synchronously, when the pharyngeal primordium is

established, the atrial primordium also appears at the site immediately posterior to the pharyngeal primordium. The wall of this atrial primordium is derived from the wall of the primary cavity, which extends, as a slit, posteriorly through the median part of the pharyngeal primordium. The further extention of the slit wall establishes eventually the atrial wall. Later, the slit within the pharyngeal primordium becomes enlarged as the definite pharyngeal lumen.

In the prepharyngeal piece, the wall of the primary cavity consists of the regenerative tissue derived from the posterior cut-surface of the median intestinal tract. The sequence of the formation of the pharyngeal part in this piece is essentially the same as in the postpharyngeal piece.

In the pharyngeal piece, the regenerative cells derived from the anterior cut end of the old atrial wall take part in the formation of cavity comparable to the primary cavity, behind which the MR-cells accumulate to form the new pharynx, as in the pre- and the postpharyngeal pieces. The formation of the atrial primordium and the pharyngeal lumen are the same as in the pre- and the postpharyngeal pieces except for some minor points.

The direction of migration of the MR-cells and the IR-cells runs parallel with that of the nerve growth.

Pharyngeal formation occurs approximately at a definite site in the piece, because in the postpharyngeal piece the extent of so-called bottle-shaped area is always uniform, and the new pharynx develops at the bottom of the bottle, while in the prepharyngeal piece the extent of the regeneration blastema from the posterior cut-surface at the time when the pharynx appears is the same in all pieces, and the new parynx always differentiates in contact with this regeneration blastema.

Epithelial, nervous and intestinal cells are transformed into regenerative cells which form again a respective syncytial tissue, but these cells redifferentiate into the definitive tissue depending upon their original specificity. Similarly, the mesenchymal cells cannot differentiate into any other tissues than the mesodermal, though they have been transformed also into migratory regenerative cells.

Mitosis is scarcely found. Therefore, it seems likely that the initial part of regeneration is performed by the reconstitution of the old cells before their proliferation.

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PLATE I

Explanation of the figures

Microscopical representation of the regenerating feature in the postpharyngeal piece of *Dugesia gonocephala*.

A. Photomicrograph of the sagittal section, showing a thin epithelium, indicated by arrow, covering incompletely over the wound surface, at four hours after cutting. I : intestinal tissue indicating the disarrangement of cells, MC : disintegrated mesenchymal components, MO : motile feature of mesenchymal tissue.

B. Spindle-shaped large cells, indicated by arrow, appeared beneath the epithelium, at four hours after cutting. I : original intestinal tract.

C. Syncytial tissue, surrounded by dotted line, derived from the original nerve (N). Note round and less stainable nuclei (NC). MR : deeply stainable MR-cells of the mesenchymal origin.

D. Enlargement of the syncytial tissue (SY) of the intestinal origin at eight hours after cutting. Note large nuclei with eosinophilic nucleolus. MP : vacuolated mesenchyme without fibrous net-work.

E. "streaming pathway" (S) from the syncytial tissue of the intestinal origin located between the lateral intestinal tracts (I), at twelve hours after cutting. DS : distal end of "streaming pathway".

F. New slender transverse nerve, indicated by arrow, lying between masses (NM) of the syncytial nervous tissue at twelve hours after cutting. Note this mass originated apparently from the cut end of the old nerve (N). Other abbreviations are the same as above

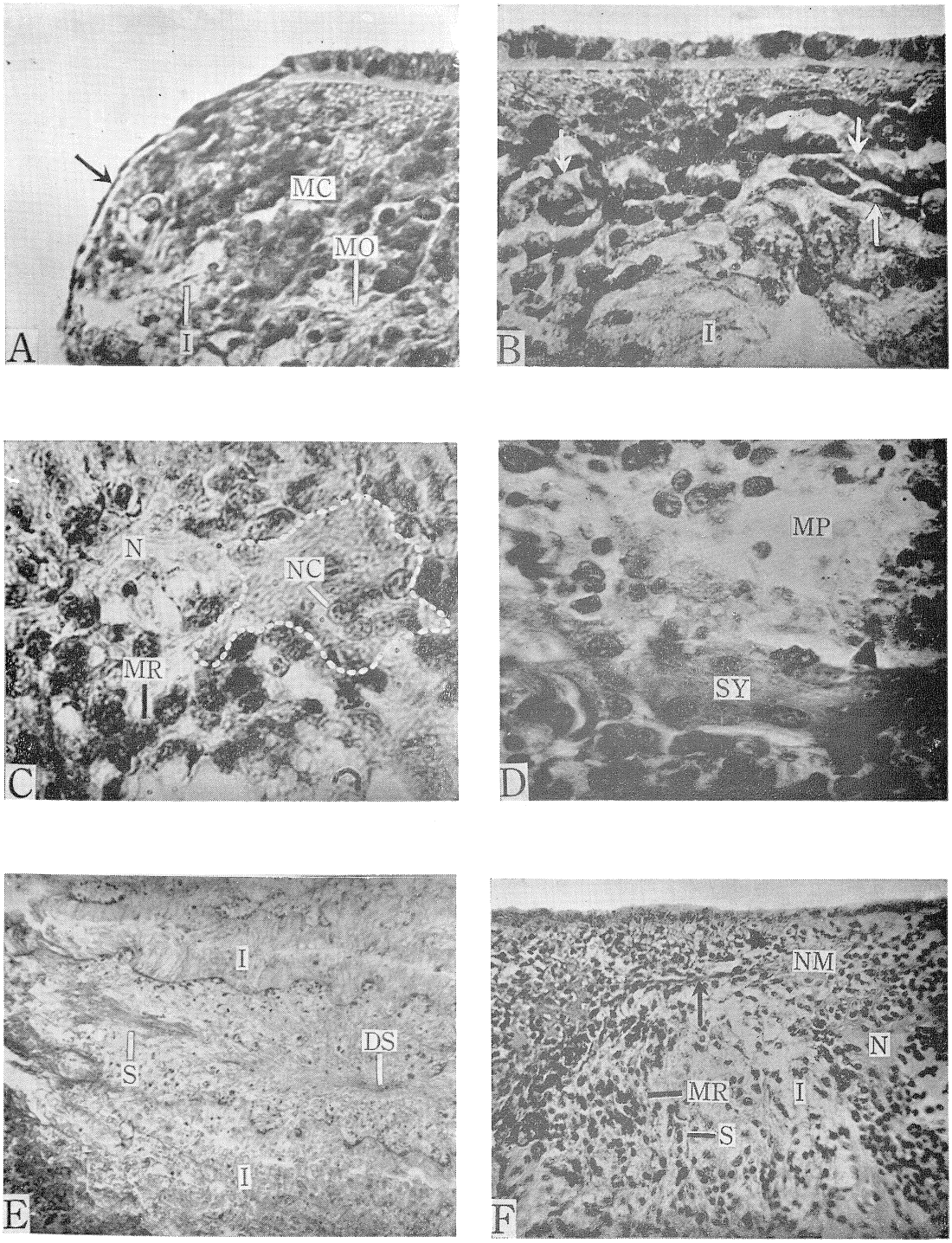
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PLATE II

Explanation of the figures

Microscopical representations of the pharyngeal regeneration in the piece taken from different levels of *Dugesia gonocephala*. G-H, postpharyngeal piece. I, prepharyngeal piece. J-L, pharyngeal piece.

G. Pharyngeal primordium (PH) produced between the anterior primary (IC) and the posterior atrial (A) cavities, at twenty-four hours after cutting. MR-cells, deeply stained, can be seen migrating along both sides of the primary cavity.

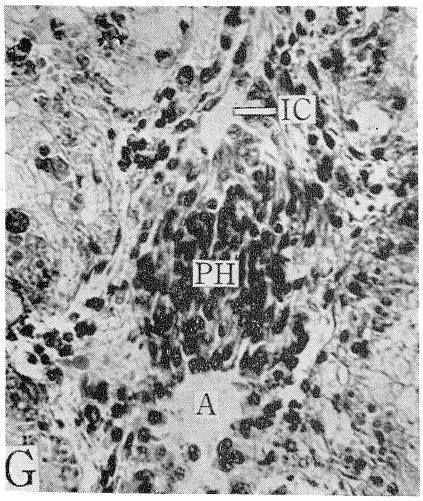
H. The cavity (CS) formed in the "streaming pathway" (S) of the syncytial tissue derived from the lateral intestinal tracts (I), at thirty hours after cutting. RB : regeneration blastema.

I. New pharynx formed in contact with the regeneration blastema (RB) at the posterior cut end, at twenty-four hours after cutting. A : new atrium, I : original intestinal tract, IB : old intestinal branch prepared to form the lateral intestinal tract in the postpharyngeal region, IC : primary cavity, PH : new pharynx.

J. Rejuvenated tissue (RA) and disturbed arrangement (D) of cells of the original atrial wall, at eight hours after cutting. PC : cavity constricted from the original atrial cavity.

K. Accumulation of the MR-cells (MR) for the pharyngeal primordium around the cavity (IC) of the atrial origin at twenty hours after cutting. OA : original atrium.

L. Lumen (LS) produced in the "streaming pathway" and extending from the place where the lateral intestinal tracts (I) fuse each other, at thirty-eight hours after cutting. A : new atrium, PL : canaliculus in the pharyngeal primordium (PH), IC : cavity of the atrial origin, OA : original atrium.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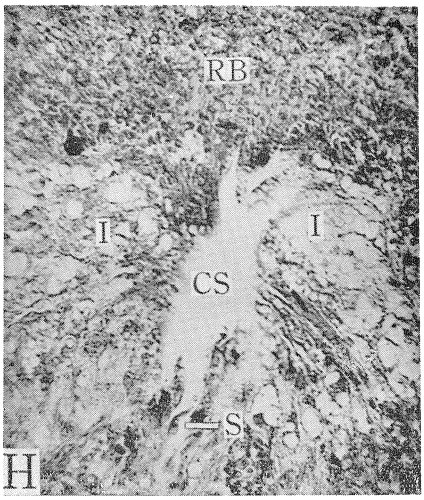
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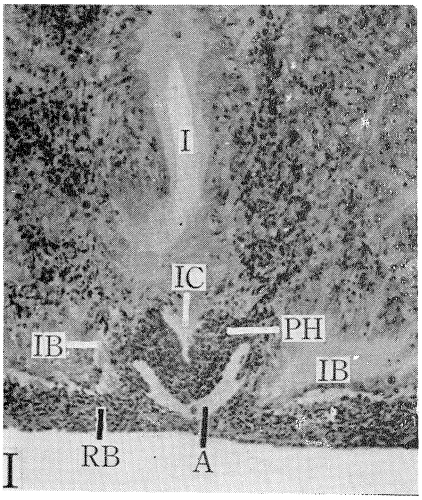
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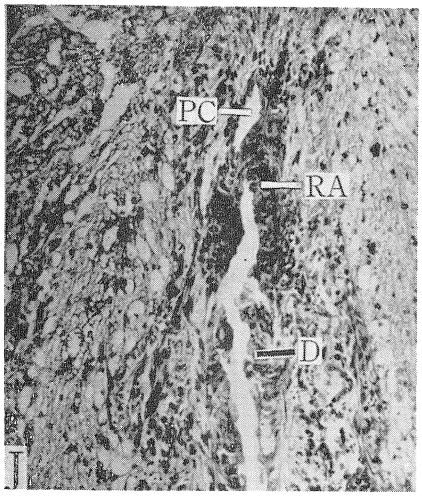
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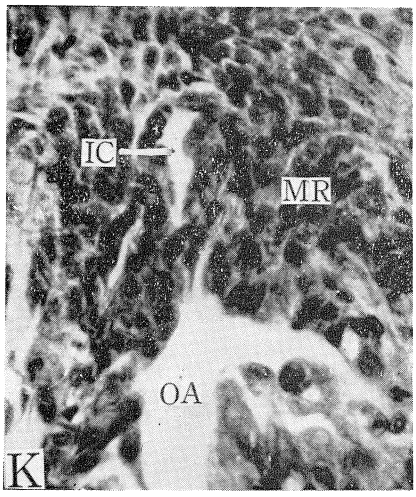
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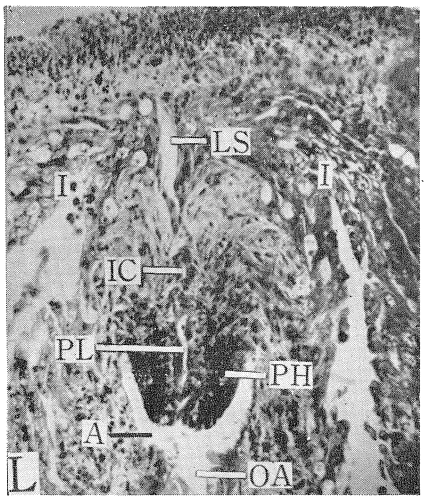
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