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

II. Histological Observation in the Abnormal Regenerates produced experimentally

By

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The information on the morphogenetic causality involved in planarian regeneration has been scarce except for the works of Child and his associates. Since these authors did not refer to histological investigation at all, the writer (1961) recently has carried out the histological study, and obtained the following results : (1) Regardless of the level from which the regenerate is taken, a new pharynx is always formed mainly from MR-cells that are derived from the mesenchymal cells and gather together at a certain site where a cavity is to be formed within a mass of cells of the intestinal origin, (2) the nerve contributes something to the establishment of this mass of the intestinal cells and to the migration of MR-cells, and (3) in addition to MR-cells, the epithelial, intestinal and nerve cells transform themselves into the regenerative cells after transection, and some of them participate in the formation of regeneration blastema and a new pharynx. But they seem to redifferentiate into their respective tissue according to their own origin.

These findings of the writer are, however, obtained from the normal process of regeneration occurring at the transected surface of the worm. In order to gain additional evidence for the findings, the following histological observation was performed on the new pharyngeal formation at the abnormal site where it was experimentally settled.

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Material and Method

Material used was asexual form of *Dugesia gonocephala* collected in the vicinity of Kanazawa City. Prior to operation the worms were starved for more than seven days. Choosing the worms 16–18mm long, they were anaesthetized in aqueous solution of chloreton (0.2–0.4%) and put on a glass plate which was previously seated with a piece of filter paper wetted with Murray's isotonic solution (Murray, 1931). Operation was carried out with a sharp scalpel for ophthalmic use under the binocular dissecting microscope.

In the first experiment, the pre- or postpharyngeal piece was cut into left and right halves along the median line of the worm, while in the second experiment the insertion method was adopted which had been first introduced to planarian operation by the writer (Kido, 1957) to see the behavior of nerve and other regenerative cells in the operated specimen. The procedure consisted of the following parts : 1) a fine slit was made at a given site on the dorsal epidermis of the anaesthetized worm, 2) from this opening a fine needle was inserted beneath the epidermis and moved gently in order to separate the epidermis from the underlying mesenchyme, and 3) into a pocket thus prepared a small piece taken from a certain level of another worm was introduced. After operation, according to Okada and Sugino's technique with a slight modification (Okada and Sugino, 1937), the worm was coated with a piece of thin and porous paper and tapped gently along its outline by a nib of scalpel. Next, the specimen together with an underlying piece of filter paper was transferred to a watch glass which in turn was placed in moist chamber with Murray's solution mixed with penicillin and mycillin. These glasses were kept in darkness and at low temperature ranging from 7 to 10°C for 30 hours. After these treatments, the worms were cultured by ten to each Petri dish 10cm in diameter and 2cm high. Temperature in the room was regulated to maintain 20–30°C throughout the experiment.

Histological preparation was made by the ordinary way of paraffin sectioning, and stained with Heidenhain's or Delafield's haematoxylin with counterstaining of eosin. Sometimes Mallory's triple staining was also applied. Prior to histological preparation, the external observation was made on the worms treated with 1 per cent solution of formic acid in order to make them transparent.

Observations and Results

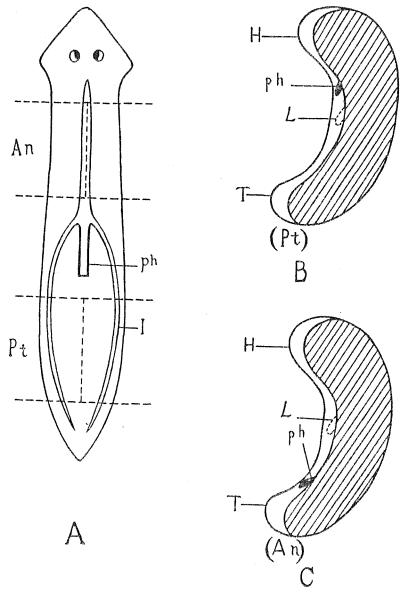
Experiment 1. *Regeneration of the longitudinally halved piece*

Since the regeneration of the previous, transected piece takes place at the narrow anterior or posterior cut-surface, the feature of the wound area is rather intricate due to various regenerative components. This complexity will be relieved in the present regenerate having a long, longitudinal cut-surface. In addition, the pharyngeal formation in the

postpharyngeal piece which has one, not two, lateral intestinal tract is to be of special interest by reason of Bardeen's statement that the fusion of two lateral intestinal tracts is indispensable factor for the pharyngeal formation in the postpharyngeal piece (Bardeeen, 1903), although the writer has pointed out that such a fusion is one factor but not always requisite for the pharyngeal formation (Kido, 1961). These are the reason why the writer extends his study to the regeneration of the longitudinally halved piece.

a). *Regeneration of longitudinally halved piece taken from the postpharyngeal region*

The worm was first cut transversely at the level slightly posterior to the distal end of the pharynx and a rear piece thus made was then cut off from a tip of tail. Finally it was divided along the median line into left and right halves (Pt in Text-Fig. 1, A).



Text-Fig. 1, A. Scheme, showing the places from which the longitudinally halved pieces were taken. An : prepharyngeal, halved piece, I : intestinal tract, ph : pharynx, Pt : postpharyngeal halved piece. B and C : regeneration of the longitudinally halved piece. B : postpharyngeal piece (Pt), C : prepharyngeal piece (An). H : new tissues of head, L : light colourless portion, ph : new pharynx, T : new tissues of tail.

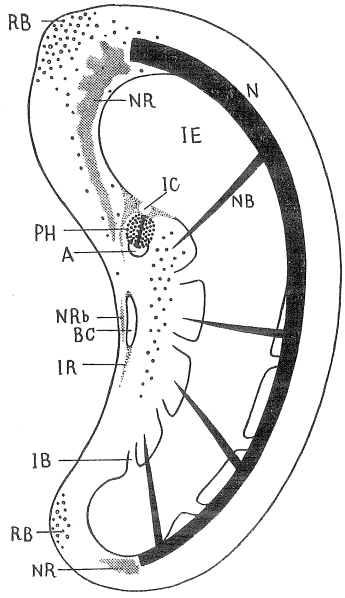
Immediately after cutting, the piece bent towards the longitudinal cut-surface (Text-Fig. 1, B). At three or four days after cutting, a little amount of new tissue was found at the anterior and posterior cut-surfaces, but not yet at the longitudinal cut-surface. One or two days later, a light and colourless portion appeared first at the approximate middle of the longitudinal cut-surface, i. e., near the bottom of the concave surface. Thereafter, the new tissue at the anterior cut end of the piece extended posteriorly along the longitudinal cut-surface, while the new tissue at the posterior cut end of the piece extended scarcely towards the anterior direction. At any rate the new tissue also appeared later along the whole length of the longitudinal cut-surface. The regeneration was less speedy at the longitudinal cut-surface than at the anterior and posterior cut ends.

The new pharynx began to be visible on the 7th or the 8th day after cutting.

As is shown in Text-Fig. 1, B, the pharyngeal formation was initiated at a site anterior to the concave bottom of the longitudinal cut-surface where the light and colourless portion had appeared and at the border between the old and new tissues (ph in Text-Fig.

1, B). The distance from the anterior cut end of the piece to the new pharynx was nearly equal to that in the piece to which only a transection was applied, although the accurate measurement was not made. These situations of pharyngeal formation seem to indicate that the internal conditions for the determination of the initial site of the pharyngeal formation had been already established prior to its external appearance. The histological investigation will elucidate this internal conditions.

Immediately after operation, a lateral intestinal tract and nerve cord were of course curved parallel to the curvature of the whole regenerate, and eventually the intestinal and nerve branches tended to point to the middle of the longitudinal cut-surface (Text-Fig. 2).



Text-Fig. 2. Scheme, showing the pharyngeal formation in the postpharyngeal, longitudinally halved piece. A : new atrium, BC : intestinal primordium formed first near the middle of the longitudinal cut surface, IB : original intestinal branch, IC : primary cavity formed in regenerating intestinal tissue, IE : enlarged part of the lateral intestinal tract, IR : intestinal regeneration from the intestinal primordium, N : original nerve cord, NB : original nerve branch, NR : syncytial nervous tissue, NRb : a mass of regenerating nervous tissue, PH : pharyngeal primordium. RB : regeneration blastema.

At four hours after cutting, a mass of syncytial nervous tissue appeared in front of the cut end of the nerve cord, although continuity between them was obscure. The nuclei in this tissue were round in shape and hardly stainable with haematoxylin, as has already been pointed out by the writer (Kido, 1961), whereas the nuclei of the original nerve cells were smaller and of spindle or elliptical shape, as described by Castle (1920). Soon later, the anterior and posterior parts of the lateral intestinal tract became enlarged, especially, in the anterior part (IE in Text-Fig. 2, IE in Fig. A on Plate I). Cell-arrangement of the inner layer of these enlarged parts of intestine became irregular, and some cells were even destroyed sometimes.

At twelve hours after cutting, the new epidermis spread already over the anterior and posterior wound surfaces, but not yet over the longitudinal cut-surface. There was a small accumulation of MR-cells under the new epidermis of each anterior and posterior cut end of the piece. These MR-cells were also found scattered near the cut ends of the nerve cord and along the nerve branches which were pointed to the middle of the longitudinal

cut-surface. From an aforesaid nervous syncytium fine processes sprouted out and infiltrated into the anterior accumulation of MR-cells, while the fine processes from the posterior cut end of the nerve cord began to extend towards the posterior cut-surface of the piece (NR in Text-Fig. 2). At the same time there occurred a small mass of syncytial tissue derived from the end of both enlarged parts of intestine.

At twenty-four or thirty hours after cutting, the longitudinal cut-surface was covered by the new epidermis coming mainly from the anterior cut end of the piece, leaving only a small naked wound near the posterior cut end.

When the infiltration of the syncytial nervous tissue came to a level of the posterior end of the anterior enlarged part of the lateral intestinal tract, the nervous growth ceased temporarily. Numerous MR-cells were found along this extending nervous tissue. On the other hand, the syncytial tissue of intestinal origin which had appeared at the anterior end of the enlarged intestine grew also posteriorly along this enlarged part. There occurred such syncytial tissues of nervous and intestinal origin in relation to the posterior enlarged intestine but they were less marked, as is seen in Text-Fig. 2.

At two or three days after cutting, the longitudinal cut-surface was already covered completely by the new epidermis, and the regenerative nervous tissue extended farther posteriorly, and the MR-cells migrated also farther, keeping the pace with the nervous extention under the new epidermis covering the longitudinal cut-surface. Nerve fibres became first visible in the syncytial nervous tissue (Fig. B on Plate I). The syncytial, intestinal tissue projected from its posterior end in the posterior direction, as if it was a branch of the original lateral intestinal tract (Text-Fig. 2), and a small cavity occurred in it. This cavity had already a connection with the enlarged intestinal lumen (IC in Text-Fig. 2). The cavity is a primary cavity according to the writer's terminology. The MR-cells accumulated surrounding the posterior part of the primary cavity and grew into the pharyngeal primordium (PH in Text-Fig. 2, PH in Fig. D on Plate I). They had been coming from all surroundings along the old nerve branches which ran towards the primary cavity. The blastema formation at the longitudinal cut-surface seemed to get contribution of MR-cells mainly from the anterior part of the piece. On the other hand, slightly prior to the pharyngeal appearance, there appeared a remarkable feature, that is, the appearance of another syncytial tissue of the intestinal origin as a rudiment of the new lateral intestinal tract (BC in Text-Fig. 2, BC in Fig. C on Plate I). Slender nerve fibres were also found closely near this new intestinal rudiment. They were derived presumably from the cut end of the original nerve branches concentrated there.

Thereafter, the tissue strand coming from the primary cavity-wall and passing through the median part of the pharyngeal primordium produced a canaliculus within it and another cavity immediately behind the pharyngeal primordium. This cavity is an atrial primordium. Therefore, the sequence of the pharyngeal development is similar to that already described in the previous paper (Kido, 1961).

The new intestinal primordium and the nerve fibres which had appeared near the concave bottom of the longitudinal cut-surface extended later posteriorly but not anteriorly.

The nervous connection between these fibres and the aforesaid extention of the nerve infiltrated into the area beneath the longitudinal cut-surface was established by the farther extention of the latter. Thus, the nerve cord which had been previously lost by cutting was restored. A part of the syncytial tissue of the intestinal origin which had projected from the posterior end of the anterior enlarged part of the original lateral intestinal tract extended to the area between the longitudinal cut-surface and the pharyngeal primordium, and eventually fused with the anterior end of the newly restored lateral intestinal tract mentioned above (BC in Text-Fig. 2). Thus, the lateral intestinal tract lost by cutting was completely restored.

The nervous tissue under the anterior cut-surface bending towards the side became more distinct, thick and ramified, fusing with the anterior cut end of the original nerve cord. Later it developed a horseshoe-shaped cephalic ganglion in closely front of the anterior enlarged intestine.

Syncytial regenerative tissues which arose from both posterior ends of the posterior enlarged part of the lateral intestinal tract and of the nerve cord were smaller in amount than those from the anterior ends. These regenerative tissues extended towards the posterior blastema. It is sure that they will, in due course, establish the intestine and nerve in that part respectively. The posterior blastema was first smaller than the anterior one, but grew rapidly in size after the pharyngeal formation was completed.

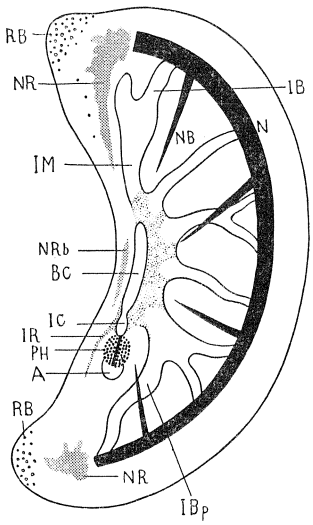
b) *Regeneration of longitudinally halved piece taken from the prepharyngeal region*

As is shown in Text-Fig. 1, a decapitated prepharyngeal piece was first transected at the level immediately anterior to the pharynx and then divided into right and left halves.

Curving of the piece, sequence of the regeneration at the anterior and posterior cut-surfaces and the first appearance of the light colourless portion in the approximate middle of the longitudinal cut-surface were almost the same as in the case of the similar halved piece taken from the postpharyngeal region, despite of the 2–3 days delay in the appearance of the new tissue. The new pharynx appeared at the site anterior to the posterior cut end of the piece on the border line between the old tissue and the new one regenerated along the longitudinal cut-surface. Precisely speaking, it was posterior to the portion in which the light colourless spot was first noticed from outside C in Text-Fig. 1).

A remarkable feature revealed by the histological observation was that a median intestinal tract was divided into fragments of various sizes by longitudinal cutting. At four hours after cutting, several syncytial tissues derived from these intestinal fragments were found here and there as small masses. Since reconstitution of the fragmented intestine occurred rather slowly, complete rearrangement of the intestine was not yet found at twenty-four hours after cutting. On the other hand, the epithelial and nervous regeneration as well as blastema formation at the anterior and posterior cut ends of the piece, proceeded steadily as in the case of the postpharyngeal halved piece.

At two or three days after cutting, a rudiment of intestinal lumen, from which a main part of the median intestinal tract develops later, appeared near the middle portion of the longitudinal cut-surface (Fig. E on Plate I). The new epidermis did not cover complete-



Text-Fig. 3. Scheme, showing the pharyngeal formation in the prepharyngeal, longitudinally halved piece. IBp : lateral intestinal tract formed by a remoulding of the original branch, IM : median intestinal tract restored by a transformation of the original branch. Other abbreviations are the same as in Text-Fig. 2.

ly the longitudinal cut-surface. The MR-cells were not found along this surface, but they were scattered, though a few in number, in the area between the rudimentary, intestinal lumen and the original nerve cord. However, it was obscure whether or not they migrated along the nerve branches as in the case of the postpharyngeal halved piece, because the intestinal arrangement was so disturbed by cutting that it was difficult to trace the running of the nerve branches from the original nerve cord. As was mentioned in the postpharyngeal halved piece, the nervous tissue and MR-cells were not found to infiltrate into the area of the longitudinal cut, unless the new epidermis could not cover the cut-surface.

At four or five days after cutting, the new epidermis had already covered the longitudinal cut-surface and the regenerative nervous tissue and MR-cells infiltrated beneath the new epidermis. The intestinal lumen developed distinctly along the longitudinal cut-surface. It is obvious that this state of the intestine lets this part of the body be light and colourless in the external appearance. At any rate the intestinal syncytium ran radially from the wall of this lumen towards the original lateral surface to meet the original intestinal branches (Text-Fig. 3).

The syncytial intestinal tissue projected also posteriorly from the posterior wall of the lumen along the longitudinal cut-surface. The lumen invaded in this posterior projection was the primary cavity.

Simultaneously, the MR-cells accumulated immediately behind the primary cavity as a pharyngeal primordium. The subsequent development of the pharyngeal part was the same as in the postpharyngeal halved piece (Text-Fig. 3).

When the new pharynx was formed, as is seen in Text-Fig. 3 and Fig. F on Plate I, the median intestinal tract was also formed by intervention of the syncytial tissue between the anterior old branch and the posterior intestinal lumen developed along the longitudinal cut-surface (IM and BC in Text-Fig. 3, IM and BC in Fig. F on Plate I). One of the lateral intestinal tract was formed by a remoulding of the original branch running posteriorly from the original intestinal tract (IBp in Text-Fig. 3, IBp in Fig. F on Plate I) and the other lateral intestinal tract of the opposite side was a new formation from the syncytial tissue of intestine which extended into the area between the new pharynx and the longitudinal cut-surface (IR in Text-Fig. 3, IR in Fig. F on Plate I).

Consideration

It has been revealed from the present observation that the time required for the pharyngeal formation in the longitudinally halved piece is always longer than that in the transected piece. This fact may be related to the delayed covering of epidermis over the longitudinal cut-surface and severer destruction of the nerve and intestine in the area faced to this cut-surface.

In the postpharyngeal halved piece, the posterior projection of the syncytial tissue regenerated from the anterior enlarged part of the original lateral intestinal tract behaves itself like that produced from both the anterior cut-surfaces of the lateral intestinal tracts in the transected piece, i. e., the primary cavity is first produced in it, and then the MR-cells accumulate behind the cavity to form a pharyngeal primordium. In the prepharyngeal halved piece, the median intestinal tract which was fragmented by cutting is first repaired from a point near the middle of the longitudinal cut-surface. From this median intestinal tract the intestinal syncytium extends posteriorly, and later a slit-like lumen from a median intestinal tract invades into this tissue as the primary cavity. The new pharynx is formed behind this primary cavity from the MR-cells accumulated there.

In these pieces the primary cavity is also indispensable for the pharyngeal formation, so that the retarded formation of the primary cavity can cause obviously the delayed formation of pharynx.

Whenever the regeneration is commenced from the cut end of the original median or lateral intestinal tract, the syncytial tissue is produced and the primary cavity generally develops in it. But, when the nervous stimulation seems not to be so powerful as to induce the large intestinal syncytium from the intestinal cut end, the primary cavity can not be produced in such tissue, even if the regeneration initiates from the end of the lateral or median intestinal tract. When the primary cavity is absent, the MR-cells are small in number. This is the reason why the pharyngeal formation cannot occur in relation to the tissue regenerated from the posterior cut end of the original lateral intestinal tract in the postpharyngeal halved piece. In other words, the regenerative condition in this part is not sufficient enough to form a pharyngeal primordium.

The cut ends of the nerve branches pointing to the middle of the longitudinal cut-surface are considered to contribute to the formation of the rudiments of the new lateral and median intestinal tracts. Moreover, the MR-cells to form a new pharynx also come together along the nerve branches and the new nervous tissue regenerated from the anterior cut end of the nerve cord.

To sum up, the pharyngeal formation in the longitudinally halved piece taks place in the same way as in the transected piece.

Experiment II. *Implantation of the tissue fragment beneath the dorsal epidermis*

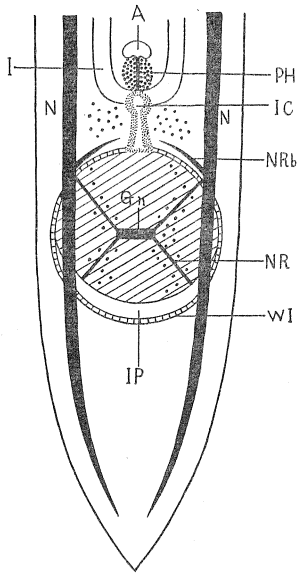
It is established fact that a piece from the cephalic or postcephalic region can induce the pharynx, when it is transplanted into the postpharyngeal region (Santos, 1929, 1931 ;

Steinmann, 1936 ; Okada and Sugino, 1934, 1937 ; Miller, 1938 ; Okada and Kido, 1943). In this induction the important role of nervous tissue was insisted by the writer on the basis of his experimental results that the postcephalic piece with nerve cords was shown to be a more powerful inductor for the pharyngeal formation than the piece without nerve cords (Kido, 1953). Moreover, previous histological observation has revealed that the nervous element was indispensable for the pharyngeal formation (Kido, 1961). But the test of various killed tissues of planarian was unsuccessful in inducing the pharyngeal formation (Kido, 1957). These informations seem to suggest that the factor(s) involved in the pharyngeal induction may be somewhat different from that of the embryonic induction in Amphibia, in which various killed tissues are proved to be quite effective.

So far, we have had no histological information about the pharyngeal formation induced by the transplanted tissue. Therefore, the histological investigation was performed in order to elucidate the mechanism of the induction of pharynx. But the routine method of transplanting the piece into desired place is unsuitable for this purpose, because the donor tissues come to contact too quickly with the host tissues to allow us to study closer relationship between them. In the present experiments, therefore, the insertion method devised by the writer was adopted.

a) *Insertion of the cephalic piece into the postpharyngeal region*

At two days after operation, the pocket made for insertion was communicated with main intestinal lumina, and therefore, the insert was surrounded mainly by the intestinal cells of the host. The insert lost its original histological organization : the epidermis was broken into separate pieces, a cephalic ganglion began to elongate and the mesenchymal cells became motile, free and often in cotact with dorsal or ventral side of the host. But no intestinal cells of insert-origin was found in the piece perhaps due to absorption in or assimilation with a host intestinal tissue.



Text-Fig. 4. Scheme, showing the pharyngeal formation at the anterior side of the insert and the mode of nerve extension from the implanted cephalic ganglion in the postpharyngeal region. Gn : cephalic ganglion, IP : insertion-pocket, NR: new nerve branch extending from the cephalic ganglion to the host tissue, NRb : new nerve branch arising from the junction point of nerves of host and insert, WI : wall of insertion-pocket. Other abbreviations are the same as in Text-Figs. 2 and 3.

At five days after operation, from a band-like cephalic ganglion a few stripes extended to the ventral side of the host, along the band and stripes the MR-cells were disposed (Fig. G on Plate II). Text-Fig. 4 shows schematically the topographical relationship among the nerve-extention, pharyngeal formation and intestinal regeneration in the specimen in which one pharynx appeared. The extending nerves reach eventually to the host tissue and communicate with the nerve cords of the host. From the position near this communication, other nerves sproute out and extend medially along the outer wall of the insertion-pocket, although the extents of the nerves are not necessarily the same (NRb in Text-Fig. 4, NRb in Fig. H on Plate II). Syncytial intestinal tissue regenerated from the median wall of the insertion-pocket extends anteriorly into an injured portion between the two lateral intestinal tracts. This syncytial tissue makes a bottle-like mass. A primary cavity appears within this mass of the syncytial tissue. The MR-cells are being accumulated behind this cavity as a pharyngeal primordium (PH in Text-Fig. 4, PH in Fig. H on Plate II).

Table 1
Results of insertion of various pieces into postpharyngeal region of the body

Implanted pieces	Number of implants survived			Total
	implants absorbed	Pharynx developed	Pharynx undeveloped	
Cephalic	6	5[1]	13	24
Postcephalic	3	1[2]	3	7
Postpharyngeal	6	0	4	10

1) In one case two new pharynges appeared each at the anterior and posterior sides of the insertion-pocket.
2) Rudimental appearance.

However, as was shown in Table 1, the new pharynx was only formed in 5 cases, in one of which the new pharynx was formed at both anterior and posterior sides of the insert. Histological examination reveals that the contact of the insert with the host was important of the pharyngeal formation. When the contact was incomplete and the extending nerve was short, no pharynx appeared (Fig. I on Plate II), to say nothing of the case in which the insertion-pocket was too large for the insert to come in contact with host tissue.

As to the origin of the MR-cells, nothing can be said from the present experiment. But both the insert and host will furnish these cells.

The posterior wall of the primary cavity projected as a narrow tubule and passed through the median part of the pharyngeal primordium. The caudal end of this tubule developed into another cavity immediately behind the pharyngeal primordium. This cavity gives rise later to an atrium.

At ten or fourteen days after operation, the insert which had lost its histological organization indicated no sign of the re-establishment of its original structure ; i. e., the original epidermis became ring-like mass that was surrounded by mesenchymal cells and the MR-cells migrated out into the host tissue through the part fused with the host tissue. The wall of insertion-pocket was lined with the cells of intestinal origin and took a feature of intestine, which was associated with the primary cavity. MR-cells increased in number in the area between the insert and the new pharynx. The area may be equivalent to the new tissue in the previous experiment of Okada and Sugino (1934, 1937).

b) *Insertion of the postcephalic piece into the postpharyngeal region*

The insert remained unabsorbed but disintegrated in 4 out of 7 cases, and this disintegration was followed by the extention of new nerve and appearance of the MR-cells, although these phenomena were less conspicuous than in the case of previous series. In one out of above 4 cases a new rudimental pharynx was formed. This specimen was very successful case due to well-contact of the insert and host which was necessary for the extention of the original nerves from insert to host (Fig. J on Plate II). Remaining three cases failed to develop a new pharynx apparently due to the lack of such intimate contact between the insert and host.

c) *Insertion of the postpharyngeal piece into the postpharyngeal region*

As is shown in the lowest row of Table 1, the inserts were very often absorbed. When they were left unabsorbed, disintegration occurred scarcely, and a few MR-cells appeared only in a part where the insert continued with the host tissues. The slight increase occurred in the size of the graft and its nerve tended to extend to the host tissue (Fig. K on Plate II). In no case, however, the pharyngeal formation was found in this series.

Consideration

The observations of the present series indicate that there exist many similarities in the sequence of pharyngeal formation between the case in which the formation is induced by the insertion of a cephalic or postcephalic piece in the postpharyngeal region and the case of the ordinarily transected piece. Namely, 1) syncytial intestinal tissue derived from the wall of the insertion-pocket, the cells of which is of intestinal origin, makes a bridge between the destroyed parts of the intestinal tracts of the host, and a primary cavity appears within this bridge, 2) the pharyngeal primordium arises from the accumulation of the MR-cells surrounding the projected part of this cavity, 3) numerous MR-cells are also found along the nerve extending to the cavity from the nerve cords.

The first point to be discussed is that why the syncytial tissue derived from the wall of the insertion-pocket can be utilized as a fusion of the destroyed parts of the lateral intestinal tracts. According to the writer's previous view (Kido, 1961) derived from the observation of the transected piece, fusion of the cut ends of two nerve cords is the

initial step which, in turn, stimulates the fusion of the cut ends of the lateral intestinal tracts. In the present specimens, however, the ventral nerve cords of host could not be cut at the time when the insertion-pocket was produced. Therefore, the fusion of the nerve cords was unable to occur so easily as in the transected piece. This phenomenon was substituted by the extention of new nerves sprouted out from the point where the nerve of graft had come in contact with the host's old nerve cords. These new nerve branches extended along the wall of the insertion-pocket. These branches may cause the formation of the syncytial tissue from the wall of the insertion-pocket. The wall is previously lined with the intestinal cells, so that the syncytial tissue coming from it is also intestinal in nature. This may be the reason why the lateral intestinal tracts are connected by this syncytial tissue.

The present experiment indicates that the cephalic ganglion has the greatest innervating ability and the postcephalic nerve cord comes next in this phenomenon. In transplantation experiment (Okada and Sugino, 1934, 1937 and Okada and Kido, 1943) a graft taken from prepharyngeal region can promote the pharyngeal formation in the postpharyngeal region, but the graft from the postpharyngeal region can not. According to Child's view, such difference of morphogenetic activity is due to the difference of the physiological gradient between the two grafted pieces. The gradient, if we use this terminology, is clearly manifested in the phenomenon of innervating ability in the present experiment.

In short, the processes of the pharyngeal formation brought about by the implantation of the cephalic or postcephalic piece are essentially the same as those in the ordinary, transected piece. In both cases, the nervous extention ranks the first, the formation of the primary cavity in the regenerating intestinal tissue is the second and the migration of MR-cells behind this cavity is the third in the sequence of the pharyngeal formation.

Lender (1954) reported with *Polycelis* that in worms from which the cephalic ganglion had been previously removed the eyes were induced numerically more in a culture medium containing the worm-extract that in the medium without extract. Buchanan (1938) stated with *Euplanaria dorotocephala* that head extract was able to increase the head forming frequency in the regenerating piece. These results seem to show that devitalized substance or substances exert the effect as an inductor upon the eye- or the head-formation, but so far as the writer is aware, there is no information that such substance(s) can induce directly the new pharynx. The writer (Kido, 1957) reported in the implantation experiment of *Dugesia gonocephala* that the worm-extract and the killed tissues by such treatments as heat, decication and alcohol were all proved to be ineffective in inducing the pharyngeal formation. It is safe, therefore, to state that only living tissues are effective, so far as the pharyngeal formation is concerned. The situation may become clearer in the light of the present result that the migration of the MR-cells is prerequisite to the new pharyngeal formation and that this migration of cells is, in advance, brought about by some unknown factor emanating from the extending, live nerve. It will sometime become probable, however, that some devitalized substance(s) will be proved to be effective in inducing the migration and differentiation of these MR-cells.

Summary

The present experiments with *Dugesia gonocephala* were carried out to gain some additional evidence for the writer's view of the pharyngeal formation, presented in the previous paper. Firstly, the pharyngeal formation in the post- and prepharyngeal pieces that were halved longitudinally in addition to transection was respectively observed, and secondly, the same formation was examined in the case where the other piece was inserted under the epidermis of the postpharyngeal region. The following is the outcome of these observations.

1. It takes longer time for the initial formation of the new pharynx in the two kinds of the longitudinally halved pieces than in the normal piece cut only transversely. Syncytial tissue derived from the cut end of the lateral or reconstituted median intestinal tract is always formed at a certain site, toward which the cut ends of the nerves are converged or the regenerating nerve branches are extending most actively. When a primary cavity is formed in this syncytial tissue, accumulation of the MR-cells to develop the new pharynx occurs behind this cavity. The primary cavity is, therefore, prerequisite to the pharyngeal formation. Migration of the MR-cells towards the prospective site of new pharynx and the regeneration blastema is in intimate relation to the direction of nerve regeneration.

2. When a cephalic piece is inserted into a pocket produced under the dorsal epidermis in the postpharyngeal region, the insert loses frequently its original, histological organization ; the cephalic ganglion elongates into a stripe and communicates with the ventral nerve cords of both sides of the host. New nerves sprout out from the points of union of these nerves and extend medially along the wall of the insertion-pocket. Along these nerves many MR-cells migrate towards the portion between two lateral intestinal tracts which have been destroyed at the time of insertion. Syncytial tissue derived from the wall of the insertion-pocket produces a bridge connecting between the destroyed ends of the two lateral intestinal tracts of the host. Within this bridge a small primary cavity is established. Subsequently, MR-cells accumulate behind this cavity, and develop into a pharyngeal primordium. Immediately behind the pharyngeal primordium, another cavity is formed in the tissue which comes down through the primordium from the posterior wall of the primary cavity. This cavity develops later into an atrium.

3. When a postcephalic or a postpharyngeal piece is inserted into the postpharyngeal region, the nervous growth from the implanted cords is not so extensive as in the case of cephalic piece. These inserts frequently remain unchanged. Only in one of the former case a rudimental pharynx occurs, but none in the latter case.

4. To sum up, the sequence of the pharyngeal formation in the present experimental conditions of pieces is quite identical with that in the ordinary, transected piece.

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PLATE 1

Explanation of Figures.

Photomicrographs showing the regeneration of longitudinally halved piece of *Dugesia gonocephala*. A - D : postpharyngeal piece, E - F : prepharyngeal piece.

A. Holizontal section, showing the anterior enlarged part (IE) of the lateral intestinal tract, at fourty-eight hours after cutting. IR : initiation of the intestinal regeneration, MR : MR-cells derived from mesenchymal cells, N : original nerve cord.

B. New nerve fibres (NR) extending from the original nerve cord (N) to between the enlarged intestine and cut-surface, at fourty-eight hours eight cutting.

C. Holizontal section, showing the initial state of the intestinal primordium (BC) produced at an approximate middle of the longitudinal cut-surface. It is formed by restoration of the original intestinal tract damaged by the longitudinal cutting, at about fifty hours after cutting.

D. Holizontal section, showing the pharyngeal primordium (PH) and intestinal regeneration (IR) from the posterolateral side of the enlarged part of the lateral intestinal tract towards intestinal primordium (BC), at three days after cutting. A : new atrium, I : original intestinal tract, IC : primary cavity.

E. Holizontal section, showing the rudiment of the median intestinal tract restored from the damaged original tract, at fourty-eight hours after cutting. IB : original intestinal branches converging towards the middle of the longitudinal cut-surface.

F. Holizontal section, showing the new pharynx (PH) established immediately behind the lumen of the newly restored median intestinal tract (BC), at six days after cutting. A : new atrium, IB : original intestinal branch, IBp : new lateral intestinal tract reconstituted from the old branch of the original intestinal tract, IM : new median intestianl tract restored by means of transformation of the old branch of the original median intestinal tract, IR : lateral intestinal tract regenerated from the median intestinal tract (BC).

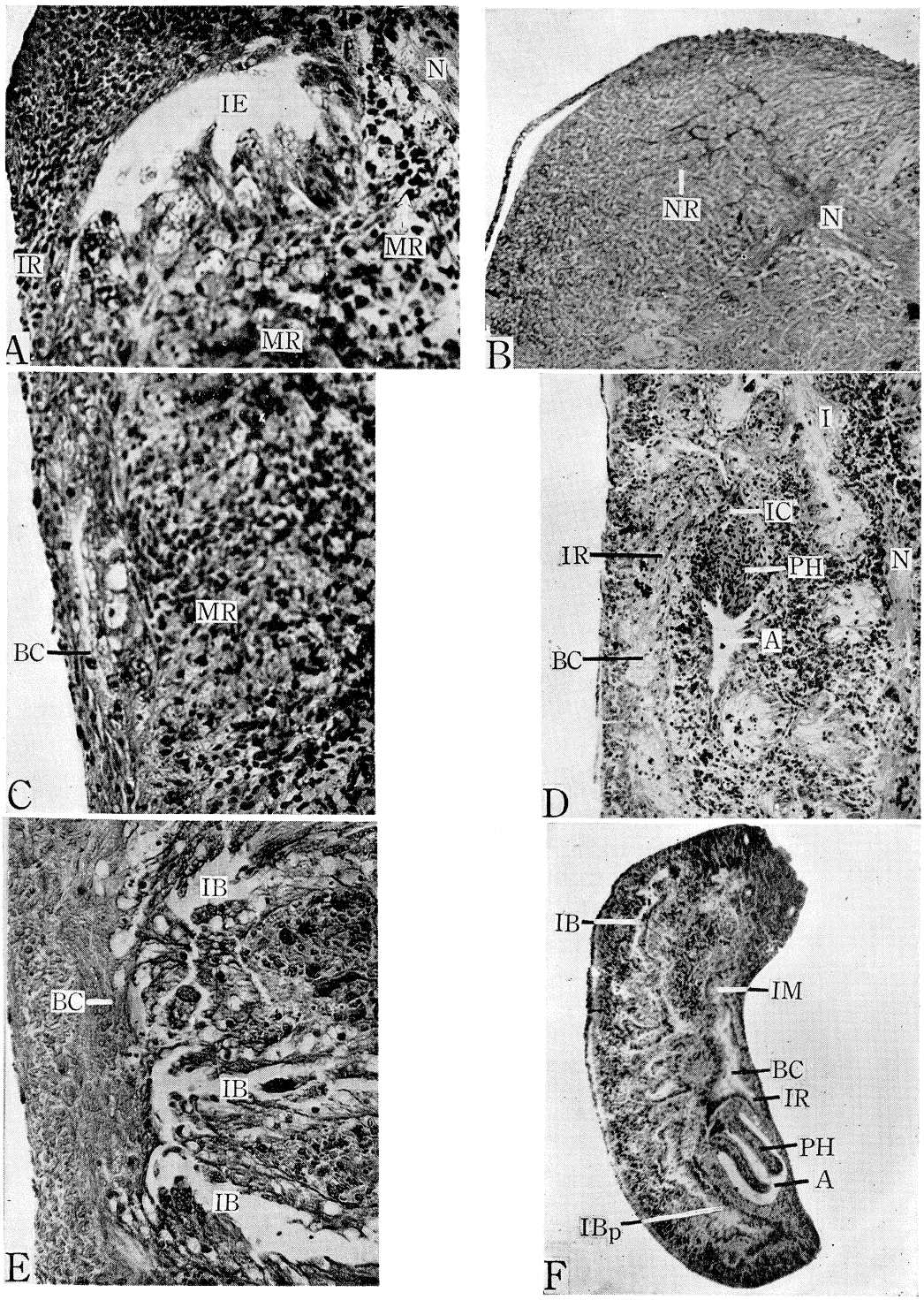
A
IE
N
MR
IR
MR
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PLATE II

Explanation of Figures

Explanation of photomicrographs of the specimens belonging to the insertion experiment. G-I : Insertion of cephalic piece, J : insertion of postcephalic piece, K : insertion of postpharyngeal piece.

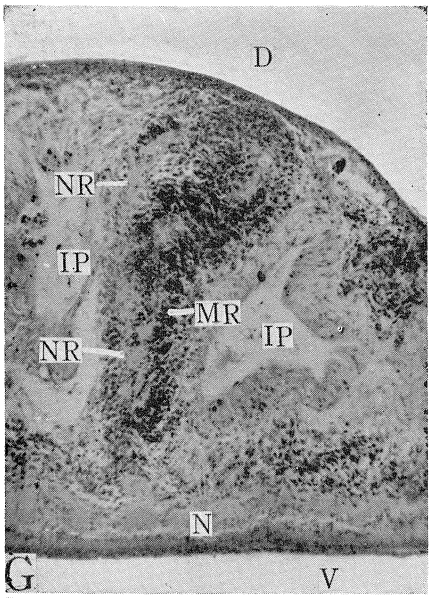
G. Sagittal section through the cephalic insert, showing the new branch (NR) of the cephalic ganglion extending to the ventral part of the host to fuse with the host nerve (N), at eight days after operation. D : dorsal side of the specimen, IP : insertion-pocket, MR : MR-cells, V : ventral side of the specimen. Cephaic ganglion cannot be seen in the picture.

H. Holizontal section, showing the new formation of pharynx at seven days after insertion. E : eye of the insert, I : lateral intestinal tract of the host, IC : primary cavity, NR : regenerating nerve branch of the cephalic ganglion extending to the host tissue, NRb : nerve branch extending medially along the outer wall of the insertion-pocket. PH : newly induced pharynx.

I. Sagittal section, showing no pharyngeal formation due to incomplete union of the tissues between the insert and the host, at ten days after insertion. G : cephalic ganglion of the insert.

J. Sagittal section, showing a rudimental new pharynx induced at the posterior side of the insert, at twelve days after insertion. EP : epidermis of the insert.

K : Sagittal section, showing a slight growth of insert and no practical extention of the nerve branches from the nerve cord (NR) of the insert, at nine days after insertion.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IP
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N
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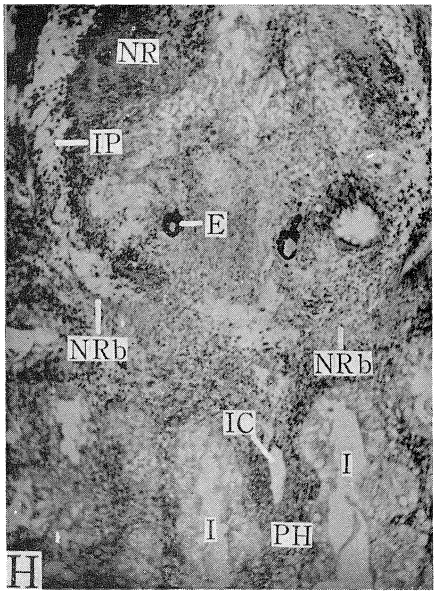
G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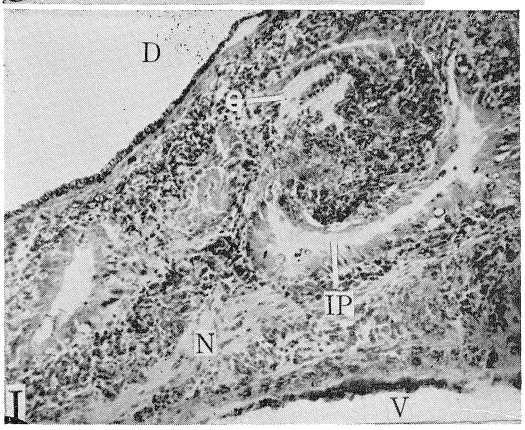
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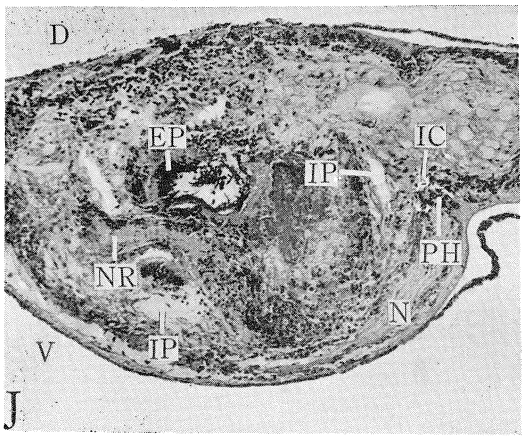
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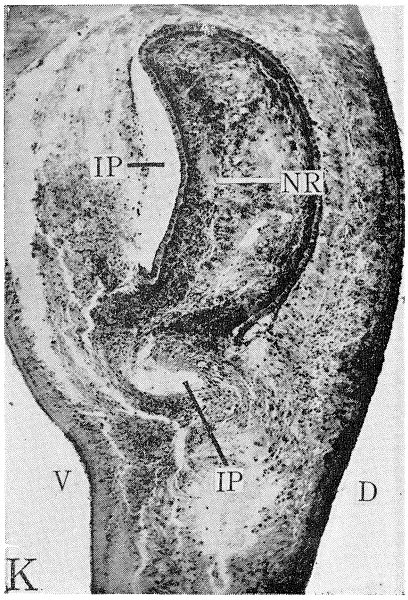
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