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Remarks on the so-called Induction of the Pharynx in Planaria

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When a piece removed from the ganglionic or postcephalic region of Planaria is transplanted into the postpharyngeal region, a new pharynx is formed at the level of anterior or posterior to the site of implantation. On the basis of this fact, many investigators are of the opinion that the grafted piece of Planaria acts as an organizer in inducing the pharynx (Santos, '29, '31 ; Steinmann, '33 ; Okada and Sugino, '34, '37; Miller, '38). According to the gradient theory of Child, however, formation of pharynx is ascribed to the change of gradient produced by transplanting the ganglionic piece into the host. In this line of thought, as is pointed out by Child ('41), it is a matter of question whether formation of planarian pharynx can be compared to the embryonic induction of amphibian neural tissue. Here, however, granted that formation of new pharynx is carried out, as suggested by Okada and Sugino ('37), Okada and Kido ('43), in the same manner as the embryonic induction, much has been left to be examined in order to elucidate that the grafted piece of Planaria acts just as the dorsal lip of the blastopore of amphibian embryo does. In amphibian embryo inducing action of the organizer is demonstrable even after it is devitalized. Therefore, it is a question to be solved, whether the killed tissue of Planaria is still capable of inducing new pharynx or not. The present study was performed to solve this problem.

Material and Method

As material, an asexual form of *Dugesia gonocephala* ranging from 16 to 18 mm in size was used. After about one week's starvation, the head pieces of the worms were cut out. They were then subjected to one of the following treatments.

1) Immersion in 96 per cent alcohol for 4 minutes with subsequent rinsing in distilled water for 2 hours.

2) Immersion in hot water of 60°C. for 15 minutes.

3) Desication at a temperature of 30°C.

4) About one hundred pieces were crushed with sand grains and filtered with distilled water. The filtrate was soaked with agar piece.

Each testing material thus prepared was cut into small square pieces ranging from 0.5 to 1.0 mm in size. Then they were inserted each into a pocket of the worm previously

made between the dorsal epidermis and underlying mesenchymal tissue at the postpharyngeal level. As a control experiment, a living head piece of equal size was inserted in the same manner. The experimental animals were fixed 10 to 14 days after the operation, serially sectioned and stained with Haematoxylin and eosin for microscopic observation.

Experimental Results

Implantation of the alcohol treated piece……In all 8 cases the inserted piece was completely absorbed by the host, and no formation of pharynx was observed. In one case at the dorsal and ventral sides of the operated area, slight elevation of the epidermis occurred. The elevation existed, as is shown Fig. I, during 14 days after the operation till the specimen was fixed. Macroscopical and microscopical observation of this specimen showed that the elevation was established with intestine-like tissue, but no new tissue was found to be produced. Presumably the elevation is due to direct damage of the operation.

Implantation of the heat treated piece……In all the operated specimens, complete absorption of the graft occurred without producing any morphological change in the operated area.

Implantation of the desicated piece……In one of 11 successful operations of this series, the grafted piece remained an amorphous mass of cell debris. In other two cases, epidermal elevation was found at the site where the graft was located. But in them, neither the grafted piece, nor the new tissue was found microscopically in the operated area. In the remaining cases, the graft was absorbed completely and no morphological change was found in the host. Consequently, no formation of the new pharynx was obtained in this series, too.

Implantation of the agar piece soaked with extract of the head piece……In 3 out of 6 available specimens the inserted piece was completely absorbed, but in the remaining 3 cases it was found in the inserted site. In the latter case slight elevation of epidermis occurred without, however, accompanying formation of new tissue. Thus, in the case of the present operation, all the specimens failed also to induce formation of new pharynx. The results of implantation of the killed pieces above described is arranged into a form of table.

Table.

Implant treated with	Available cases	Cases of ectodermal elevation	Cases where implant was absorbed	Cases where pharynx was formed
Alcohol	8	1	8	0
Heat	7	0	7	0
Desication	11	2	10	0
water (Extract)	6	3	3	0
Total	32	6	27	0
Implantation of living head piece	8	6	2	2

As is shown in the table, implantation of the piece killed in any way of planarian head always failed to produce the formation of new pharynx. A simple morphological change was elevation of the host epidermis which was brought about by the operation. The elevation contained no new tissue, though it was formed always in the area where the graft was inserted and mostly in those cases where the grafted piece persisted without being absorbed till the time of fixation.

Implantation of the living head piece……In 2 out of 8 cases the graft was completely absorbed without accompanying any morphological change. In the other cases insertion of the head piece always followed by dorsal elevation of the host epidermis. In 2 of these 6 cases formation of new pharynx actually took place (Fig. II A). In the 2 specimens dorsal elevation was marked after the operation, but it became a little reduced within the first 3 or 4 days, and afterwards it grew again conspicuous. Microscopical examination of these specimens revealed that the epidermal component of the grafted piece accumulated to form a large irregular mass, while the mesenchymal component scattered around the epidermal mass (Fig. II, B). As is shown in Fig II, B, the cerebral ganglionic cells of the graft elongated to connect with the nerve cord of the host. Along the elongated ganglionic cells were found a mass of cells provided with a large nucleus each. Undoubtedly, this mass of cells represents the new tissue already pointed out by Okada and Sugino ('37) and Okada and Kido ('43). In their experiments the same situation was always obtained in the transplantation of the head piece. On the other hand, in 6 cases of the present experiment in which new pharynx failed to appear, it was a rule that no connection between the cerebral ganglion of the graft and the nerve cord of the host was established, and subsequently that formation of new tissues was in conspicuous.

Consideration

In the present experiments above mentioned, new formation of the pharynx was not brought about by implanting the killed tissues of the planarian head. Recently, however, Lender ('56) pointed out that induction of eyes occurs if the worms were immersed in the medium containing planarian tissue extracts. According to this fact, he advocates a view that this is the same phenomenon as the embryonic induction in amphibian embryos, and further he assumes that induction of the pharynx may be possible when appropriate agents are used. If the assumption of Lender holds true, our failure of the pharyngeal induction may be referable to either one of the following possibilities ; 1) the inserted piece was absorbed too fast to exert its effect upon the host tissue ; 2) inductive effect from the inserted piece was interrupted by the intestine-like tissue formed in the surrounding of the graft ; 3) reacting potency of our planaria, *Dugesia gonocephala* is too weak to response the effect exerted from the inserted piece ; 4) effective substance of killed tissue became lost or ineffective by the operative procedures.

In the transplantation of the living head piece, it has been clearly established that the new tissue appeared between the grafted head piece and the old tissue of host in a majority

of cases. In such cases, however, the grafted tissue did not lie under the host tissue, so that the former was not in contact with the latter in the same sense as in the case of induction with the organizer of the amphibian embryo. Therefore, even if formation of new pharynx is realized through the effect of the grafted head piece, the mechanism operating in the case of Planaria may not be identical with that in amphibian embryo.

On the other hand, it has been demonstrated that the new formation of the planarian pharynx is intimately connected with the presence of the nervous tissue. As already pointed out by the present authour ('52) implant of a piece removed from the ventral side of planarian body and containing the nerve cord can induce formation of the new pharynx more frequently than that taken from the dorsal side and lacking nervous component. The similar phenomenon found also in the present experiments ; i. e., the elongation of cerebral ganglionic cells of the implant is necessary for the formation of the new tissue and accordingly for the formation of new pharynx. Under these situations, I am inclined to assume that the nervous component of the implanted piece plays the most important role in the formation of new pharynx of Planaria.

Summary

Implantation of the head pieces previously killed by alcohol, heat or desication was carried out into the postpharyngeal region of *Dugesia gonocephala* in order to test their faculty of new pharynx. In any case of the operations, neither pharyngeal induction nor formation of new tissue was found to occur. Water extract of the head piece could show nothing which has the inductive effect of the new pharynx. On the other hand, by implanting living head piece, pharyngeal induction is found to be always anticipated by the elongation of the ganglionic cells in the implanted piece. From these results, importance of the nervous tissue is assumed for the formation of pharynx.

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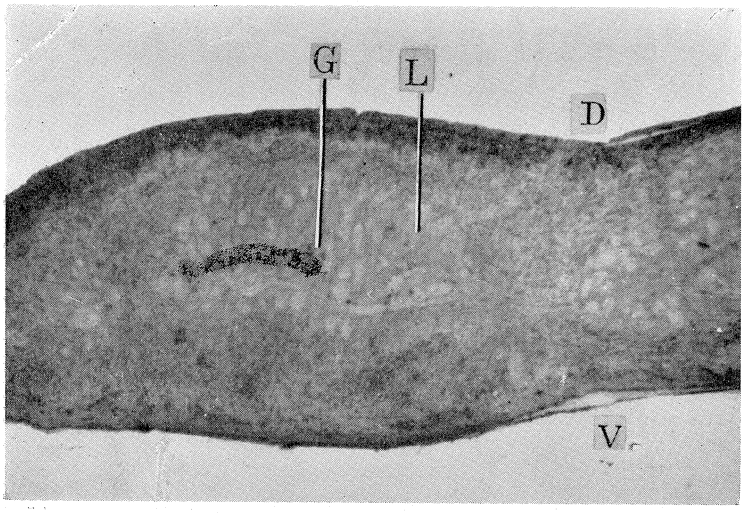
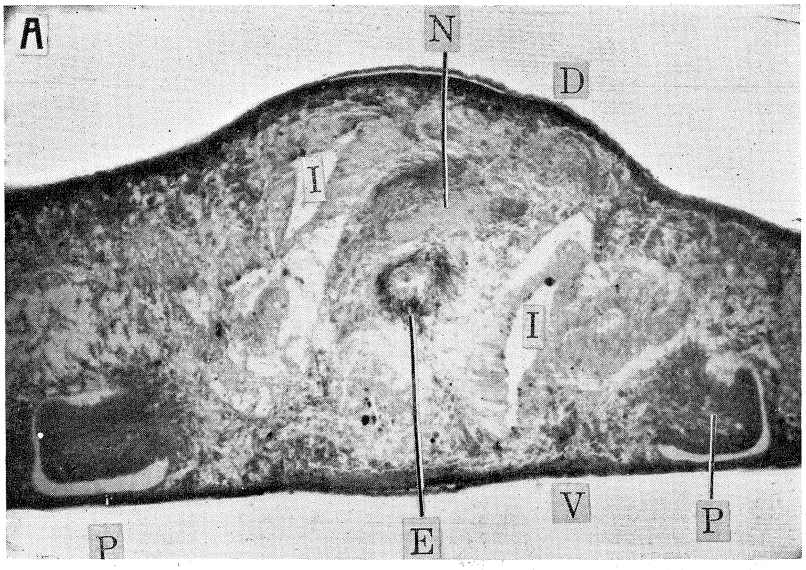


Fig. I. Photografic representation through the implant of killed tissues of head piece fixation at 14 days after operation.
D ; dorsal. G ; area of insertion. V ; ventral.
L ; intestine-like tissue.



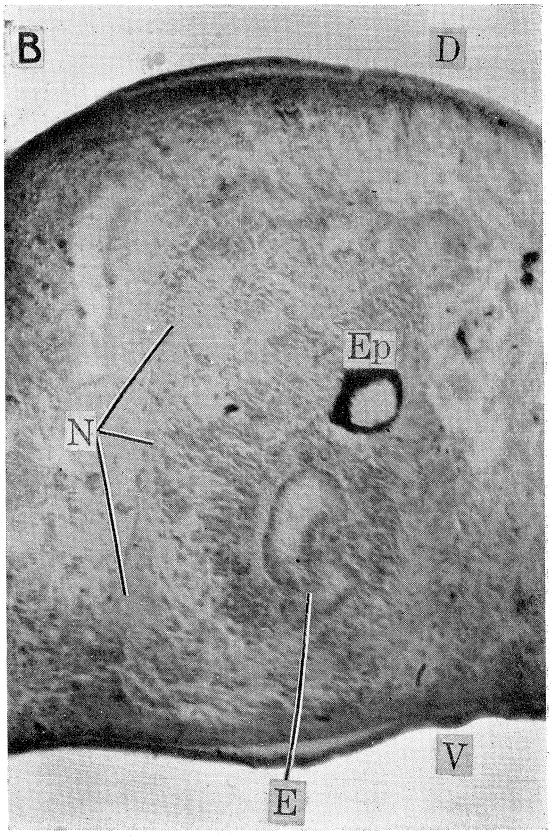


Fig. II. A ; formation of new pharynx induced by a living head piece fixed 14 days after the operation. B ; elongation of the inserted ganglion fixed 14 days after operation. E ; epidermis of the inserted head. Ep ; eye-pigment of the inserted head. I ; intestine of the host. N ; nerve of the graft. P ; pharynx newly formed.