

Experimental Studies on the Differentiation of  
the Endodermal Organs in Amphibia  
III. The Relation between the Differentiation of Pharynx  
and Head-Mesenchyme

By

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Experimental works so far carried out by previous investigators (Holtfreter, '31, '33a, '33b, Stableford, '48) concerning the differentiation of the endoderm in amphibian embryos have suggested commonly the precocious determination of the respective endodermal organ-areas. Holtfreter pointed out that a piece of presumptive endoderm of the early stage as blastula can self-differentiate according to its presumptive fate. In the explantation experiments of the present author, however, this was found to be not necessarily the case. As reported in our previous publications (Okada, '53, '54a, '54b), the explanted pieces of the presumptive endoderm taken from gastrula actually showed differentiation only when they were surrounded by the mesenchymal tissues. In the absence of these tissues the explanted endoderm always failed to differentiate. Apparently, therefore, presence of the mesenchymal tissues is considered to be indispensable for the differentiation of the endoderm. In order to search for further evidences to confirm our conclusion the present investigation was undertaken.

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**Material and Method**

The material used was embryos of *Triturus pyrrhogaster*. Explantation of a piece of endoderm was carried out by inserting it into a cranial piece of an embryo. At first, cranial part of the neurula at Okada & Ichikawa's st. 16 was cut at the level of anterior one third of the total length, and the endodermal components included within it were thoroughly removed. Next, into this endoderm-free cranial piece of neurula the endodermal piece to be tested was inserted. The inserted endoderm was taken from two different regions, i. e., (1) from the region corresponding to the antero-lateral and basal walls of fore-gut (this region will be designated as the *anterior endoderm* in the following description) and (2) from the

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region corresponding to the anterior part of the floor of mid-gut (designated as the *middle endoderm*). The developmental stages of the donor embryos from which the endodermal piece was taken were varied from late gastrula (st. 14) to late neurula (st. 20). To compare the experimental results, explantations of the cranial piece alone without inserting an endodermal piece and also of the endodermal piece wrapped by the epidermis of the belly of neurula were carried out. Cultivation of the explants was done in Holtfreter's solution for about four weeks.

### Experimental Results

#### (1) *Explantation of the endodermal piece wrapped by the epidermis*

From the outward appearance, the explants always formed the small shriveled masses consisting of the endodermal and epidermal components. In all cases of this series (in 58 cases), the explants, irrespective of the *anterior endoderm* and *middle one*, showed no sign of differentiation even after the cultivation of about four weeks (Fig. 1). They remained as the amorphous masses of undifferentiated endodermal cells designated as "yolk-mass" in the previous papers (cf. Okada, '53, '54a).

#### (2) *Explantation of the cranial piece of the neurula from which the endoderm was removed*

All the eight cranial pieces exhibited good development into a nearly normal head structure, although a large oedematous swelling was found in its ventro-posterior part. It was provided with eyes, balancers and mouth equipped with teeth and visceral cartilages. The differentiation of the neural tissues contained within the explant was perfect, the divisions of the brain being well developed. Beneath the hind-brain the anterior-most end of notochord was found. A large abundance of head-mesenchyme was also included. Only a difference of the present explants from the normal head was the complete absence of the endodermal tissues. Hence, the results indicate that after the removal of the presumptive endoderm from neurula, endodermal transformation from the mesodermal or ectodermal tissues never occurs in the course of the explantation.

#### (3) *Explantation of the anterior endoderm by inserting into the cranial part of a neurula*

Most of the present explants (18 out of 22 cases) were as well developed as the explants of the previous experiment did. In spite of the presence of the inserted piece of endoderm, the mesodermal and ectodermal tissues of the cranial part were formed quite normally. The inserted piece of endoderm was found at the site a little posterior to the mouth, always imbedded within the head-mesenchyme. Generally, under such conditions the endodermal piece performed the complete differentiation into a pharynx, which was very frequently equipped with the branchial pouches and visceral cartilages (Fig. 2, cf. Table). In most cases, the pharynx connected with the oral invagination of the cranial ectoderm.

Table, Endodermal tissues formed from the explanted endoderm by inserting into the cranial piece

Pieces explanted	Dev. stages of donors	No. of cases	Endodermal tissues found			
			Pharynx	Intestine	Liver	Yolk-mass
Anterior endoderm	14	8	6	0	2	3
	17	10	8	0	1	3
	20	4	4	0	0	0
	Total	22	18(82%)	0	3 (14%)	4 (18%)
Middle endoderm	14	18	12	1	1	5
	17	15	9	0	1	5
	20	21	7	2	2	13
	Total	54	28(52%)	3 (6%)	4 (7%)	23 (43%)

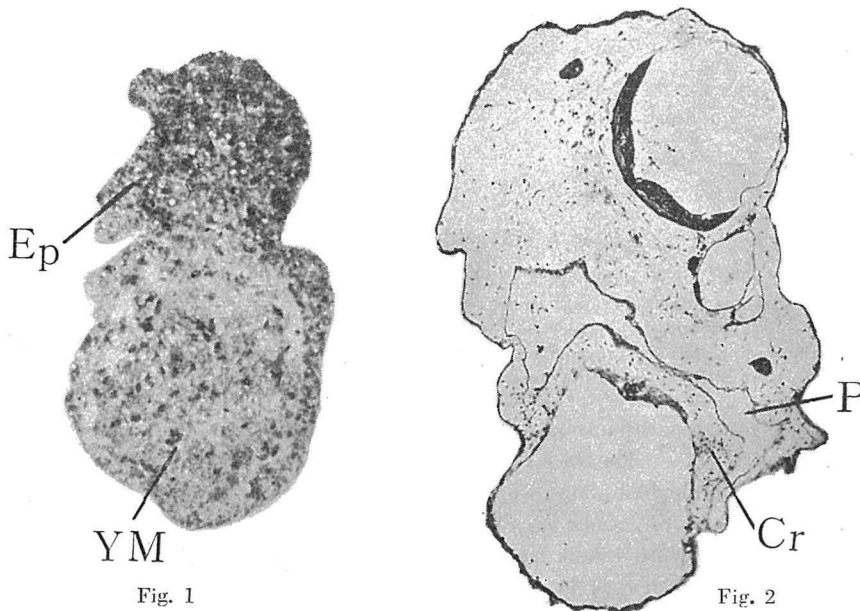


Fig. 1. Explantation of the *anterior endoderm* within the epidermal vesicle. The endodermal piece remained as "yolk-mass" (YM) without showing any sign of differentiation. Ep; atypical epidermis.

Fig. 2. Differentiation of pharynx (P) from the *anterior endoderm* which was explanted by inserting it into the cranial part. Cr; visceral cartilage.

But, even in three cases without mouth, the inserted endoderm differentiated towards pharynx. Accordingly, it seems apparent that the differentiation of pharynx can take place without any connection with the mouth. In the other three cases, the liver, adding to the pharynx, was encountered. The liver, if

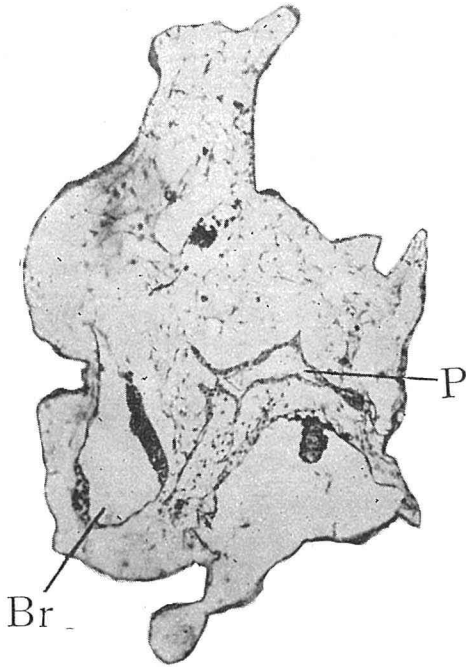


Fig. 3

Fig. 3. Differentiation of pharynx (P) from the *middle endoderm* which was explanted by inserting it into the cranial part. Br; brain.

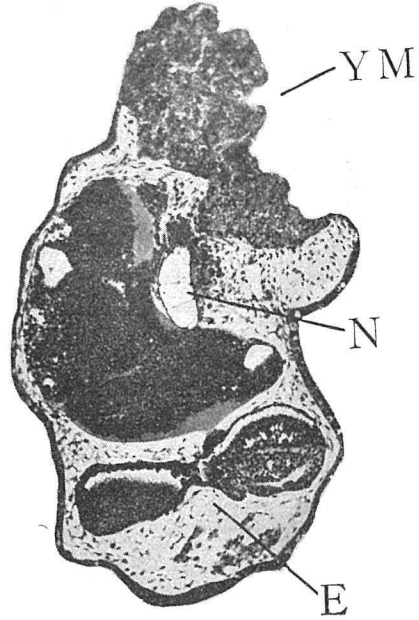


Fig. 4

Fig. 4. The *middle endoderm* inserted into the cranial part was protruded from the latter. The endoderm shows no sign of differentiation, remaining as "yolk-mass" (YM). E; synophthalmic eye, N; notochord.

present, was situated separately from the pharynx at the ventro-posterior site of the explanted head. The heart or pericardial tissue was formed near it. The same results as described above were obtained in most of the present experiments, notwithstanding the difference of the developmental stages at which the inserted endodermal pieces were taken from the embryo.

There were four cases in which the explanted cranial part failed to form the normal head structure. They were merely amorphous compact masses after the cultivation. In these cases, the mesenchymal tissue was hardly included within the explants, and the inserted piece of endoderm did not succeed in the differentiation towards any recognizable tissue.

(4) *Explantation of the middle endoderm by inserting into the cranial part of a neurula*

In nearly all the explants of this series (51 among 54 cases) normal head structure was established, with the exception of three cases where the explanted cranial part remained as an amorphous mass. The inserted piece of endoderm

succeeded in the differentiation into the endodermal tissues such as pharynx, intestine and liver. Of these endodermal tissues, pharynx was formed most frequently (Fig. 3, cf. Table). In comparison with the pharynx produced in the previous experiment, the tissue found here was more frequently in a state of an imperfect differentiation, retaining still numerous yolk-granules in cells, but it was provided with the branchial pouches and visceral cartilages. The pharynx, even under the explanted condition, was found to lie at the normal site ventral to the brain: and it was completely surrounded by the head-mesenchyme. In a few cases the inserted endoderm was found coated by the mesodermal tissue other than mesenchyme, such as the pericardial or mesenterial tissue. Such endoderm resulted in the production of intestine or liver. There were some other specimens in which the inserted endoderm was not in any mesodermal surroundings, but in contact directly with the epidermis. In extreme cases, it was separated from the ectodermal envelope of the cranial piece (Fig. 4). In these abnormal locations, the explanted endoderm showed no sign of differentiation, remaining as "yolk-mass" in the author's terminology. As shown in the Table, such a failure of differentiation was mostly found in the experiments where the explanted endoderm was taken from late neurula.

### Discussion

In the present experiments, the endodermal pieces were taken from two different areas of late gastrula or neurula. According to the "Anlagenplan" presented by Balinsky ('47) and by Nakamura & Tabara ('54), there are clear distinction between these two areas with respect to their presumptive fates; the *anterior endoderm* is destined to form pharynx, liver and stomach, whereas the *middle one* to develop into intestine. In our explantation experiments where these endodermal pieces were inserted into the cranial piece respectively, both pieces equally produced the pharynx (cf. Table). The results indicate that the presumptive organ-forming areas of fore-gut are not yet determined or, if at all, are still in a state of labile determination at neurula stage.

Such undetermined endoderm, if cultured within the epidermal envelope without being accompanied by any mesodermal components, could not differentiate into any recognizable tissue, remaining as "yolk-mass". Consequently, it may be supposed that the presumptive endoderm is incapable of self-differentiation, and that for the differentiation of the endoderm, the influence from the mesodermal tissues should be taken into consideration. Actually in the experiments, if the endodermal piece was explanted by inserting it into the cranial piece including the mesodermal tissues, it could undergo the differentiation into the definitive tissues. But, the endodermal pieces succeeded in its differentiation within the cranial piece, only when it was situated amidst the free-mesenchyme of the head. No successful differentiation of the inserted endoderm took place, when the mesen-

chyme was absent around the endoderm. These results must give an evidence to the author's earlier assumption (Okada, '54a) that the differentiation of the presumptive endoderm can be realized only in the mesenchymal surroundings.

In our present experiments where the endodermal differentiation occurred amidst the mesenchyme, the explanted endoderm gave rise exclusively to pharynx, production of the other endodermal tissues being hardly met with. One of the author's previous results (Okada, '54b) had also demonstrated that even the posterior-most part of the presumptive endodermal area of gastrula can differentiate towards pharynx, in case it was found amidst the free-mesenchyme. All these results must denote that the development of the undetermined endoderm amidst the mesenchymal surroundings resulted in the realization of only pharyngeal differentiation, excluding the realization of any other tissues. Consequently, we can assume that which part of the digestive tract is realized from the endoderm of an early embryo is varied according to the surroundings within which the presumptive endoderm develops. Actually, the mesodermal tissue other than mesenchyme, as will be given in the next paper, bears a significance in the production of intestine.

### Summary

The present investigation was performed to see what effect is exerted from the mesodermal or ectodermal tissues of the cranial part upon the differentiation of the presumptive endoderm, using the late gastrula or neurula of *Triturus pyrrhogaster* as material. For this purpose, the *anterior* or *middle endoderm* was explanted by inserting it into the endoderm-free cranial part of another neurula. As the result, both *anterior* and *middle endoderms* generally showed differentiation into pharynges, as far as they were found to be located amidst the head-mesenchyme. But, any kind of definitive tissue was not produced from the endodermal piece which lay apart from the mesenchymal surroundings. These results indicate that each area of the presumptive endoderm is neither irrevocably determined, nor self-differentiating in neurula stage. The significance of the mesenchymal tissue in the differentiation of pharynx was discussed.

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