

Experimental Studies on the Differentiation of the Endodermal Organs in Amphibia

I. Significance of the Mesenchymatous Tissue to the Differentiation of the Presumptive Endoderm

By

Tokino S. OKADA

Zoological Institute, University of Kyoto

(Received May 16, 1954)

By means of explantation, the author found that the presumptive endoderm of amphibian gastrula can differentiate into the definitive tissues and organs so far as it is surrounded by the mesenchymatous tissue (Okada, '53)⁽¹⁾. But the tested part of the presumptive endoderm was limited only to an anterior part of the endodermal area of early gastrula, and the other parts remained to the further study. In the present experiments, various parts of the presumptive endoderm were examined. Results of the experiments were in good agreement with those obtained in the preceding experiments, and they will be described in the following.

The operation was carried out on early gastrulae (Okada & Ichikawa's st. 11)⁽²⁾ and early neurulae (st. 16) of *Triturus pyrrhogaster*. The endodermal pieces to be explanted were taken from the anterior^(a) (part 1 in Fig. 1), middle (part

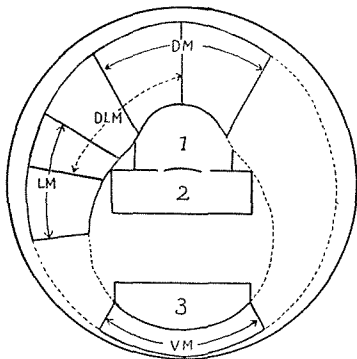


Fig. 1. Ventral view of the early gastrula showing the positions of the explanted pieces.

1; anterior endoderm
2; middle endoderm
3; posterior endoderm

DM, DLM, LM and VM represent the areas from which the mesodermal pieces to be added to the endoderm were taken.

(a) This part was designated as "dorsal" with respect to the position before gastrulation in the preceding experiments.⁽¹⁾

2) or posterior (part 3) part of the presumptive endodermal area of the early gastrula. The mesodermal tissue to be added to the endodermal piece was taken from the dorsal (DM in Fig. 1), lateral (LM), dorso-lateral (DLM) or ventral (VM) part of the marginal mesoderm of another embryo in the same developmental stage. Each isolated endodermal piece was enveloped alone or together with the mesodermal piece by a ventral epidermis cut out from the early neurula (st. 16). These explants were kept in Holtfreter's solution for about four weeks.

Before going farther, the author takes pleasure in expressing his hearty thanks to Prof. M. Ichikawa for his kind guidance, and he owed also to valuable criticisms from Dr. H. Takaya and Mr. N. Ikushima during the course of the study.

Experiment

Series 1. Explantation of the anterior part of the endoderm.

Among the explants of the anterior endoderm alone, 10 cases were small solid masses consisting of the endodermal and atypical epidermal components, and the other 18 were large swollen vesicles which contained the mesenchymatous tissue between the ecto- and endodermal tissues. In the former, the endodermal component showed no sign of differentiation, only remaining each as a "yolk-mass" (cf. Okada, '53^c; see Fig. 2), while in the latter, there occurred sometimes well defined endodermal tissues and organs. Closer examination of these swollen explants revealed the existence of the mesenchymatous tissue within them. Especially in 11 out of these 18 swollen cases, the mesenchymatous tissue was present abundantly and the good differentiation of the endoderm was always observed, as is shown in Fig. 3, while in the remaining 7 cases in which the mesenchymatous tissue was scarce, the endodermal differentiation failed to take place.

When the mesodermal piece was added to the explanting endoderm, a number of explants contained the marked mesenchymatous tissue (75 out of 84 cases). Moreover, the mesodermal organs such as the notochord, muscle, pronephros or heart were produced very frequently (66 cases out of 84 explants). Which of these mesodermal organs had developed seemed to vary in accordance with the area from where the mesodermal piece was cut out. Of the above 75 cases in which the mesenchymatous tissue was found, as many as 60 showed the definitive endodermal tissues or organs, and 54 out of these 60 cases indicated the mesodermal organs as well. As to the endodermal organs, the pharynx was produced always, and decreasingly followed by the stomach, intestine, liver and pancreas in this order. Out of 24 cases that failed in the endodermal differentiation, 9 cases contained no mesenchymatous tissue and the remaining 15 cases included it very scarcely. But the mesodermal organs such as notochord and muscles were found in some of these 24 cases (see Fig. 4).

These facts mentioned above indicate probably that it is the mesenchymatous

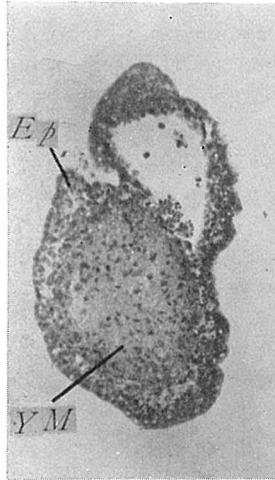


Fig. 2.

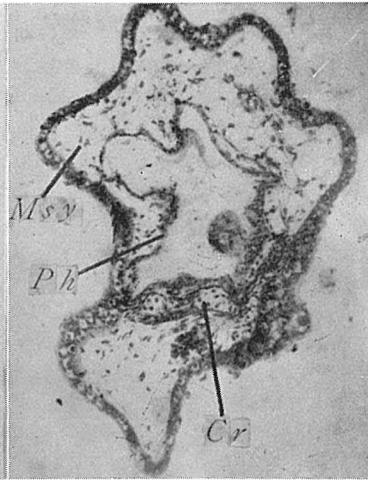


Fig. 3.

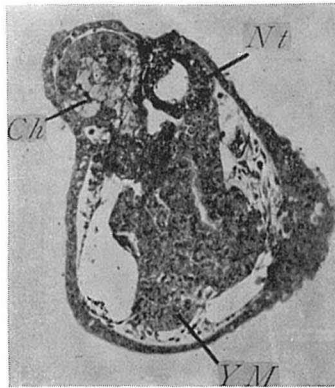


Fig. 4.



Fig. 5.

- Fig. 2. The explanted anterior endoderm remaining as "yolk-mass" (YM) enveloped with atypical epidermis (Ep).
- Fig. 3. The explanted anterior endoderm differentiated into pharynx (Ph) surrounded by the free mesenchyme (Msy); the visceral cartilage (Cr) was observed.
- Fig. 4. The explanted anterior endoderm remained as "yolk-mass" (YM) in the presence of the notochord (Ch) and neural tube (Nt); the mesenchymatous tissue contained was very scarce.
- Fig. 5. The explanted posterior endoderm differentiated into the intestine (It) surrounded by the mesenterial tissue (Mt).

tissue, but not the mesodermal organs, that is responsible for the differentiation of the explanted endoderm.

Series 2. Explantation of the middle part of the endoderm.

Explantation of the endoderm alone or combining with DLM was made. Among 44 explants of this series, differentiation of the endodermal tissues and organs was found in 22 cases. In these successful cases the mesenchymatous tissue was always abundant within the explants. In the explants of endo- plus mesoderm, the mesodermal organs were frequently formed, but they bear no direct connection with the differentiation of the endoderm, i. e., it occurred even in their absence.

As to the endodermal organs, the pharynx, stomach, intestine, liver and pancreas were produced.

Series 3. Explantation of the posterior part of the endoderm.

Explantation of the endoderm alone or together with the mesodermal piece such as DM, DLM or VM was performed. When the mesodermal piece was added, the production of the mesodermal organs such as notochord, muscle, pronephros and heart was of frequent occurrence. Among 84 available explants of this series, 52 cases succeeded in the differentiation of the explanted endoderm and contained the mesenchymatous tissue abundantly. Of these 52 cases, 44 formed the mesodermal organs, and other 8, mostly belonging to the explantation of the ventral endoderm alone, formed none of the mesodermal organs, the mesenchymatous tissue alone being found as the mesodermal component of the explants. In 32 cases which failed in the endodermal differentiation, the mesenchymatous tissue was hardly present and, if any, it was scanty. Even in these cases, however, the mesodermal organs appeared in 12 of them.

As to the endodermal organs, the intestine was formed in all explants which showed endodermal differentiation (Fig. 5). Only in rare specimens the pharynx or stomach was formed in addition to the intestine. It was a characteristic feature of this series that the mesenchymatous tissue frequently became a thin layer like a mesentery surrounding the intestinal tissue, as is shown in Fig. 5. Differentiation of the intestinal tissue generally was more perfect when it was surrounded by such mesenterial tissue.

Discussion

Explantation of the presumptive endoderm has been carried out by Holtfreter ('38a,⁽³⁾ '38b,⁽⁴⁾ '39a,⁽⁵⁾ '39b⁽⁶⁾). He explanted various parts of the presumptive endoderm of early gastrula or late blastula, and obtained well differentiated tissues or organs. Also in our experiments, the explanted endoderm generally showed the differentiation into definitive tissues or organs. This was a rule in all series of our present experiments regardless of the endodermal part where it was taken from. According to the various localities of the endodermal parts tested, no marked difference in the percentages of the successful differentiation was encoun-

tered; i. e., 63 per cent in the anterior, 50 per cent in the middle and 62 per cent in the posterior part of the endoderm. Thus, in general, our results are in good agreement with those of Holtfreter in the point that every part of the presumptive endoderm of early gastrula has the capacity to undergo its differentiation *in vitro*. But in 40 per cent of the operated specimens the endoderm failed in differentiation, and remained as "yolk-mass" even after the cultivation as long as about four weeks. It should be mentioned that this failure was limited only to those cases in which the mesenchymatous tissue was absent or very scarce within the explants. The endodermal differentiation occurred always in the explants containing the mesenchymatous tissue. Throughout the whole series of the present experiments, this was a rule which permitted no exception. In the mesenchyme-containing explants there existed generally a wide space which separated the endoderm from the overlying ectoderm. But the space in itself seemed to have no significance to the endodermal differentiation, because the explanted endoderm always failed to differentiate in the presence of the space, if the mesenchymatous tissue was scarce within this space. Further, our results verified that even when the mesodermal organs such as notochord and muscle were present within the explants, the endoderm always failed to differentiate so far as the mesenchymatous tissue was absent or very scarce. On the contrary, under the presence of the mesenchymatous tissue, no other mesodermal tissues or organs having been found, complete differentiation of the endoderm occurred. From these facts in consideration, it seems evident that the explanted endoderm can undergo its differentiation only when it is surrounded by the mesenchymatous tissue.

Concerning the rôle of the mesenchymatous tissue in the development of the endoderm, Holtfreter ('39a⁽⁶⁾, '39b⁽⁶⁾) has assumed that the tissue provides only a mechanical substrate for the developing endoderm, but has nothing to do with the process of differentiation. From his preceding experiments, however, the author had arrived at the conclusion that the mesenchymatous tissue bears an important significance, not merely of providing the mechanical substrate, but also of promoting the differentiation of the endoderm (Okada, '53⁽¹⁾). But in those experiments only the anterior part of the endoderm had been tested, while in the present, all the parts of the presumptive endodermal area were examined with the same results that the explanted endoderm could differentiate when they were surrounded by abundant mesenchymatous tissue, but that when the latter was absent, the endoderm could not differentiate into any tissues and organs. However, whether the surrounding mesenchymatous tissue behaves as an indispensable realizer for the endodermal differentiation or merely acts as a promotor in the process of its differentiation is a question still to be investigated.

At any rate, it should be mentioned that the differentiated tissues of the endoderm vary in their qualities according to the regions from where the explanted endoderm has been taken. As is above given, the anterior part of the

endoderm produces the pharynx, while the posterior part forms the intestine without exception. Details concerning the tissues and organs produced in the explants will be given in the following paper.

Summary

The explantation of the anterior, middle or posterior part of the presumptive endoderm alone or along with the various part of the marginal mesoderm was performed, using the early gastrulae of *Triturus pyrrhogaster* as materials. The results showed that well defined tissues or organs were produced from every part of the endoderm. But such differentiation occurred only when the explanted endoderm was surrounded by the mesenchymatous tissue, and when the latter was absent, the endoderm remained as a "yolk-mass". The presence of the definitive mesodermal organs derived from the added marginal mesoderm apparently has nothing to do with the endodermal differentiation.

References

- (1) Okada, T. S., '53 Mem. Coll. Sci., Univ. Kyoto, B 20 (3).
- (2) Okada, Y. K. & M. Ichikawa, '47 Jikken Keitai-gaku Nenpo, 3.
- (3) Holtfreter, J., '38a Roux' Arch., 138.
- (4) ———, '38b Roux' Arch., 138.
- (5) ———, '39a Roux' Arch., 139.
- (6) ———, '39b Arch. f. exper. Zellforsch., 23.