

## Experimental Studies on the Differentiation of the Endodermal Organs in Amphibia

### II. Differentiating Potencies of the Presumptive Endoderm in the Presence of the Mesodermal Tissues

By

**Tokino S. OKADA.**

Zoological Institute, University of Kyoto

(Received May 16, 1954)

As to the mesodermal influence upon the differentiation of the endoderm, the author has pointed out that the presence of the mesenchymatous tissue is indispensable for the complete differentiation of the explanted endoderm (Okada, '53a<sup>(1)</sup> & '54<sup>(2)</sup>). But the question is still open whether the mesodermal tissues exert an inductive influence upon the differentiating endoderm. In order to solve the question, the explantation of the endoderm was carried out by combining with the mesodermal piece taken from the various parts of the marginal area.

In the present experiments, the early embryos of *Triturus pyrrhogaster* were used as materials. The endodermal tissue to be explanted was taken from the anterior or posterior part of the presumptive endodermal area of the early gastrula. The mesodermal piece to be added to the endoderm was cut out from the dorsal, dorso-lateral, lateral or ventral part of the marginal area in early gastrula<sup>(a)</sup>. The endodermal piece alone or combining with one of these mesodermal pieces was inserted into the ectodermal vesicle and kept in Holtfreter's solution for about four weeks. Of the specimens thus operated the positive cases in which the differentiation of the definitive endodermal tissues was successful will be described, whereas other negative cases in which the explanted endoderm remained as "yolk-mass" will be omitted in this occasion (cf. Okada, '54<sup>(3)</sup>).

Taking this opportunity, the author wishes to express his hearty thanks to Prof. M. Ichikawa, under whose direction the present studies are progressing.

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(a) The positions of the explanted meso- or endodermal piece were the same with those represented in Fig. 1 in the previous paper (Okada, '54).

### Experimental Results








To the explants of the anterior part of the endoderm was added the mesodermal piece which was taken from the dorsal, dorso-lateral, lateral or ventral area. To compare the results, the explantation of the endoderm alone was made. The tissues and organs produced within the explants are enumerated in the first column of Table 1. As is seen in the Table, the mesodermal organs varied according to the different mesodermal pieces that were employed. Notochord and muscle were very frequently obtained from the dorsal, dorso-lateral

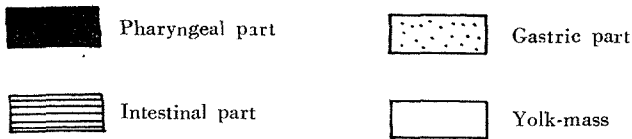
Table 1. Organs and tissues derived from the meso- and endoderm of the explants.

Kind of the explantations. Positive cases	Anterior endoderm						Posterior endoderm				
	alone	DM	DL	LM	VM	total	alone	DM	DL	VM	total
Organs and tissues differentiated	10	8	18	25	9	70	5	20	18	6	49
Pharynx	10 (100)	8 (100)	18 (100)	25 (100)	9 (100)	70 (100)	0	0 (5)	2 (11)	1 (20)	4 (8)
Stomach	5 (50)	3 (38)	14 (77)	11 (44)	4 (44)	37 (52)	0	1 (5)	3 (17)	1 (20)	5 (10)
Intestine	4 (40)	1 (13)	11 (61)	8 (32)	3 (33)	27 (38)	5 (100)	20 (100)	18 (100)	6 (100)	49 (100)
Yolk-mass	8 (80)	8 (100)	10 (55)	20 (80)	7 (77)	53 (74)	4 (80)	15 (75)	8 (44)	5 (80)	32 (64)
Liver	2 (20)	1 (13)	8 (44)	4 (16)	2 (22)	17 (24)	0	0	1 (6)	0	1 (2)
Pancreas	2 (20)	0	4 (22)	2 (8)	1 (11)	9 (13)	0	1 (5)	1 (6)	0	2 (4)
Notochord	1 (10)	7 (88)	18 (100)	21 (84)	2 (22)	49 (69)	0	16 (80)	17 (95)	1 (20)	34 (68)
Muscle	1 (10)	7 (88)	18 (100)	22 (88)	3 (33)	51 (71)	1 (20)	17 (85)	17 (95)	1 (20)	36 (72)
Pronephros	0	0	7 (39)	8 (32)	3 (33)	18 (25)	0	0	8 (44)	0	8 (16)
Heart	1 (10)	0	7 (39)	6 (24)	3 (33)	17 (24)	0	0	3 (17)	1 (20)	4 (8)
Mesenchymatous tissue	10 (100)	8 (100)	18 (100)	25 (100)	9 (100)	70 (100)	5 (100)	20 (100)	18 (100)	6 (100)	49 (100)

Number in parenthesis indicates percentages to the total positive cases of each kind of the explantations.

Table 2. Differentiation of the digestive tract from the explanted endoderm.

Kind of the explantations	Anterior endoderm					Posterior end.			
	alone	DM	DL	LM	VM	alone	DM	DL	VM
	10	8	18	25	9	5	20	18	6
(a) 	5	5	4	14	5	0	0	0	0
(b) 	1	2	3	3	1	0	0	0	0
(c) 	2	1	3	3	1	0	1	1	1
(d) 	2	0	8	5	2	0	0	1	0
(e) 	0	0	0	0	0	0	0	1	0
(f) 	0	0	0	0	0	4	14	7	3
(g) 	0	0	0	0	0	1	5	8	2



and lateral mesoderm, but the ventral mesoderm produced these organs only in rare cases. Although pronephros and heart were never found in the explantation of the dorsal mesoderm, they appeared in some of the other mesodermal pieces. In the explants of the dorso-lateral mesoderm, frequently the four mesodermal organs above enumerated were simultaneously found in a single specimen (see Fig. 3). On the other hand, in the explants of the endoderm alone, generally no formation of the mesodermal organ was encountered, but the mesenchymatous tissue was found in all the positive cases.

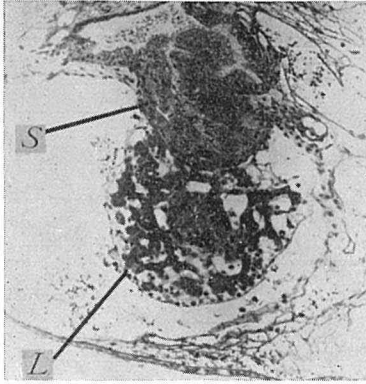


Fig. 1

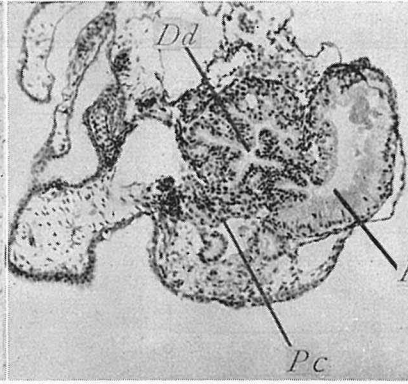


Fig. 2

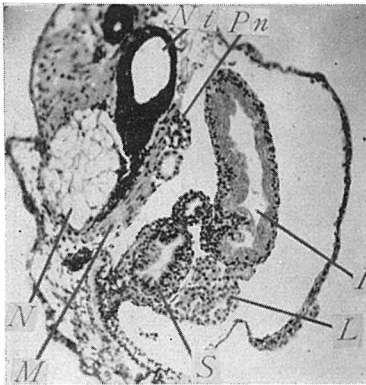


Fig. 3

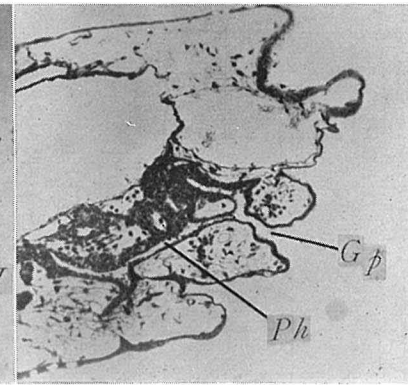


Fig. 4

Fig. 1; Differentiation of gastró-hepatic region from the explant of the anterior endoderm alone.

L: Liver, S: Stomach,

Fig. 2; Differentiation of intestinal region from the explant of the anterior endoderm plus dorso-lateral mesoderm.

Dd: Duodenum, I: Small intestine, Pc: Pancreas,

Fig. 3; Differentiation of gastro-intestinal region in the presence of the mesodermal organs the explant made of the anterior endoderm plus dorso-lateral mesoderm.

L: Liver, S: Stomach, I: Small intestine, M: Muscle,

N: Notochord; Nt: Neural tube, Pn: Pronephros,

Fig. 4; Differentiation of branchial region from the explant made of the posterior endoderm plus dorso-lateral mesoderm.

Ph: Pharynx, Gp: Branchial pouch,

With respect to the endodermal differentiation, pharynx was found in all explants irrespectively of the part of the added mesoderm where it was taken from. The pharynx obtained was often equipped with the branchial pouches

and visceral cartilages. In the explants of the anterior endoderm, however, there was no case in which the pharynx alone was formed, but other tissues of the endoderm were always produced in addition to the pharynx. Actually, stomach, intestine or "yolk-mass" was found (see Figs. 1, 2 & 3). These tissues appeared in the explants in such combinations as followings (cf. Table 2); pharynx and "yolk-mass" (a), pharynx, stomach and "yolk-mass" (b), pharynx, stomach, intestine and "yolk-mass" (c) and finally pharynx, stomach and intestine without "yolk-mass" (d). No other combination besides these four was entailed. This result probably indicates that the differentiation of the gastric part is possible only in the presence of the pharyngeal one, and that the differentiation of intestinal part occurs when both pharyngeal and gastric parts are formed in the explant. As far as the present results were concerned, the differentiation of the gastric or intestinal part was found in all kinds of the explants, although added mesoderm was taken from different parts (cf. Tables 1 & 2). But the frequency of appearance of these caudal parts of the digestive tract varied in accordance with the mesodermal piece added. As shown in Table 2, the occurrence of c and d types was most frequent and as high as 60 per cent of the explants with the dorso-lateral mesoderm, while it was only 12 per cent of those explants with the dorsal mesoderm. When the lateral or ventral mesoderm was added to the endoderm, this frequency came between the two cases; i. e., it was 33 per cent respectively. As described above, when the dorso-lateral mesoderm was added, there occurred the frequent production of the various mesodermal organs such as notochord, muscle, pronephros and heart. In such explants, the stomach and intestine were generally formed (see Fig. 3). On the other hand, when the dorsal mesoderm was added, the differentiation of the mesodermal organs was limited to the notochord and muscle, and the endodermal tissue was only pharyngeal. These results seem to indicate the existence of some relation between the differentiation of the caudal parts of the digestive tract and the kinds of the mesodermal organs. But, it is still difficult to state that each mesodermal organ exerts specific influence upon the differentiation of the endoderm, because there were several cases in which the well differentiated pharynx, stomach and intestine were produced in spite of the absence of any mesodermal organ (see Fig. 2). In these cases, however, it was a rule that large amount of the mesenchymatous tissues was found around the endodermal tissues. The mesenchymatous tissues in these explants were of two types. The first was loosely scattered cells of free-mesenchyme found around the pharynx, while the second was a thin layer of the mesenterial character which existed surrounding the intestine.

Besides the endodermal organs mentioned above, liver (Figs. 1 & 3) and pancreas (Fig. 2) were found sometimes (cf. Table 1). Although there were many cases in which the differentiation of the liver and the heart occurred simultaneously (11 cases), there were still other cases (6 cases, see Fig. 1) in which

the liver was formed in the absence of the heart. The influence from the heart upon the differentiation of liver was pointed out by Balinsky ('49<sup>(3)</sup>) and others, but this finding is an apparent objection to this view.

In the *explantations of the posterior part of the endoderm*, the endodermal tissues markedly varied as compared with those in the explantations of the anterior endoderm (cf. Tables 1 & 2). In the present explants the intestine alone was generally formed, and only in 5 cases pharyngeal and gastric tissues in addition to the intestinal were produced (see Fig. 4). In a nearly half of the explants in which the intestine alone was formed, a part of the explanted endoderm was undifferentiated and remained as "yolk-mass" (cf. Table 2). In the other half, the entire endoderm underwent the complete differentiation of the intestine (g). It goes without saying that the presence of the mesenchymatous tissues was found in all explants of the present explantations (cf. Okada, '54<sup>(2)</sup>). Generally, these mesenchymatous tissues formed thin layers surrounding the intestine. Apparently the tissue was mesenterial in its character. But in 5 cases in which the pharynx and stomach were present, the pharyngeal part was surrounded by the loosely scattered cells (see Fig. 4), and the stomach and intestine were coated by a thin layer of the mesenterial tissue.

In these cases where the dorsal, dorso-lateral or ventral piece of the mesoderm was added to the explanted endoderm, various mesodermal organs were formed in accordance with the presumptive fate of the added piece (cf. Table 1). But the differentiation of the endoderm was common to all explants, irrespective of the part of the mesoderm employed.

### Discussion

The endodermal tissues explanted in the present experiments were taken from the anterior and posterior regions which located separately in the presumptive endodermal area of the early gastrulae. Nevertheless, the anterior and posterior pieces produced similar parts of the digestive tract in explantation. As is described above, the parts of the digestive tract actually obtained were pharyngeal, gastric and intestinal parts. That such nearly all parts of the digestive tract were produced from the restricted region of the endoderm, apparently indicates the fact that the presumptive endoderm does not consist of the irrevocably determined parts in the gastrula stage. The same conclusion has already been drawn from the defect experiments of the author (Okada, '53b<sup>(4)</sup>). The transplantation experiments carried out by Balinsky ('38<sup>(5)</sup>, '49<sup>(3)</sup>) also showed that the determination of the endoderm is not established even in the neurula stage. On the contrary, Holtfreter ('38a<sup>(6)</sup>, '38b<sup>(7)</sup>, '39<sup>(8)</sup>) considered that the presumptive endoderm of the early gastrula was determined in mosaic fashion. Such interpretation was drawn from the results of his explantation experiments, in which

the anterior endoderm differentiated into the pharynx, while the posterior one into the intestine. No other tissues of the endoderm were formed from each piece. In our experiments there were cases in which the anterior piece produced merely pharynx and the posterior, only intestine. But, it should be pointed out that all these cases were limited to those explants in which the mesodermal tissues were formed only in small quantity. When large amount of the mesodermal tissues was present, various tissues of the endoderm were produced always. Consequently, the differentiation of many kinds of the endodermal tissues from the explanted piece may be attributed to the presence of large amount of the mesodermal tissues within the explants.

Further, the endodermal differentiation probably has no relation with the presence of the respective mesodermal organ, as is shown by the following facts. In our experiments, it was a rule that the pharynx was always formed from the anterior endoderm, whereas the intestine, from the posterior. This was common regardless of the part of the added mesoderm. Therefore, it is apparent that the special part of the added mesoderm exerts no influence, at least, upon the pharyngeal and intestinal differentiation from the anterior and posterior parts respectively. As described above, the gastric and intestinal tissues were formed from the anterior endoderm in addition to the pharyngeal tissue in some specimens, and the pharyngeal and gastric tissue were formed, besides the intestinal, from the posterior endoderm. But the differentiation of such various tissues of the endoderm was met with in all kinds of the explants in which the dorsal, dorso-lateral, lateral or ventral mesoderm was added to the endoderm (cf. Table 2). Moreover, there were several cases in which gastric and intestinal differentiation occurred from the anterior endoderm even when any mesodermal organ was not found within the explant. But it was a common feature that the large amount of the mesenchymatous tissues was always found in all of these explants. From these fact, it is natural to surmise that the presence of the mesenchymatous tissues is the factor essential for the endodermal differentiation, while the presence of the particular organs of the mesoderm apparently plays no significant rôle. Here, it should be pointed out that the mesenchymatous tissues showed the different characters according to the place of appearance where they were found together with the pharyngeal or the intestinal tissue. The mesenchymatous tissue found together with the former was loosely scattered, resembling to the so-called "free-mesenchyme", whereas the tissue found together with the latter represented the mesenterial character. Presumably, there is an intimate relation between the character of the mesenchymatous tissues and the differentiated endoderm.

However, the experimental results of the anterior endoderm reveal that the formation of the stomach and intestine occurred very frequently when the mesodermal organs such as the pronephros and heart were encountered in the explants, while it occurred rarely when these mesodermal organs were absent. In our experiments, the presence of the pronephros and heart was necessarily accom-

panied with the appearance of the mesenterial tissue. On the other hand, in the absence of these organs, the mesenterial tissue was formed in a few cases, and the stomach and intestine were found exclusively in these cases. Therefore, the frequent occurrence of the caudal organs of the digestive tract such as stomach and intestine in the presence of the pronephros and heart can be attributed to the presence of the mesenterial tissue coexisting with these mesodermal organs.

### Summary

The anterior and posterior endodermal pieces taken from *Triturus-gastrulae* were explanted after combining with the mesodermal piece cut out from the various parts of the marginal area. Regardless of the parts from where the mesodermal piece was removed, the anterior endoderm always differentiated into pharynx, whereas the posterior one, into intestine. But stomach and intestine in addition to the pharynx were formed frequently from the anterior endoderm, while the pharynx and stomach were produced from the posterior endoderm in addition to the intestine. Then, the differentiation of nearly all parts of the digestive tract was obtained from each of the small explanted pieces of the endoderm, when the large amount of the mesenchymatous tissues was contained within the explants.

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