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Rôle of the Mesoderm in the Differentiation of Endodermal Organs

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In the differentiation of the ectodermal organs, an inductive influence of the mesoderm is generally shown. But, in the endodermal differentiation no such influence of the mesoderm has been evidenced.

Holtfreter ('34, '38a, '38b, '39a, '39b), Stableford ('48), Takaya ('52) and Mikami and Murakawa ('52) have pointed out the mesodermal influence upon the endodermal organs, but it is yet open to question whether or not the inductive influence is exerted from the mesoderm upon the endodermal differentiation. Some experiments were carried out with the aim of solving this question. The results will be described in the following.

Before going further, the author wishes to express his hearty thanks to Prof. M. Ichikawa, under whose direction the present experiments were carried out.

Material and Method

Materials used were early embryos of *Triturus pyrrhogaster*. The experiments were performed by means of explanting the presumptive materials of the endoderm alone or together with the mesoderm. In one series (series A), a piece of the presumptive endoderm alone was taken out from the dorsal portion of the invagination groove, including the invaginated portion, of the early gastrula (Okada and Ichikawa's stage 11) as represented by the shaded area in Fig. 1. In the other series (series B), a piece of the dorsal marginal

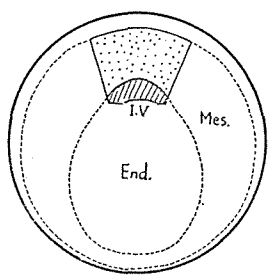

Fig. 1, Ventral view of the early gastrula showing the area from which the explanted piece was taken. Shaded area indicates the endodermal piece, and dotted, the mesodermal.

I. V.: Invagination groove, End.: presumptive endoderm, Mes.: presumptive mesoderm.

mesoderm (Fig. 1, dotted area) was added to this piece of the endoderm. In order to avoid the premature disintegration, the pieces to be explanted were enveloped with the scrap of the ectoderm cut out of the ventral region of the late gastaulae or early neurulae (st. 14~16), and they were cultivated in Holtfreter's solution for about 20 days. For the microscopical observations, sections were cut in the ordinary way, and stained with borax-carmine and "picro blue-black".

Experimental Results

Series A., Explantation of the presumptive endoderm alone. From external appearance of the explants, two types were distinguished. One was a compact mass covered with more or less wrinkled epidermis (9 out of 23 specimens), and the other, a swollen vesicle of thin epidermis (14 out of 23). In the former type of the explants, the endodermal component was often extruded from the ectodermal envelope, whereas, in the latter, the endoderm was always enclosed in the ectodermal envelope. In several cases of the latter type, balancers and pigment cells were produced.

Microscopical observations of the explants revealed that in the former type, there was none in which any mesodermal tissues were detected, while in the latter type, the mesenchymatous tissue was always found in the cavity between the ecto- and endodermal components. Further, the presence or absence of the mesenchymatous tissue yielded the striking difference in the differentiation of the explanted endoderm (compare the first row with the second of the table). The explants without mesenchyme did not show any sign of endodermal differentiation even after 20 days of cultivation, e. i., the endoderm remained as an amorphous mass of large cells heavily laden with yolk. This state of endoderm will be brifley designated as "yolk-mass" in the following. On the other hand, the explants containing the mesenchymatous tissue showed, as a rule, the far more advanced endodermal differentiation. In all of these explants, the pharyngeal tissue was produced as is shown in Fig. 2 (PH), and in 6 of them, evagination of this tissue was found towards the ectoderm, apparently showing the gill-pouches (Fig. 2, GP). The gill developed in one of them. The stomodeal invagination of the outer epidermis was often found to communicate with the endodermal layer, making a thin membrane

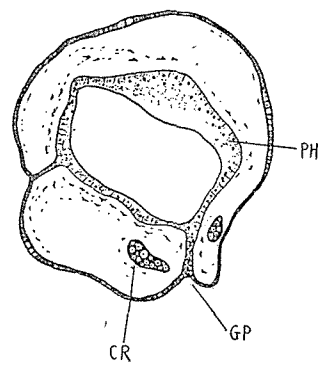

Fig. 2, Formation of pharynx (PH) equipped with gill-pouches (GP) and cartilages (CR).

like an oral plate. In the vicinity of these stomodeal and pharyngeal tissues, visceral cartilages were formed in 3 cases (Fig. 2, CR). In addition to the endodermal organs, "yolk-mass" was present in all cases. Even the neural tissue was met with in several of the explants in which the endodermal differentiation was good.

Series B., Explantation of the presumptive endoderm together with the dorsal marginal mseoderm.

All the explants developed into swollen vesicles of comparatively large size. Addition of the marginal mesoderm to the endoderm apparently favoured the development of the explants, so that such organs as balancers, gills, limb-like-protuberances and pigment cells appeared in most of the specimens.

Histological observations of the explants showed that the definite mesodermal organs and mesenchymatous tissue were produced between the ecto- and endodermal components. In most specimens examined (15 out of 17), the pharynx was well developed, provided with the gill-pouches and visceral cartilages. The mouth together with its cartilaginous components was found in 10 cases, and in 5 of them the teeth and the ectodermal collar cells were found too. Differentiation of the liver and intestine occurred in a few explants (Fig. 3, LV and INT), admitting that these tissues were small and fragmental as is shown in Fig. 3. The "yolk-mass" (Fig. 3, YM) was also found in nearly all of the cases.

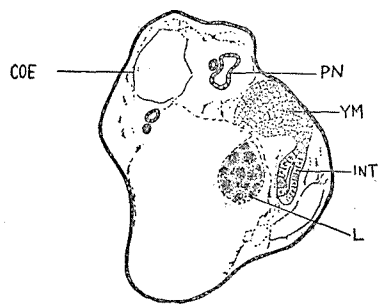

Fig. 3, Formation of liver (L) and intestine (INT) accompanied with pronephros (PN), coelom-like-cavities (COE) and "yolk-mass" (YM).

In almost all of the cases of the present explants, the notochord and myotomes were formed apparently from the explanted mesoderm. The pronephri (Fig. 3, PN) and coelomic cavities lined with pericardial tissue (Fig. 3, COE) were found in 5 cases.

It was noted that there exists close relation between differentiation of the endoderm and that of the mesoderm; the more the mesodermal organs were formed, the more the endodermal organs and tissues were produced. For instance, formation of liver and intestine occurred only when the pronephri and coelomic cavities were formed in the explants. Further, when both mesodermal and endodermal organs developed well, their disposition was found

Table. Showing the organs and tissues appeared in the explants.

	Series A		Series B	Total
Available cases	23		17	40
Presence of mesenchyme	without mesenchyme 9, 38%	with mesenchyme 14, 62%	with mesenchyme 17, 100%	with mesenchyme 31, 78%
Mouth	0	4, 28%	10, 60%	14, 35%
Pharynx	0	11, 77%	15, 90%	26, 65%
Gill-pouch	0	6, 42%	12, 72%	18, 45%
Liver	0	0	2, 12%	2, 5%
Intestine	0	0	2, 12%	2, 5%
Yolk-mass	9, 100%	14, 100%	16, 96%	39, 69%
Gill	0	1, 7%	7, 42%	8, 20%
Cartilage	0	3, 21%	11, 66%	14, 35%
Notochord	0	1, 7%	16, 96%	17, 43%
Muscle	0	2, 14%	17, 100%	19, 48%
Pronephros	0	0	4, 24%	4, 10%
Coelom	0	0	5, 30%	5, 13%
Balancer	0	2, 14%	12, 72%	14, 35%
Pigment cell	0	3, 21%	12, 72%	15, 38%
Neural organ	1, 11%	4, 28%	10, 60%	15, 38%

to be comparable with the cranio-caudal arrangement of these organs in the normal embryo. In some explants, neural organs such as brain, eye, ear, neural tube and free lens were also found. However, between the differentiation of the endoderm and these neural organs, there was no apparent correlation which suggested the inductive influence of the former upon the latter. But, free lenses were always formed in intimate contact with or embedded in the endodermal tissues. The fact may indicate that the endoderm possesses the faculty of inducing the lens from the ectoderm.

Discussion

Explantation of the presumptive endoderm has been tried by Dürken ('25), Küsche ('29), Mangold ('36) and Stableford ('48). These authors failed in obtaining the differentiated organs or tissues from the explanted endoderm.

However, Holtfreter ('38a, '38b, '39a), who also tried explantation of the endoderm, found that the definitive tissues were formed from the explanted endoderm and that these tissues varied in accordance with the prospective fate of the explanted pieces, even when they were taken from early gastrulae. And he pointed out in his case that the explanted endoderm was influenced by the mesodermal organs or mesenchymatous tissue. According to him, this influence is of such nature that it offers a mechanical support for the endodermal tissues to develop their proper shape. The influence of the mesenchymatous tissue was also apparent in our experiments, but the influence seems to be different nature from that pointed out by Holtfreter. Evidences that the explanted endoderm showed differentiation only when it was surrounded by the mesenchymatous tissue, and that in the absence of this tissue no differentiation of the endoderm was found in any case, show that the mesenchymatous tissue bears some influence upon the histological differentiation of the explanted endoderm. Holtfreter pointed out also that even when the mesenchymatous or mesodermal tissues were absent, full differentiaiton of the endodermal tissues occurred in several cases, but that these tissues did not show the definitive shape as the endodermal organs. Standing on this fact, he denied naturally the significance of the mesenchymatous or mesodermal tissues upon the histological differentiation of the presumptive endoderm, only assuming the mechanical influence of the former upon the latter. However, it should be mentioned here that his argument is based mostly upon the results with anuran embryos. In our experiments with urodelan embryos, no such case of endodermal differentiation was obtained, i. e., endodermal differentiation was possible only in existence of the mesodermal components. Therefore, there seems to exist the difference in the differentiation of the endoderm between anuran and urodelan embryos. Holtfreter's observation of "die besten Ergebnisse bei den Anurenversuchen von weniger gut an Triton und die Schlechsten an Axolotl" ('38a, p. 570) suggests this difference apparently. From these considerations, we may be safe to state that at least so far as the urodelan embryos are concerned, differentiation of the explanted endoderm is possible only when the mesenchymatous tissue is present in the explants. The mesenchymatous tissue exerts presumably the influence of favouring or promoting the endodermal differentiation.

Addition of the dorsal part of the marginal mesoderm to the explanted endoderm (in series B of the experiments), is followed by the production of the notochord and myotomes. In these cases, differentiation of the endoderm took place quite sufficiently. It is, however, the increased amounts of the mesenchymatous tissue and not the presence of the notochord and myotome that is significant for the endodermal differentiation, because there were the specimens in which the differentiation of the endoderm occurred only in the presence of the mesenchymatous tissue, even when the notochord and myotomes

were not formed. The fact leads us to assume that the mesenchymatous tissue bears greater influence than the notochord and myotomes.

The endodermal tissues produced in explants presented mostly pharyngeal or oral character. But in a few cases of series B, liver and intestine were also obtained. Such production of liver and intestine as well as pharyngeal and oral tissues from the explant may naturally be expected from the results of vital staining observations of Balinsky ('47), i. e., according to his "Anlageplan", our explants were taken from the presumptive areas of these endodermal organs. Nevertheless, the pharyngeal and oral tissues were always produced in the cases of the successful differentiation of the endoderm, but the liver and intestine were formed in a few cases in which the mesodermal tissuses such as coelom-like-cavities or pronephros were produced within the explants in addition to the notochord, myotomes and mesenchyme. Therefore, an interaction seems to exist between the mesodermal differentiation and the endodermal one. Experiments to verify this supposition is now in progress.

Summary

In order to test the rôle of the mesoderm in the differentiation of the presumptive endoderm, the explantation experiments were carried out, using the early gastrulae of *Triturus* as materials. The explantation of the presumptive endoderm alone is compared with that of combining it with the dorsal marginal mesoderm (see the accompanying table) with the results that the differentiation of the isolated endoderm is possible only when the mesenchymatous tissue is present. The notochord and myotomes are demonstrated not to exert any essential influence on the differentiation of the endoderm, but the mesenchymatous tissue bears an important significance of promoting the endodermal differentiation.

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