# REGENERATION OF THE ECTODERM AND ENDODERM IN HYDROIDS

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# REGENERATION OF THE ECTODERM AND ENDODERM IN HYDROIDS[1]

SEIEI AIZU[2]
会津清英
*Biological Institute, Tôhoku University, Sendai, Japan*

Although many studies have been published on the morphogenesis of hydroids, the emphasis has largely concerned with the problem of polarity. In this study, the nature and the ability of the ectoderm and the endoderm were investigated.

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## MATERIAL AND METHOD

Two species of colonial hydroids, *Obelia plana* and *Coryne pusilla* were used as materials. They were collected in Matsushima Bay, Miyagi Prefecture. They were cultured in the laboratory at 10°C by feeding *Artemia* larvae. The coenosarcs were taken from the periderm by squeezing the pieces of the hydrocaulus. Then, the ectoderm was isolated from the endoderm with a fine glass knife or needle. The endodermal layer of the fed colonies was easily distinguished from the ectoderm by its pink color. Further, the ectoderm was distinguished from the endoderm, as the latter contained the granules of a remarkable birefringence.

## OBSERVATIONS AND CONSIDERATIONS

### I. REGENERATION OF THE ISOLATED ECTODERM AND ENDODERM

#### 1. *Obelia plana*

a. Regeneration of the ectodermal tissue fragments.

The pure ectodermal cells fused readily and formed a smooth spherical mass. In the center of the mass a core of cells mass was formed by splitting from the surrounding ectoderm. After five days, the stem bud having a hydranth budded at its tip began to grow. The complete hydranth was formed after six to seven days.

1) Contributions from the Marine Biological Station of Asamushi, Aomori Ken, No. 359
2) Present address: Department of Anatomy, Tôhoku University School of Medicine, Sendai.

b. Regeneration from the endodermal tissue fragments.

The endodermal fragments were cultured in the same way as the ectodermal fragments. All of the endodermal fragments rounded up into balls within several hours after isolation and remained in this form until they disintegrated twenty-four to thirty-six hours later. No sign of differentiation was observed.

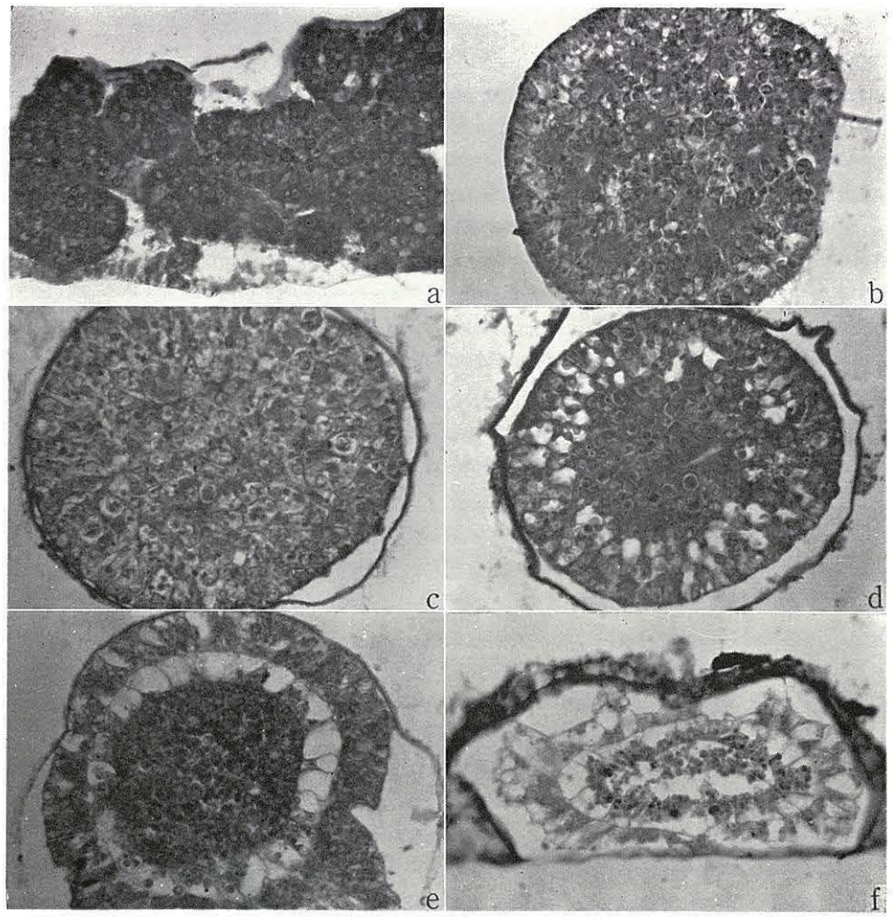


Fig. 1. Photomicrographs of sections showing the regeneration process of the ectodermal fragment of *Obelia*.
a, Section of the ectodermal tissue fixed immediately after isolation. b, The same, after twelve hours. c, After twenty-four hours. d, After three days, showing the porous structure in the ectoderm. e, After four or five days, showing the connection between the outer and the inner part. f, Complete coenosarc regenerated from the ectodermal fragment six days after isolation.



c. Histological observations.

The arrangement of the cells of the fragments fixed immediately after isolation was complete showing contraction. The tissue fragments consisted of many epithelio-muscular cells, interstitial cells and nematocysts, whereas no endodermal cells which contained many small inclusions was observed (Fig. 1a). In the specimens of the isolates fixed six to twelve hours after isolation, the tissue fragments became tightly rounded and were covered with a thin periderm (Fig. 1b). After twelve to thirty-six hours the external cells became columnar, forming a layer of cells on the surface. The cells in the central area were degenerating (Fig. 1c). Many pores were arranging continuously in a circle under the external columnar cells. A few remained forming the thin bridge of the cytoplasm connecting the external columnar cells and the internal degenerating cells (Fig. 1d). After three to five days, the connecting cytoplasm became thick incorporating the particles which were derived from the degenerated cells (Fig. 1e). At the time of hydranth formation, the connecting cells became large and contained many inclusions. These cells were considered to become the endodermal cells from the fact that they contained many basophilic particles. Thus, the endodermal cells were newly formed (Fig. 1f).

2. *Coryne pusilla*

a. Regeneration of the ectodermal tissue fragments.

The isolated ectodermal tissue fragment rounded up very quickly, usually into a spherical mass, and began to adhere to the substratum. At first, the whole sphere was translucent. Soon, the center of the mass became opaque. At the same time, circulation of the hydroplasm was observed in the mass. Three to four days after isolation the stolon began to elongate from the ectodermal tissue fragment. Then, the hydranth bud was formed on the stolon near the tip. After ten to fourteen days the complete hydranth with many tentacles was formed.

b. Regeneration of the endodermal fragments.

All of the endodermal fragments rounded up into ball form within six hours. But they became disintegrated twelve hours later. In addition, the endodermal tissue mass which was enveloped in the mesogloea membrane also showed no further differentiation.

c. Histological observations in regeneration of ectoderm.

The components of the ectodermal cells fixed immediately after isolation were nearly normal showing contraction. The tissue fragment consisted of many epithelio-muscular cells with large nuclei, nematocysts and a few small interstitial cells (Fig. 2a). In the specimens of the isolates fixed after twelve to twenty-four hours after isolation, the whole tissue masses rounded up tightly and were covered with a new thin periderm. The epithelio-muscular cells arranged

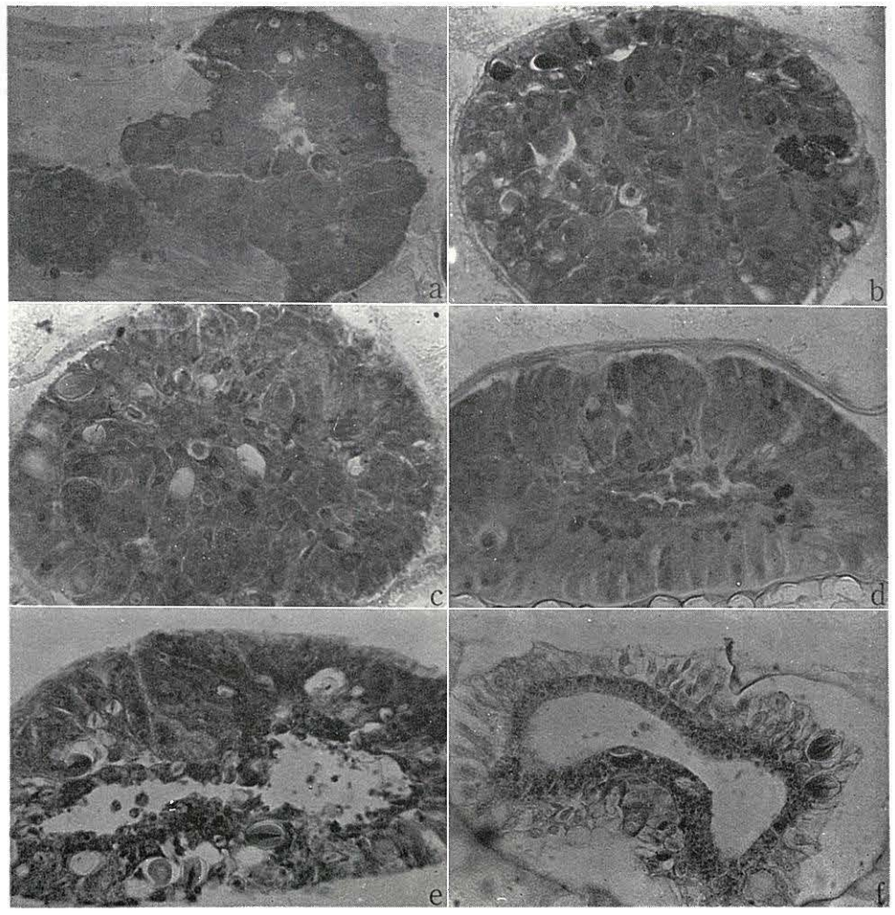


Fig. 2. Photomicrographs of sections showing the regeneration process of the ectodermal fragment of *Coryne*.
a, Section of the ectodermal tissue fixed immediately after isolation. b, The same, after twelve hours. c, After twenty-four hours, increasing in number of the interstitial cells. d, After thirty-six hours, the interstitial cells appearing along the inner margin of the external cell layer. e, Showing the extensive opening. f, The complete coenosarc regenerated from the ectodermal fragment.

around the periphery of the tissue mass (Fig. 2b). The cells of the central area remained contracted. The interstitial cells increased in numbers (Fig. 2c). After twenty-four to thirty-six hours, the intercellular space was formed by the degeneration of the central cell mass. Thirty-six to fourty-eight hours after isolation the external cells became columnar, forming a layer of cells in the surface. Many interstitial cells appeared along the inner margin of external cell layer (Fig. 2d).



Then, the space became extensive. Further, many small particles began to appear in the cytoplasm of the external columnar cells (Fig. 2e). After two to three days the cytoplasm of the interstitial cells became large and began to incorporate many inclusions. These cells were considered to become the endodermal cells (Fig. 2f). As mentioned above, the isolated ectoderm in *Coryne* was able to regenerate the lost endoderm and to produce the new hydranth as in *Obelia*. The transformation of the ectoderm to the endoderm in hydroids has been known in *Cordylophora* (Zwilling, 1963). But it was not clear what kind of the cells in the ectodermal layer transformed to the endodermal cells. The results of the present study indicate that the interstitial cells transformed to the endodermal cells.

## II. The relation between the ectoderm and the endoderm.

### 1. *Contact of the ectodermal and the endodermal fragments.*

When the endodermal fragment came into contact with the ectodermal fragment of *Coryne*, in the case in which the former was smaller than the latter, it was incorporated by the ectodermal fragment. After six hours it became located in the center of the ectodermal fragment. Hale (1964) described that the initiation of a hydranth was possibly due to a concentration of the ectodermal cells, and that the ectodermal cells continued to migrate up the stalk. From these facts, it can be suggested that the ectodermal cells are active in morphogenetic movement.

### 2. *The relation between the regeneration index and the ratio of the ectodermal and the endodermal masses.*

The isolated ectodermal and the endodermal tissue fragments were minced to obtain many small fragments of 25, 50, 75 and 100 microns in diameter, respectively. These ectodermal and endodermal fragments came into contact in combinations as shown in Table 1. The regeneration from the tissue fragment could be arranged in a graded series, ranging from tissue fragment to complete hydranth. In order to obtain a numerical measure of the grade of regeneration, arbitary numerical values were assigned to six grades of regeneration (Fig. 3). The regeneration index is calculated after the formula

Table 1. The combination of the ectodermal and endodermal fragments.

| Diameter of ectoderm in micra | Diameter of endoderm in micra |
|---|---|
| 100 | 100 |
| 50 | 100 |
| 50 | 50 |
| 25 | 50 |
| 25 | 25 |
| 25 | 75 |
| 25 | 100 |

$$\text{reg. index} \frac{100 \cdot n_1 + 80 \cdot n_2 + 70 \cdot n_3 + 50 \cdot n_4 + 30 \cdot n_5 + 10 \cdot n_6}{N}$$

where $n_1$, $n_2$,....$n_6$ are the number of the cases of the different grades of regeneration and N is the total number of the masses. Fig. 4 is a record of the regeneration index of various combined masses. The combined masses 100–100, tissue mass consisting of the ectodermal fragment having 100 microns in diameter and the endodermal fragment having 100 microns in diameter, produced the complete hydranth on the tenth day in more than ninety per cent of the cases. The masses 50–50 produced the hydranths in forty per cent. In the masses 25–25, no hydranth was differentiated. From this, the more the volume of the tissue mass, the faster the regeneration of the mass, in the case that the volume of the ectodermal fragments were equal to the endodermal fragment. Neither 25–100 mass nor 25–75 mass differentiated any organs, though the volumes of both masses were more than 50–50 masses. This suggested that an excess of the endodermal cells hindered the regeneration of the mass.

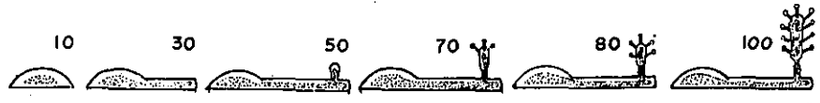


Fig. 3. Six grades of regeneration to which arbitary numerical values were assigned.

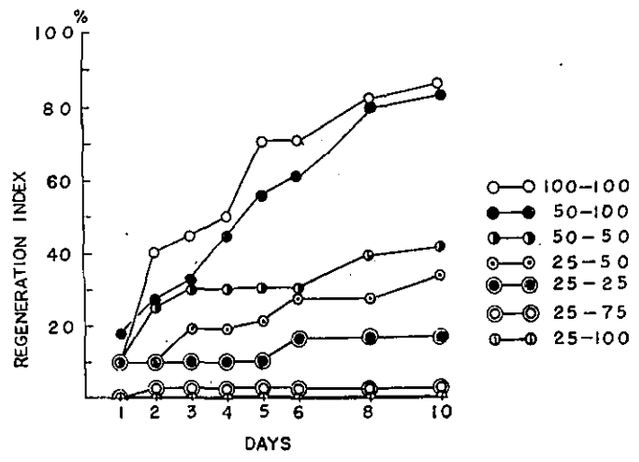


Fig. 4. Record of the regeneration index of various combined masses.

## SUMMARY

1. The ectodermal fragment of *Obelia plana* and *Coryne pusilla* regenerated the endoderm and formed the complete hydranth.



2. Histological observations indicated that the new endodermal cells originated from the interstitial cells.

3. The endodermal fragment disintegrated after isolation.

4. Mesogloea membrane is not important for the regeneration of the endodermal fragment.

5. When the endodermal fragment came into contact with the ectodermal fragment, the former was incorporated by the latter.

6. An excess of the endodermal cells hindered the regeneration of the tissue mass.

7. In conclusion, it was shown that the ectodermal cells have a high morphogenetic potency in comparison with the endodermal cells.

## REFERENCES

HALE, L.J., 1964. Cell movements, cell division and growth in the hydroid *Clytia johnstoni*. J. Embryol. exp. Morph., **12**; 517–538.

ZWILLING, E., 1963. Formation of endoderm from ectoderm in *Cordylophora*. Biol. Bull., **124**: 368–378.