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The Fate of Aggregates formed by Two Species of Hydra (*Hydra magnipapillata* and *Pelmatohydra robusta*)¹⁾

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(With 2 Text-figures, 3 Tables and 1 Plate)

Since the classical work by Wilson (1907) in sponges, reconstitution of a new whole animal by reaggregation of isolated cells has been the subject of many investigators' interest. However, no one has ever been successful in obtaining similar results with other animals, although the phenomenon bears an obvious relationship to the reaggregation of embryonic cells in higher organisms. Instead, in hydra reunion of small cut pieces has been studied by several investigators (Issajew, 1926; Weimer, 1934; Papenfuss, 1934), partly because the culture technique of isolated hydra cells has not been developed yet. One of these workers further attempted to obtain regenerates from a mixture of tissue fragments that originated from two different species (Papenfuss, 1934). She reported that tissue fragments of the green and brown hydra unite but that all the reunion masses eventually degenerated.

The present author re-examined Papenfuss' experiment, using *Hydra magnipapillata* and *Pelmatohydra robusta*, and obtained the results somewhat different from hers. The purpose of this report is to describe the morphogenetic process through which the tissue aggregates develop into a new organism and the fate of tissue fragments of different origins after the reunion.

Materials and Methods

The animals used in this experiment were from clones of *Hydra magnipapillata* and *Pelmatohydra robusta*. All the specimens were cultured by the method of Loomis and Lenhoff (1956) and fed daily with *Artemia salina* nauplii. The temperature of the culture solution was maintained at approximately 18°C. Well-fed hydra, 24 hours after feeding, were used as materials in the present study. For this experiment, two-hundred such individuals of each species were used. To exclude too much specialized tentacles from tissue masses, the hypostome

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including all the tentacles was removed prior to preparation of tissue fragments. Also, peduncle and basal disk were removed. The remaining tissues of both species were cut with a pair of scissors to many small fragments that usually contained epidermis, gastrodermis and mesoglea while they were kept in the physiological salt solution of Haynes and Burnett (1963), or the "HB solution". Such fragments were separated to two groups by sifting them through meshes of stainless steel of two different sizes; 100–150 μ and 200–500 μ in diameter, respectively. They were then transferred to a conical centrifuge tube filled with the HB solution and left there for 30 minutes allowing them to aggregate at the bottom of the tube. Then, gentle agitation of the solution was made with a small pipette in order to loosen the aggregate's adhesion to the centrifuge tube, and they were transferred to a petri dish filled with the HB solution. In twenty hours, they were again transferred into the bicarbonate-versene-tap water (BVT) (Loomis and Lenhoff, 1956). Observation was made twenty hours after aggregation and thereafter once a day for 77 days. Newly formed animals were fed with newly hatched *Artemia* nauplii every other day since the fourth day of aggregation.

For histological examination, animals were fixed in Bouin's solution for 4 hours. Serial paraffin sections of 5 μ in thickness were made parallel or perpendicular to the long axis of hydra and were stained with modified Azan (Noda, 1968).

Observation

The species of origin of each aggregated tissue fragment was determined by the features of two species of hydra shown in Table 1.

1. *Mosaic animals in living state.* Within thirty minute, five to twelve tissue fragments from the two species adhered to each other to form a tissue-mass, in 20 hours, with a hollow within it. For the following observation, a hundred such aggregates were selected at random. Pink color of the *magnipapillata* tissues and yellowish green color of the *robusta* tissues, due to color of the food inclusions in the digestive cells of both species were well preserved so that each tissue fragment was easily distinguished under a binocular dissecting microscope by its color. The border line between the tissue fragments of different origins became even clearer by feeding. The tissue-masses regenerated three or more tentacles within 2–3 days after aggregation. One or two days later they adhered to the bottom of the dish. The body column was 600–1200 μ in elongated condition. The *robusta* tissue always participated in the formation of hypostome and tentacles (Text-fig. 1). There was, however, an exceptional case where the formation of hypostome and tentacles was performed solely by the *magnipapillata* tissue and where the *robusta* tissue formed peduncle and basal disk. Sometimes two or three individuals were formed from one tissue-mass. When they were fed with *Artemia* nauplii on the fourth day for the first time one-fifth of the population failed to ingest the food. However, on the sixth day all the specimens succeeded in ingestion of *Artemia*.

Table 1

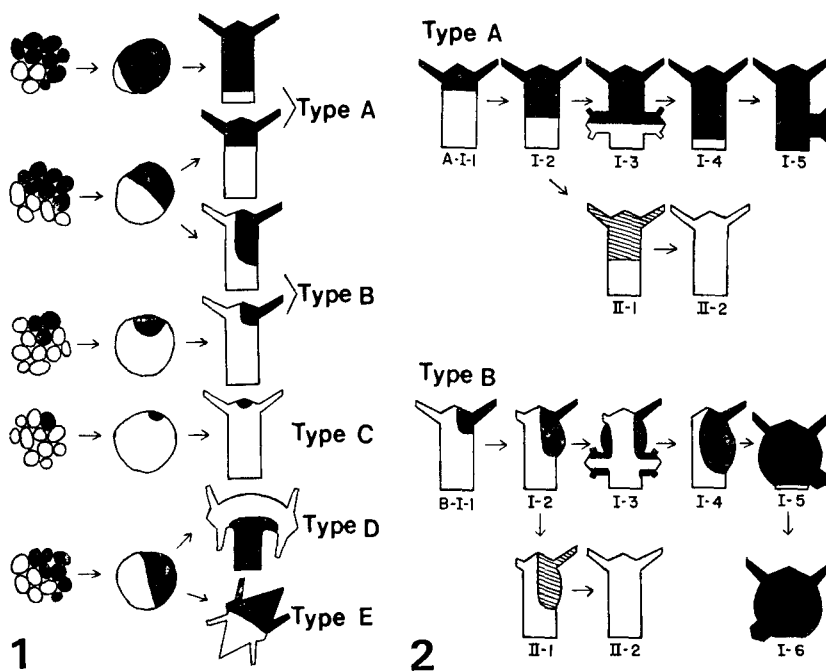
Species		<i>Pelmatohydra robusta</i>	<i>Hydra magnipapillata</i>
Epithelio-muscular cell		Cytoplasm extremely vacuolated, with mucous granules stained with aniline blue.	Cytoplasm not highly vacuolated, containing mucous granules stained with aniline blue and azocarmine.
Glandulo-muscular cell		Containing mucous granules stained with aniline blue.	Containing mucous granules stained with azocarmine.
Streptline glutinant (Ito, 1947 a, b).		The thread coils longitudinally.	The thread occupying the upper half of the capsule coils about four times transversely, while the remaining lower half is entangled.
Food inclusions in living state		Droplets of the food inclusions are yellowish green to green in <i>robusta</i> and rather pink in <i>magnipapillata</i> . Besides these droplets, both species have red, irregular shaped inclusions in digestive cells. The yellowish green color of <i>robusta</i> and the pinkish color of <i>magnipapillata</i> , therefore, depend on the color of food inclusions contained in the digestive cells.	
Histology of food inclusions in	hypostome-budding region	9.2-10.8 μ in diameter	5.3-6.7 μ in diameter
	peduncle	3.3-4.2 μ in diameter and concentrated in the apex of digestive cells.	2.5-4.2 μ in diameter and dispersed throughout digestive cells.
	basal disk	About 2.5 μ in diameter	Granular food inclusions are seldom observed in digestive cells of basal disk.

From 100 mosaic masses 128 individuals developed, and they were divided into five groups on the basis of the combination pattern of the tissues originated from the two species (Text-fig. 1). Type A (forty animals): At least, the tentacles, hypostome and subhypostomal region were formed by the *robusta* tissues. Type B (fifty-two animals): The tissue of *robusta* occupied a part of hypostome and subhypostomal region. Type C (twelve-eight animals): The tissue of *robusta* occupied only the hypostome including one or two tentacles. Type D (one animal): The two heads made of the *magnipapillata* tissue regenerated adjacent to the *robusta* tissue. Green colored *robusta* tissue formed the peduncle and the basal disk by which it attached to the bottom of a petri dish. This was the only case in which the tissue of *robusta* did not form the hypostome. Type E (three animals): Two heads developed in the area where the tissues of the two species united.

When the time needed for formation of the hypostome and tentacles of mosaic animals was compared to that of animals developed from the aggregates made up

of only *robusta* or *magnipapillata* tissues, the former in Groups B, C, and E was longer than the *robusta* aggregates and shorter than the *magnipapillata* aggregates (Table 2). To see the fate of these mosaic animals, fifty animals (twenty-five animals each from Types A and B) were selected at random and examined.

In twenty-four Type A specimens, pink-colored (*mag*) part gradually moved downward and it disappeared completely within 24–55 days (Text-fig. 2). In twenty Type B specimens, pink-colored (*mag*) tentacles began to contract since about 14 days after the aggregation and disappeared within 20–30 days. Thereafter,



Text-figs. 1 & 2. Schematic presentations of various types of aggregation of *mag* (white) and *rob* (black) tissues. Shaded is the portion of *rob* tissue which were temporarily orange in color. For further explanation, see text.

pink-colored area of the body column gradually became narrower and disappeared completely within 50–77 days. In a Type A animal and five Type B specimens the color of *robusta* tissues changed from green to orange and again to pink following feeding. After that, it was not possible to distinguish the tissues of both species by their color in live animals.

2. *Histology.* Histological observation of the total 64 specimens fixed in 3–77 days after aggregation revealed that any region of the body including tentacles

Table 2. The number of hydra, *Pematohydra robusta*, *Hydra magnipapillata* and their mosaic, which bear outpushing tentacles.

Days after aggregation	Origin of hypostome and tentacle tissue	<i>rob</i>	mosaic	<i>mag</i>
2 days	<i>rob</i>	54	39 (Type A)	—
	<i>rob & mag</i>	—	4 (Type B)	—
	<i>mag</i>	—	0	0
	non-tentacle	10	85	52
3 days	<i>rob</i>	64	40 (Type A)	—
	<i>rob & mag</i>	—	83 (Type B,C,E)	—
	<i>mag</i>	—	0	10
	non-tentacle	0	5	42
4 days	<i>rob</i>	64	40 (Type A)	—
	<i>rob & mag</i>	—	87 (Type B,C,E)	—
	<i>mag</i>	—	1 (Type D)	24
	non-tentacle	0	0	28

may be formed as tissue mosaic. Pattern of the mosaic in the gastrodermis was consistent with that observed in living animals by color, whereas in the epidermis the pattern histologically revealed and that in live specimens do not always coincide.

Four out of seven completely green specimens (Text-fig. 2, A-1-5 and B-I-6) and all of five completely pink specimens (Text-fig. 2, A-II-2 and B-II-2) had their epidermis comprising from both *robusta* and *magnipapillata* tissues, whereas the gastrodermis was made up from tissue of either *robusta* or *magnipapillata* only in all of these nine specimens. In such epidermis the interstitial cells and the developing cnidoblasts from the subordinate species decreased remarkably in number (Table 3). However, in the epidermal tissue from subordinate species mitotic figures were recognized only in epithelio-muscular cells, which were sometimes characterized by vacuolization of their cytoplasm near the corresponding cells of dominant species (Fig. 6). In many cases, the epidermis of depressed species was partly covered with that of the other species. In the specimens where the gastrodermal cells from the two species comprised part of the body, the rate of occurrence of each epidermal cell type was the same as in intact hydra.

Further attention was focused on the problem of species specificity in the tissue adhesion. Gastrodermis, or the digestive cells and muscle, of the *robusta* and the *magnipapillata* origins were connected thoroughly with each other within 20

hours after aggregation and never separated thereafter (Figs. 3-5). Epidermis from the two species at first come into contact with each other in the base of epithelio-muscular cells and epidermal muscle, but later they were merely connected by their muscle (Figs. 3-4 and 6-7). In another case, epidermis of one species was found tightly connected with gastrodermis of the other species by the muscle and the mesoglea (Fig. 6-7). Connection of mesogleas from two species took place always after the completion of union of two cell layers, and once connected the origin of mesoglea could not be determined. It was confirmed that the nematocyst (streptline glutinant) of any one of the two species could be contained in tissues from the other species.

Table 3. The number of epidermal cells comparable to 250 epithelio-muscular cells in the gastric region of *magnipapillata* (*mag*) and *robusta* (*rob.*)
Counting was made on one specimen only in each group.

Stage	Control		A-I-5		B-II-2			
Species	<i>mag</i>	<i>rob</i>	<i>mag</i>	<i>rob</i>	<i>mag</i>	<i>rob</i>	<i>mag</i>	<i>rob</i>
Number of epidermal cells	1688	1888	65	1169	1125	37	530	13
Days after becoming completely pink or green			14 days		14 days		30 days	

3. *Budding*. During the earlier stage (Text-fig. 2, A-I-2 - A-I-3 and B-I-2 - B-I-4) some mosaic (pink and green) buds were observed but later only the green colored buds were produced. A completely pink colored bud was never produced in any animal. Since stage B-I-5, animals became rather sphere in shape, and they oftenly produced buds near the basal disk. Histological observation of such animals revealed that many interstitial cells and developing enidoblasts were present among the glandulo-muscular cells of the basal disk.

Discussion

Burnett (1961, 1962) performed the grafting experiments between the two different species of hydra to study the polarity, and found that the grafted portions invariably separated. In the present study, however, the tissues of different origins in a mosaic animal never separated but formed any region of the body from tentacles to basal disk as the mosaic of tissues, although the tissue from one of the two species (mostly *magnipapillata*) of the mosaic hydra was gradually sent into a state of depression. These results suggest that there is a certain degree of compatibility between the tissues of *magnipapillata* and *robusta*.

In almost all the aggregates, the part of a mosaic that contained the *robusta* tissue formed the head region. The result is considered to be due to the difference

in their regenerative potency between the two species. The present author found that, when cut horizontally at the level just beneath the hypostome, a new hypostome and small tentacle buds were formed within 19 hours in *robusta* and in 43 hours in *magnipapillata*, respectively, at 20–22°C (Noda, unpublished). In the case where the head region was mosaic, the *magnipapillata* tissue formed hypostome and tentacles earlier than the aggregates from only the *magnipapillata* tissue did (Table 2). The result seems to suggest that the formation of hypostome and tentacles by the *magnipapillata* tissue in mosaic hydra may be promoted by the *robusta* tissue.

The reason why in most case *magnipapillata* tissue of a mosaic hydra was sent into a state of depression is unknown. However, in this connection, the author found that in a mixed culture of the two species of hydra the *magnipapillata* specimens invariably suffered (Noda, 1970).

Summary

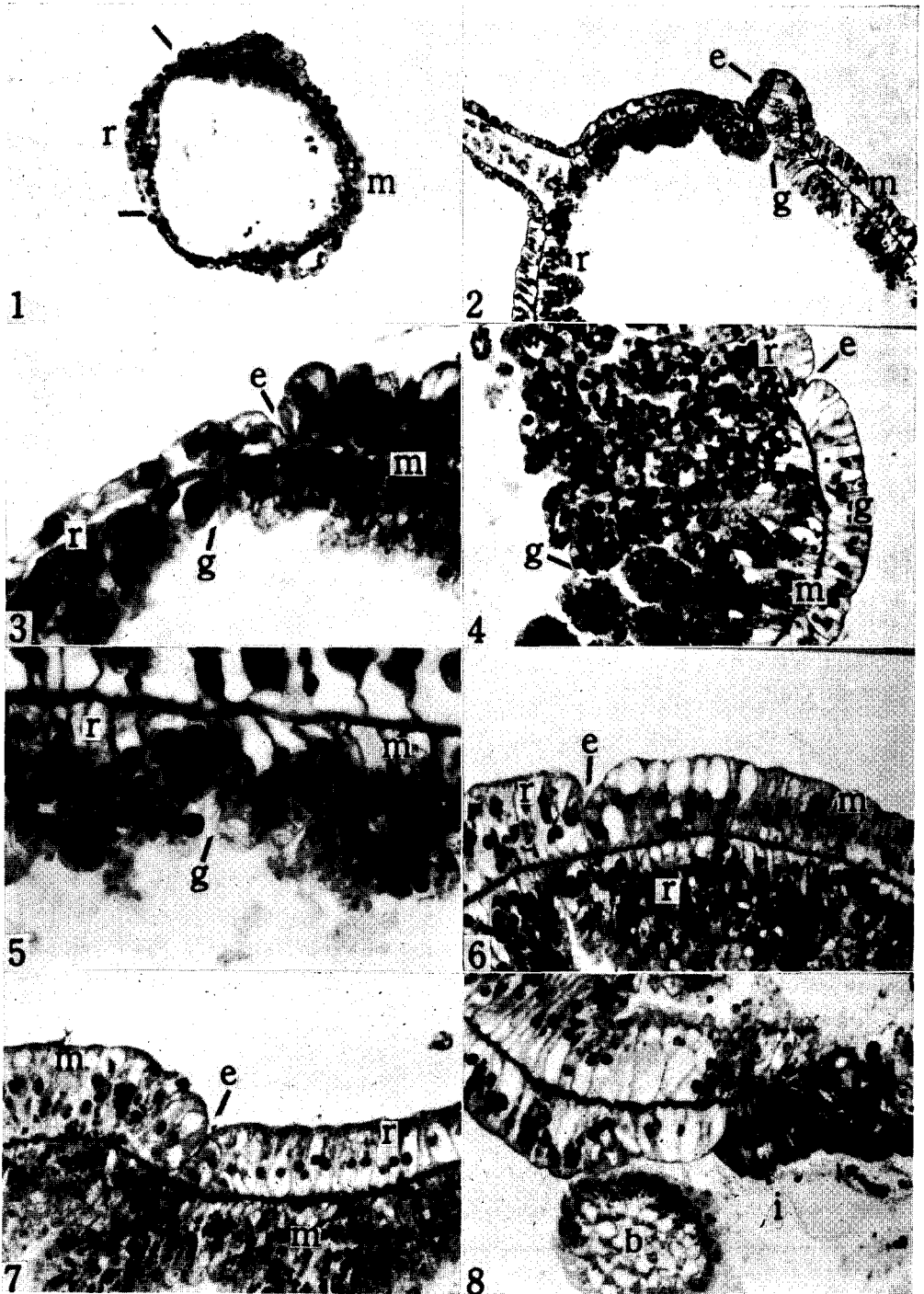
An adhesion occurred easily between the fragments of the two different species of hydra, *Hydra magnipapillata* and *Pelmatohydra robusta* and the aggregates developed into a new whole animal consisting of the tissues from the two species throughout the body column and tentacles.

Although the tissues from the two species in a mosaic hydra never separated, the *magnipapillata* tissue was sent into a state of depression in most cases. In such depressed epidermis, the interstitial cells and developing cnidoblasts decreased markedly in number and mitotic figures were recognized only in epithelio-muscular cells. At least, the nematocyst (streptline glutinant) of one of the two species could be incorporated into the tissue of the other species.

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K. Noda: Mosaic Hydra

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Explanation of Plate VI

Figs. 1-7 are photomicrographs showing aggregation of tissue fragments from two different species of hydra in various stages of mosaic formation. arows e (epidermis) and g (gastrodermis), the borders of tissues from two species. m, *magnipapillata* tissue; r, *robusta* tissue. Modified Azan stain.

Fig. 1. The tissue-mass, with a cavity within it, 20 hours after aggregation. arrow, the border of tissues from the two species. $\times 70$.

Fig. 2. The distal part of mosaic hydra 4 days after aggregation. The right tentacle is formed as a mosaic of tissues. $\times 150$.

Fig. 3. A photomicrograph of the border of tissues from the two species 20 hours after aggregation. $\times 400$.

Fig. 4. A longitudinal section of a mosaic specimen 12 days after aggregation. Gastrodermis of the two species are clearly distinguished by the size of food inclusions in digestive cells. $\times 150$.

Fig. 5. Gastrodermis in the gastric region of a mosaic hydra 24 days after aggregation. Digestive cells from the two species are thoroughly connected with each other. $\times 400$.

Figs. 6-7. Photomicrographs showing the tissue fragments from the two species on the 55th day of aggregation. Note the characteristic vacuoles in epithelio-muscular cells of *magnipapillata* (m) (Fig. 6). No interstitial cells are found in the epidermis of the species different from the one occupying the gastrodermis (m in Fig. 6, r in Fig. 7). Epithelio-muscular cells from the different species are either connected only in their base (Fig. 6) or completely separated (Fig. 7). $\times 200$.

Fig. 8. Many interstitial cells (i) are found in the basal disk of parental hydra of B-I-6. b; basal disk of bud. Modified Azan. $\times 200$.