On the Incompatibility of Two Species of Hydra, *Hydra magnipapillata* and *Pelmatohydra robusta*, in a Mixed Culture[1)]

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(With 4 Text-figures)

An adhesion occurred easily between the fragments of two different species of hydra, *Hydra magnipapillata* and *Pelmatohydra robusta*, and the aggregates developed to form a new whole animal consisting of tissues from the two species throughout the body column and tentacles (Noda, 1970). Tissues from the two species in a mosaic hydra never separated and the newly formed animal even produced mosaic buds, although in such mosaic animals tissues of one species (in most cases *magnipapillata*) were gradually sent into a state of depression and at last disappeared completely.

In order to elucidate the cause for depression of *magnipapillata* tissues in a mosaic hydra, the present author has studied the interspecific relationship between the two species of hydra in a mixed culture.

Materials and Methods

The animals used in the present study were from clones of *Hydra magnipapillata* and *Pelmatohydra robusta*. They were cultured by the method of Loomis and Lenhoff (1956) and fed daily with *Artemia salina* nauplii. Animals starved for 24 hours were selected at random from the mass cultures and were employed for experimental use. As the culture solution, BVT-solution (bicarbonate-versene-tap water), maintained at approximately 20°C, was used. In the present study, 2 ml and 6 ml of BVT were poured in small petri dishes of 2.6 cm and 4.0 cm in diameter, respectively. Observation of hydra in living state was done under the binocular dissecting microscope.

For histological study, animals were fixed in Bouin's solution for 4 hours, and serial paraffin sections of 5 μ in thickness were stained by modified Azan stain method (Noda, 1968) and in 0.5% toluidine blue solution buffered at pH 6.0. The toluidine blue stain was applied for 30 seconds and dehydrated in t-butyl alcohol.

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Experiments

At first, two species of hydra, *Hydra magnipapillata* (*mag*) and *Pelmatohydra robusta* (*rob*) were mixed in the following ratios; 1) forty *mag* and five *rob*, 2) fifteen *mag* and five *rob*, 3) ten *mag* and ten *rob*, 4) five *mag* and fifteen *rob*, 5) five *mag* and forty *rob*. They were placed in a finger bowl containing 100 ml BVT ketp at 20°C. When the tentacles of *rob* came to contact with *mag*, the latter contracted its tentacles and body column and was held in the tentacles of the former. On the other hand, *rob* appeared to be normal in all groups.

In the next, one hundred *rob* and ten *mag* or ten *rob* and one hundred *mag* were placed in a glass dish, 11 cm in diameter, containing 100 ml BVT. Remaining in the same dish, specimens of both species, however, were separated by a screen of carbasus absorbent gauze. Without affecting each other they remained there for four days at 20°C, suggesting that no chemical substance was transmitted from one species of hydra to another through the culture medium to cause the damage.

In order to see the nature of the damage inflicted in hydra in a mixed culture the following experiments were performed.

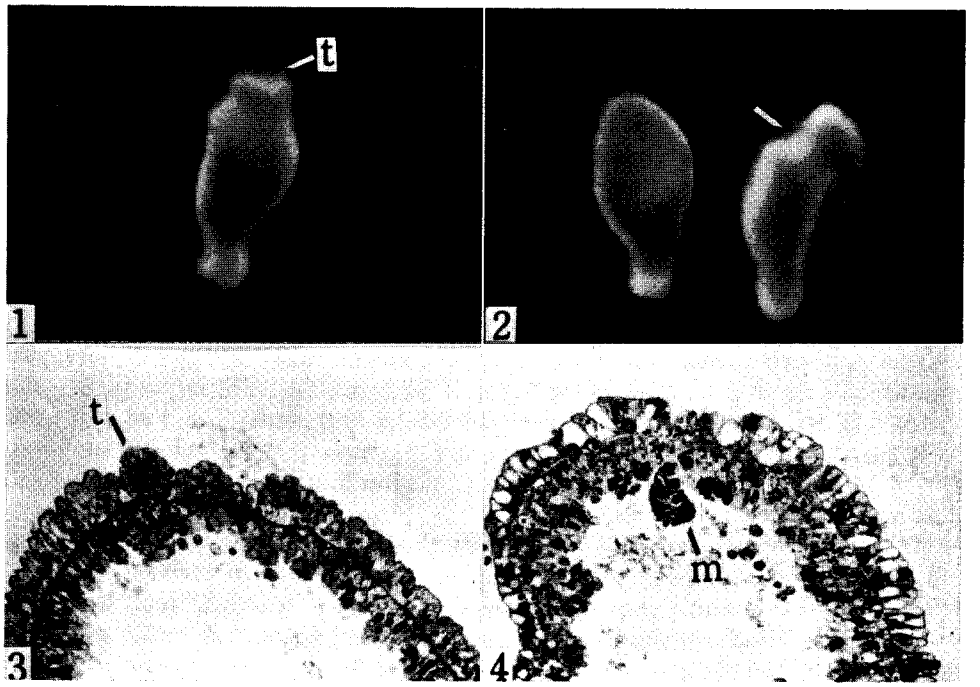
1. Mixed culture of *mag* and *rob*.

For this experiment, five intact *mag* and ten or thirty intact *rob* were put together in a small petri dish containing 2 ml BVT or 6 ml BVT, respectively. As controls, fifteen or thirty-five *mag* were put into dishes containing 2 ml and 6 ml BVT, respectively. BVT was renewed every 24 hours and the temperature was kept at 20–22°C throughout the experiment. After four days of observation, *mag* were fixed in Bouin's solution.

When the tentacles of *rob* attached *mag*, the latter contracted its body column and tentacles and was held by tentacls of the former. Usually *rob* set loose *mag* after a few minutes, but in this overcrowded condition *mag* was held soon by other *rob*'s tentacles. Such an event was repeated again and again, and within 24 hours all *mag* were sent into a state of depression, their body column and tentacles being contracted. Three specimens were even constricted in their subhypostomal region and the tentacles of eleven *mag* were retarded to merely knobs. One animal died within 24 hours. The damage of *mag* in mixed culture increased in degree with the lapse of time. After four days the body columns in ten out of thirteen survivors were irregular in shape and no tentacles were detected histologically in eight specimens. All *rob* specimens in the experimental group and *mag* in the control group did not show any damage throughout the same period of time. The degree of damage suffered by *mag* in the mixed culture was even greater when five *mag* were maintained with thirty *rob*.

Histological observation. In *mag* with degraded tentacles, no visible damage was detected histologically except in the hypostome, where cells of both layers became disorganized in their arrangement (Fig. 3). On the other hand, various degrees of

damage were seen in *mag* which lost tentacles. Developing cnidoblasts were scarce and interstitial cells were found only near the mesoglea (Fig. 4). A few mitotic figures were observed only in the interstitial cells. Hypostomal mucous cells decreased in number and their arrangement became disorganized. Mouth opening was hardly recognizable. Mesoglea in the distal part of animals often became discontinuous so that two cell layers were directly contacted with each other. In all cases the distal part suffered a greater damage than proximal part did.



Figs. 1 and 2. Unretouched photographs of *Hydra magnipapillata*, either with retarded tentacles (m) (Fig. 1) or with no tentacles (Fig. 2), which had been left in a mixed culture with *Pelmatohydra robusta*. Subhypostomal region of such animals is sometimes constricted (Fig. 2, indicated by an arrow). ×9.

Figs. 3 and 4. Photomicrographs of transverse sections of some damaged *Hydra magnipapillata*. Fig. 3. The distal (upper) region with degraded tentacle (t). The hypostomal cells are arranged irregularly. Modified. Azan. ×100. Fig. 4. Epithelio-muscular cells are highly vacuolated, hypostomal mucous cells (m) are few in number, and interstitial cells are present only near the mesoglea. Toluidine blue stain. ×100.

II. Culture of whole *mag* together with portions of *rob*.

Experiments with the rob *hypostome including tentacles and body column.* Cut horizontally at the level just beneath the hypostome with a scalpel, each of the

distal (upper) and the proximal (lower) parts of fifteen *rob* were put in a dish containing five intact (whole) *mag* in 2 ml BVT, respectively. During the observation that lasted 24 hours after amputation, the temperature was maintained at 18°C. No formation of tentacle buds was observed of the proximal part of *rob* maintained in this condition for at last 24 hours.

When *mag* came to contact with *rob*'s tentacles, the former suffered severe damage. Body columns of all *mag* were contracted and their tentacles became degraded to knobs within 24 hours.

No damage was noticed in *mag* that were maintained with lower part of *rob*. It was, therefore, surmised that all the damage in *mag* was caused by *rob*'s tentacles, possibly by their nematocysts.

Experiments with the tentacles and the hypostome without tentacles of rob. Hypostomes of *rob* obtained in the procedure just described were further cut at their tentacular joint with a scalpel under a dissecting microscope. Two hundreds tentacles and thirty hypostomes bearing no tentacles of *rob* were put in a dish containing five intact (whole) *mag* with 2 ml BVT, respectively. In order to prolong the effect of the *rob* hypostome without tentacles on *mag*, the regeneration of tentacles was delayed by keeping the hypostome tissue at 13°C.

Severe damage was inflicted to *mag* by *rob*'s tentacles alone, whereas the *rob* hypostome without tentacles did not affect *mag* tissue.

Experiment with rob*'s hypostome with tentacles that had discharged nematocysts previously.* A great number of *Artemia* was given four times repeatedly within 6 hours to the cut hypostome of *rob* including tentacles, so that almost all the nematocysts were supposedly discharged. The cut hypostome was prepared by separating the part carefully at the level just beneath the hypostome so that no supply of newly formed nematocysts to the tentacles seemed possible. The observation was made for 24 hours at 18°C.

No damage was given to *mag* by *rob*'s hypostome thus prepared. Instead, when they came to contact *mag*'s tentacles held *rob*'s hypostome yielding no visible damage on either species.

Discussion

Kato *et al.* (1962, 1963, 1967) and Chiba and Kato (1966), working on the interspecific relationship among three marine hydrozoan species, found that the regeneration from tissue pieces and the colony growth of *Cladonema radiatum* and *Clytia volubilis* were inhibited by the co-existing *Bougainvillia* sp. These authors were of the opinion that these phenomena were caused not only by the metabolite of the dominant species but also by the direct contact with the stolons of the dominant species. In the present experiments, however, no visible changes occurred in cut pieces of *mag* which were kept from direct contact with *rob* tentacles, thus producing no evidence to support the view that the metabolite of the dominant

species is responsible for inhibition of regeneration in subordinate hydra. Further works seem to be necessary for establishing this view in hydra.

Results obtained in the present investigation show that the damage made on *mag* in mixed cultures was caused by the nematocysts of co-existing *rob*. As the *rob* tissue never showed damages in the same condition, experiments are now being undertaken in our laboratory to clarify why *mag*'s nematocyst toxin is ineffective of the *rob* tissue.

The dominancy of *rob* in mixed culture was explained in terms of nematocyst toxin released in the same culture. However, the present writer (Noda, 1970) found in the mosaic aggregates of *mag* and *rob* tissue fragments that the *rob* tissue was almost always dominant, forming the hypostome and tentacles. He also found that such a combination of tissues from two species promoted the subordinate hydra's ability to form its own hypostome and tentacles. It is, therefore, suggested that the *rob* tissue has a higher basal metabolic rate than the *mag* tissue. It was not decided whether the dominancy of *rob* tissue in the aggregates and that shown by *rob* individuals in mixed cultures are more than coincidence.

Summary

In a mixed culture of *Hydra magnipapillata* (*mag*) and *Pelmatohydra robusta* (*rob*), the former always suffered varied degrees of damage, yielding degraded tentacles, constricted body column, or sometimes died. Separated *rob* tentacles alone could inflicted the damage in co-existing *mag* while the tentacles with discharged nematocysts could not. The results are discussed in relation to the author's previous work (Noda, 1970) on the development of aggregates of tissues from the two species.

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