TAIL SHORTENING OF THE LARVAE OF AN ASCIDIAN, HALOCYNTHIA RORETZI (v. DRASCHE), SEPARATED AT VARIOUS POSITIONS AFTER STIMULATION TO METAMORPHOSE

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TAIL SHORTENING OF THE LARVAE OF AN ASCIDIAN, *HALOCYNTHIA RORETZI* (v. DRASCHE), SEPARATED AT VARIOUS POSITIONS AFTER STIMULATION TO METAMORPHOSE[1)]

By

TAKAHARU NUMAKUNAI
沼宮内　隆　晴

Marine Biological Station of Asamushi, Tôhoku University, Aomori city, Japan

INTRODUCTION

Hirai (1961) reported that when the larvae of *Halocynthia roretzi* were separated into two parts at various positions before treatment to initiate metamorphosis, the posterior part showed no sign of metamorphosis. He concluded from his experiments that the adhesive papillae are the receptors of the stimulus to metamorphose. According to Oka (1961), when the tails of compound ascidian larvae were cut off immediately after stimulation, the isolated tails could contract actively. On the other hand, Cloney (1961) stated as a hypothesis on tail resorption that after the stimulus to metamorphose is received a proteolytic enzyme is secreted at the base of the tail, and it gradually moves towards the tip of the tail. Numakunai *et al.* observed that the original axes of the epidermal cells and muscle cells are oriented in the shape of a fan at the base of the tail at the beginning of the tail resorption in the larvae of *Halocynthia roretzi* (Numakunai *et al.*, 1964). This may suggest the important role of the base in the tail resorption. But to date no direct evidence has been demonstrated on this problem. The present experiments were done to confirm which part of the larva is most concerned with the tail resorption.

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MATERIAL AND METHOD

In the present experiments the larvae of *Halocynthia roretzi* were used. Adult animals were collected in the vicinity of the Marine Biological Station of Asamushi, Aomori City, in October. They were kept in glass tanks, in which the gametes were obtained by natural spawning. The embryos were kept in large Petri-dishes

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at 11–13°C. The larvae were used about 12 hours or more after hatching (Hirai, 1963). After the beginning of tail resorption the larvae were excised at four positions, Position I is anterior to the brain vesicle, Position II is posterior to the brain vesicle, Position III at the proximal region of the tail and Poisition IV at the mid part of the animal (see Fig. 1). To cause the metamorphosis the larvae were immersed in sea water to which nile blue diluted to 1:100,000 was added. Only the larvae which showed ruptures of notochordal ends within five minutes after immersion in the sea water were used. Usually it requires 20 to 30 minutes to complete the tail resorption in intact larvae after treatment to initiate metamorphosis. In the present experiments there was no difference observed between the larval length after 1 hour and after 2 hours. To express the degree of shortening of the tail the ratio of the length from the cut surface to tail tip 1 hour after cutting to that when cut was given. In Positions I, II and III excision was done when the larvae reached about 92 per cent of full length, and in Position IV two series of experiments were made. The first was performed when the larvae reached 92 per cent of full length as in Poisitions I, II and III, the second was when the larvae reached about 85 per cent of full length. As the control intact larvae were used, the ratio between length after completion of the tail resorption to full length was given. In each position ten larvae were excised. The experiments were performed at 11–13°C.

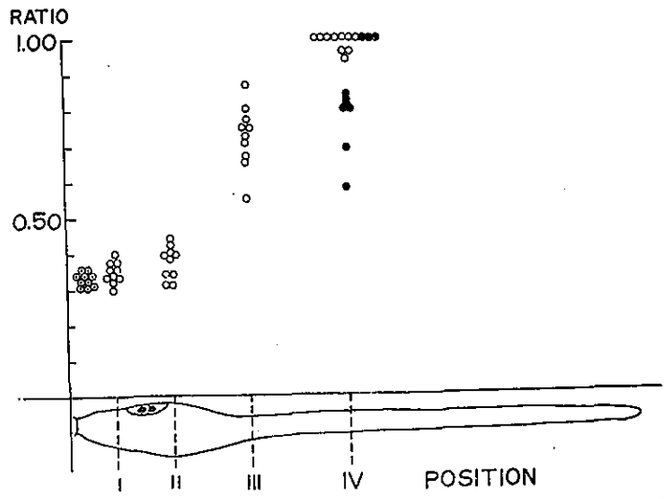

Fig. 1. The ratio of the length from the cut surface to tail tip one hour after cutting to that when cut. ⊙: Control, ○: Experiments when shortened to 92 per cent, ●: Experiments when shortened to 85 per cent in Position IV.

RESULTS

Control. All ten specimens were shortened to about 33 per cent of the original length.

Position I. The larvae were excised at a position anterior to the brain vesicle. Some of them did not shorten as much as the control, but seven out of ten larvae showed tail resorption to the same extent as the control.

Position II. The larvae were excised at a position posterior to the brain vesicle. In this position approximately the same shortening as in Position I was observed. Namely, in 4 specimens shortening proceeded to about 65 to 69 percent, but in the other six to 57 to 60 per cent of the length when cut.

Position III. The larvae were excised at the proximal region of the tail. At this position the degree of shortening decreased remarkably. The maximum shortening was about 44 per cent of the length when cut, and the minimum at 13 per cent.

Position IVA. The larvae were excised slightly posterior to Position III, when they reached about 92 per cent of full length. In this experiments the tails did not show shortening, only three out of ten shortened to about 95 per cent of the length when cut.

Position IVB. The larvae were excised at the same position as in Position IVA, when the larvae reached about 85 per cent of full length. The maximum shortening was about 40 per cent of the length when cut, but three specimens did not show any sign of shortening.

These results are summarized in Figure I. When shrotening occurred in the tails which were cut off at Positions III and IV, the sign of shortening such as thickening appeared near the cut surface and proceeded towards the distal end of the tail. Sometimes it was observed that the tail ends were pulled inward. The anterior parts which were cut off at Positions III and IV showed signs of normal metamorphosis.

DISCUSSION

The larvae which were cut off at Positions I and II showed tail resorption to the same extent as in intact larvae. There was no remarkable difference between Positions I and II. This may suggest that the region anterior to Position II has no effect on tail resorption. Though the tails which were cut off at the proximal region (Position III) shortened to some extent, they did not become as short as in Positions I and II. This may mean that some factor located in the region between Positions II and III is closely related to tail resorption. In Position IV, it was suggested that a part of the tail which did shorten a little became shorter as the tail resorption proceeded. Moreover, in the tails which were cut off at Positions III and IV the signs of shortening such as thickening appeared always near the cut

surface of the tail and moved towards the distal part. This suggests that some factor causing tail shortening passes from the region between Positions II and III to the tail end gradually as the trail resorption proceeds. Though the degree of the shortening decreased remarkably at Position III, shortening proceeded to a conspicuous extent in the present experiments. The borderline between the point at which the tail resorption is possible or impossible was not detected. This may be due to the time factor after the beginning of tail resorption. A method of initiating metamorphosis of the larvae after removal of the adhesive papillae has not been found in this species.

Cloney (1961) proposed as a tentative hypothesis on the tail resorption of the ascidian larvae that a proteolytic enzyme is secreted at the base of the tail which causes the breakdown of the notochordal sheath and intercellular cementing substance. The enzyme moves gradually towards the tip of the tail. Numakunai *et al.* (1964) observed in *Halocynthia roretzi* that the original axes of the epidermal cells and muscle cells are oriented in the shape of a fan at the region between Positions II and III. In the present experiments the substance that moved towards the tail was not detected, but it may be that the base of tail has an important role in tail resorption, some of factor which causes the tail to contract moves from this region to the distal part of the tail.

Further experiments should be carried out to discover the factor that causes the tail shrotening and moves distally.

SUMMARY

1. The larvae of *Halocynthia roretzi* were excised at four regions after the beginning of the tail resorption. These were anterior to the brain vesicle, posterior to the brain vesicle, basal part of the tail and mid way along the length of the larva. The larvae which were excised at Positions I and II showed tail resorption to the same extent as in intact larvae. In Poisition III the degree of shortening decreased considerably. In Position IV two series of experiments were done. The first was excision of the tail when the larvae had reached about 92 per cent of full length, and the second was done when the larvae had reached 85 per cent of full length. In the first experiment the tails shortened little, but as the tail resorption proceeded, shortening to some extent was observed.

2. Sign of tail shortening such as thickening appeared near the cut surface of the tail and moved distally.

3. It may be thought that the region between Positions II and III is important in relation to tail resorption and that some factor that causes tail shortening passes from the basal region of the tail to the distal part as was stated by Cloney (1961) in his hypothesis.

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