

# 琉球大学学術リポジトリ

## 1.メダカの尾鰭の再生に及ぼすヘパリンの効果について

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## Effect of Heparin on the Tail Fin Regeneration in the Medaka, *Oryzias latipes*

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Blood does not coagulate inside blood vessels, but it does outside them. It is because there is heparin in blood vessels and the heparin prevents the blood from coagulating. In consequence, heparin is widely known as a substance which retards blood coagulation.

In recent years, various studies have made it clear that heparin hinders the cell-division. For example, KATAYAMA reported that heparin is functioning as an element which checks the mitosis in the epidermis of the mouse's ear (1957). KANATANI studies the effects of heparin on the occurrence of fission in the *Planaria*, invertebrate, and he also disclosed that heparin retards the fission.

In view of the previous information, the present study was undertaken in order to determine whether or not heparin has also some effects on the tail fin regeneration, in the *Oryzias*, vertebrate.

### Material and Method

The *Oryzias latipes* used as materials were collected in rice fields in Takushi, Urasoe village.<sup>1</sup> The fish were put in a room for two or three days before an experiment, so that they may get accustomed to eating the feed<sup>2</sup> for a gold fish. The regeneration takes place the most rapidly when the tail fin is cut off with a pair of scissors at the spot, two fifths of the tail from the front. (Mori, 1942). Consequently the tail was chopped at the point, two thirds of the tail from the front, at a right angle to the tail, using a pair of Weckels' scissors. The tools which were used for an operation were disinfected by 70 % alcohol. Fish which underwent operations were kept in the disinfected water (boiled water) for a day. While the experiment is going on, one medaka was kept into each glass vessel. Service water, after it was preserved two or three days and weakened by chloroform, was used as culture medium. The experimental fish kept in the heparin solution in the culture room which is 19 degrees to 25 degrees centigrade. While the medaka is being under experiment, the medium was emptied and filled, in once every day.

During this change of the medium, the fish were given food in the water. Since the quantity of heparin was not large enough to have an effect on pH., the volume of heparin was not changed.

The following tests were made in order to decide the thickness of heparin

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<sup>1</sup> Urasoe village is adjacent to Naha which is a capital in the Ryukyu Islands.

<sup>2</sup> The feed sold on the market.

which is used for an experiment. (1) When fish were put into 1 % heparin solution, they lived for only twenty-four hours. Therefore the density of this solution seems to be too strong for the present study. (2) Next, 0.0001 % to 0.1 % heparin solution were tried. This time, the fish looked active and fine throughout the test period. The observations of the regenerated tail fin were made every seven days, and for the measurement of the regenerated tail was used a lower binocular microscope.

### Results and Discussion

I. Effect of heparin on the tail fin regeneration (Heparin was treated one day after the tail was cut off).

Table 1 Effect of heparin on the tail fin regeneration of *Oryzias latipes*, at 15°-20° C, 0.0001-0.1 % heparin was applied for 7 days after the operation. The regenerated tail fin tips were measured every 7 days after amputation. (mm)

Size of fish		Length of regenerating tail fins									
Standard length	Tail fin length	7th day					14th day				
		Control	Experimental				Control	Experimental			
			0.0001	0.001	0.01	0.1		0.0001	0.001	0.01	0.1
20.8	5.04	0.60	0.60	0.60	0.48	0.48	1.20	1.20	1.20	0.90	1.20
20.4	5.04	0.48	0.60	0.48	0.40	0.40	1.00	1.00	1.00	0.80	0.80
20.8	5.04	0.60	0.48	0.48	0.60	0.48	1.20	1.00	1.00	1.00	1.00
20.4	5.04	0.48	0.60	0.40	0.48	0.40	1.00	1.20	0.90	1.20	0.80
20.8	5.00	0.48	0.67	0.60	0.48	0.40	0.90	1.20	1.20	1.00	0.80
Average		0.53	0.59	0.51	0.49	0.43	1.06	1.12	1.06	0.98	0.92

Table 2 Effect of heparin on the tail fin regeneration of *Oryzias latipes*, at 15°-20° C, 0.0005-0.05 % heparin was applied for 7 days after operation. The regenerated tail fin tips were measured every 7 days after amputation. (mm)

Size of fish		Length of regenerating tail fins							
Standard length	Tail fin length	7th day				14th day			
		Control	Experimental			Control	Experimental		
			0.0005	0.005	0.05		0.0005	0.005	0.05
23.2	4.80	0.48	0.48	0.48	0.40	1.00	1.00	0.80	0.80
23.2	5.20	0.60	0.60	0.60	0.48	1.20	1.20	1.20	1.20
23.6	5.60	0.60	0.60	0.48	0.48	1.20	1.00	1.20	0.90
23.2	4.80	0.48	0.48	0.40	0.40	0.80	1.04	0.80	0.80
23.2	4.80	0.48	0.60	0.48	0.34	1.00	1.20	1.00	0.80
Average		0.53	0.55	0.49	0.42	1.04	1.09	1.00	0.90

In this experiment, heparin was added to the culture medium one day after the tail was amputated, and the heparin was supplied to the culture medium for seven more days. The regenerating tail tips are presented in Table 1 and 2. The table shows that the retarding function of the heparin in the regeneration of the tail is obvious in the 0.001% to 0.1% experimental solution. This function of retarding increases as the thickness of heparin increases, and the effect is particularly remarkable in the 0.1% heparin solution. However, also, in the case of 0.0001% and 0.0005% heparin solution promoted the regeneration.

II. Effect of heparin on the tail fin regeneration (heparin was treated seven days after the tail was cut off).

In the previous experiments we have learned that 0.001%—0.1% heparin solution is effective in checking the regeneration. I believe this is because heparin has worked on the amputated part of the tail and has caused it to regenerate slowly.

The second test was tried to determine whether or not heparin still acts in the following stages of regeneration. The fish, after the tail was cut off, was kept in the water seven days or fourteen days. Then heparin was added to the culture medium and observation of the regeneration was made. As to the concentration of the heparin, 0.001%—0.1% solution, which proved to be effective in the last test, was applied. The results of observations made on the seventh, fourteenth, twenty-first and twenty-eighth day are shown in Table 3 and 4. The effect on retarding the regeneration is noticeable in 0.001%—0.1% heparin solution. The above effect increases as the concentration of the heparin solution increases.

It was found that on the tail fin regeneration the retarding effect of heparin was noticeable in 0.001%—0.1% solution, but such effect was not in the solution of 0.0001% and 0.0005%.

Table 3 Effect of heparin on the tail fin regeneration of *Oryzias latipes*, at 15°–20° C, 0.001–0.1% heparin was applied for 7 days after the fish were kept in the water, non-heparin solution, for 7 days. The regenerated tail fin tips were measured every 7 days after their operations. (mm)

Size of fish		Length of regenerating tail fins								
Standard length	Tail fin length	7th day	14th day				21st day			
			Control	Experimental			Control	Experimental		
				0.001	0.01	0.1		0.001	0.01	0.1
22.4	5.00	0.48	1.00	0.90	1.00	0.80	1.60	1.40	1.40	1.20
21.6	5.00	0.44	0.80	0.80	0.80	0.80	1.40	1.40	1.20	1.40
19.2	5.08	0.40	0.80	0.72	0.72	0.72	1.40	1.40	1.40	1.20
21.6	5.00	0.44	0.80	0.80	0.80	0.80	1.20	1.20	1.20	1.40
22.4	5.04	0.60	1.20	1.00	0.90	0.80	1.60	1.40	1.60	1.60
Average			0.92	0.84	0.84	0.78	1.44	1.36	1.36	1.36

Table 4 Effect of heparin on the tail fin regeneration of *Oryzias latipes*, at 15°-20° C, 0.001-0.1 % heparin was applied for 7 days after the fish were kept in the water, non-heparin solution, for 14 consecutive days. The regenerated tail fin tips were measured every 7 days after amputation. (mm)

Size of fish		Length of regenerating tail fins								
Standard length	Tail fin length	14th day	21st day				28th day			
			Control	Experimental			Control	Experimental		
				0.001	0.01	0.1		0.001	0.01	0.1
20.4	5.04	0.90	1.62	1.40	1.40	1.20	2.00	2.08	2.00	2.04
20.8	5.04	0.80	1.20	1.20	1.19	1.20	1.90	1.92	1.80	1.70
21.8	5.60	0.90	1.40	1.40	1.40	1.20	2.20	2.20	2.08	2.00
21.6	5.60	1.02	1.60	1.60	1.60	1.20	2.40	2.00	1.92	2.20
23.2	5.44	1.20	1.80	1.72	1.60	1.40	2.40	2.20	2.00	2.20
Average			1.52	1.46	1.44	1.24	2.18	2.08	1.96	2.03

KANATANI reported that heparin produces effect upon the retarding of fission in the planaria, invertebrate. KATAYAMA also reported that heparin has effect on prohibiting the process of mitosis in the mouse ear epidermis cultured *in vitro*.

The results of the present study were supported by the results of the above investigators. In the early stage of the tail fin regeneration heparin was found to be effective. This effect of the heparin causes the cut bleeding to be active. Consequently, the regeneration of the tail fin seemed to have been delayed at the beginning of the regeneration; however the heparin had also prohibited the tail fin from regenerating in the period after the initial stage.

### Summary

The present study was made about the effect of heparin on the tail fin regeneration in the *Oryzias latipes*, vertebrate. The animals used in the experiments were cultured in the heparin solution for seven consecutive days, but the observations of the fish were made more than seven days. It was found that the regeneration in the tail fin of the fish cultured in the medium containing 0.001 %-0.1 % heparin solution had been retarded. In the case of the 0.0001 % and 0.0005 % solution, the regeneration was promoted.

The lowest possible effective concentration was found to be somewhere between 0.0005 % and 0.001 % under the conditions of the present experiments.

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