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The Effects of Denervation on Caudal Fin Regeneration in the Goldfish, Carassius Auratus (Linnaeus)

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THE EFFECTS OF DENERVATION ON CAUDAL FIN REGENERATION IN THE GOLDFISH, *CARASSIUS AURATUS* (LINNAEUS)

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Master of Science

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Janet Leigh Rowlett

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THE EFFECTS OF DENERVATION ON CAUDAL FIN

REGENERATION IN THE GOLDFISH,

CARASSIUS AURATUS (LINNAEUS)

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Directed by: Drs. Robert D. Hoyt, Larry N. Gleason and Blaine R. Ferrell.

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Goldfish of the wild-strain variety, Carassius auratus, were used in the study of caudal fin regeneration. The purpose of this study was to determine the effects of denervation on caudal fin regeneration.

In the experimental groups, it was evident that denervation did not prevent regeneration, although it was significantly reduced. Statistical analysis using the Duncan's Multiple Range Test showed a highly significant difference (0.01 % level) in the mean percentage of regeneration between the denerved group and the other three groups.

Caudal fins having proximal amputations regenerated at a faster rate than those having distal amputations, producing a higher mean percentage of regeneration.

The results of this study indicated that denervation did not prevent regeneration of the caudal fin in the goldfish and suggested the possibility that this process might be controlled by a combination of several factors.

INTRODUCTION

Since the time of Aristotle, it has been known that many animals possess the ability to repair damage to their bodies. This damage may be a result of natural or experimental causes (Schmidt 1968, Balinsky 1975). The damage may be in the form of a wound that destroys the animal's tissues or it may include the loss of a limb or an organ. The repair of this damage, if possible, is known as regeneration (Balinsky 1975).

Much of the work done in the field of regeneration has been done on amphibians, which are known to regenerate amputated appendages successfully (Liversage 1959, Goss 1969, Balinsky 1975). Regeneration of appendages is not known to occur in mammals. However, when amputation does occur, the wound heals smoothly (Goss 1969).

Teleosts are one of the most diverse groups of vertebrates, not only in body form, but also in variety of appendages, scales, barbels, and fins, which are all capable of regenerating (Goss 1969). The ability of teleosts to regenerate lost parts makes them ideal animals for the study of regeneration.

The phenomenon of regeneration of amputated appendages is very complex, and the systems influencing this process are still under investigation. One control mechanism known to affect regeneration is the endocrine system. It has been

shown to play an important role in regulating and controlling regeneration (Liversage 1963, 1967, 1973). Regeneration ceases completely in some teleosts following removal of the hypophysis (Goss 1969). However, Fortner (1979) found that removal of the hypophysis did not prevent regeneration in the goldfish (Carassius auratus), but it did reduce the rate and the amount of regeneration.

A second system influencing regeneration is the nervous system. The role of nerves has been well established as a critical feature in the process of regeneration. It has been shown that in order for regeneration to occur, there must be an adequate supply of nerves in the area of amputation to stimulate regeneration both in amphibians (Schotte and Butler 1944, Singer 1942a, 1942b, 1943, 1946, 1959, 1960, Kamrin and Singer 1955, Liversage 1959, Goss 1969, Balinsky 1975) and in teleosts (Nabrit 1929, 1931, Goss and Stagg 1957, Goss 1969). Destruction of the nerve supply in the amputated area has been shown to prevent regeneration in reptiles, amphibians, and teleosts (Goss 1954, Kamrin and Singer 1955, Holtzer 1956, Goss 1969, Geraudie and Singer 1979).

In teleosts, the rate of regeneration is proportional to the amount of amputation. The more fin removed, the greater the rate of regeneration (Goss 1969, Weiss 1972). Other factors affecting the rate of regeneration are age, size and species. The younger the fish, the faster the rate of regeneration (Tassava and Goss 1966).

The purpose of this study was to determine the effects of denervation on caudal fin regeneration in the goldfish, C. auratus.

METHODS AND MATERIALS

Seventy wild-strain goldfish were delivered to Western Kentucky University on April 23, 1980, from the Kentucky Department of Fish and Wildlife Cave Run Fish Hatchery in Morehead, Kentucky. The fish, 90-118 mm total length, were placed in plastic-lined 570 l aquaria containing conditioned water (dechlorinated) and allowed to acclimate for four weeks. Water temperatures ranged from 17-20 C throughout the experiment with an average of 18.5 C. The fish were fed a commercially produced 32% protein trout chow every second day. Excess food and excreta were siphoned from the tanks daily. Fresh conditioned water was added to each tank, and water chemistry tests were conducted weekly to monitor water conditions. Average physico-chemical determinations for the 10-week study period were: dissolved oxygen 9 mg/l, alkalinity 120 mg/l, total hardness 188 mg/l, and pH 8.0.

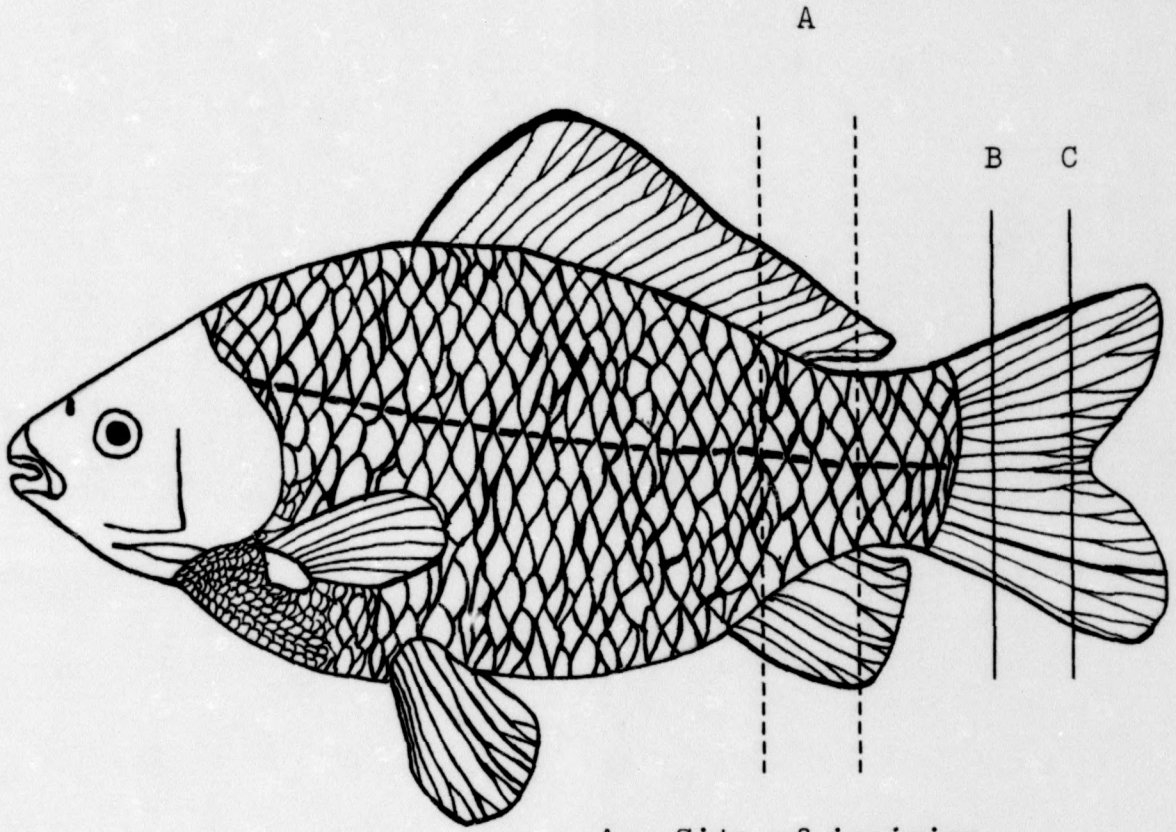
The goldfish were separated into four groups: controls, shams, 6-hydroxydopamine treated, and denerved individuals. Each group was composed of twelve similarly sized fish. Two groups of six were placed in each tank and separated by a net partition in the center of each tank. After the four week acclimation period, the fish were surgically treated on May 16, 1980. Control fish received no spinal cord operation. All fish in the remaining groups were anesthetized in a 600

mg/l solution of Chloretone in distilled water. Fish were held in the anesthesia for approximately five minutes or until opercular movements were greatly reduced.

Surgical materials used in the operation included a grooved, styrofoam dissecting board with a narrow slit in the groove to allow for drainage, scalpel with a narrow pointed blade (size 11), forceps with the tips bent outward, Ringer's solution flushing system, and a water powered aspiration device. A Dremel Model 380 variable speed (5,000-25,000 rpm) moto-tool drill with a round head (size 4) Cutwell burr was used to penetrate the neural arch and destroy the spinal cord.

Surgical procedures included taking the fish from the anesthesia, placing it on the dissecting board with the left side up, inserting the Ringer's solution tube into the opercular cavity and flushing the gills during the operation. Beginning at a point on the lateral line just below the posterior base of the dorsal fin, five to six scales were removed caudally along the lateral line. An incision approximately 10 mm long, was then made along the horizontal septum toward the midline, keeping the blade tip angled slightly dorsad (Figure 1). Successively deeper cuts were made until the blade tip came into contact with the vertebral column. Fish receiving this treatment only were referred to as shams and at this point were returned to the test tank and revived by holding the fish upright and forcing water across the gills. The fish were treated individually for approximately two minutes until opercular and fin movements were restored.

Figure 1. Planes of caudal fin amputations and site of surgical incision in the goldfish.



A-- Site of incision

B--Proximal amputation

C--Distal amputation

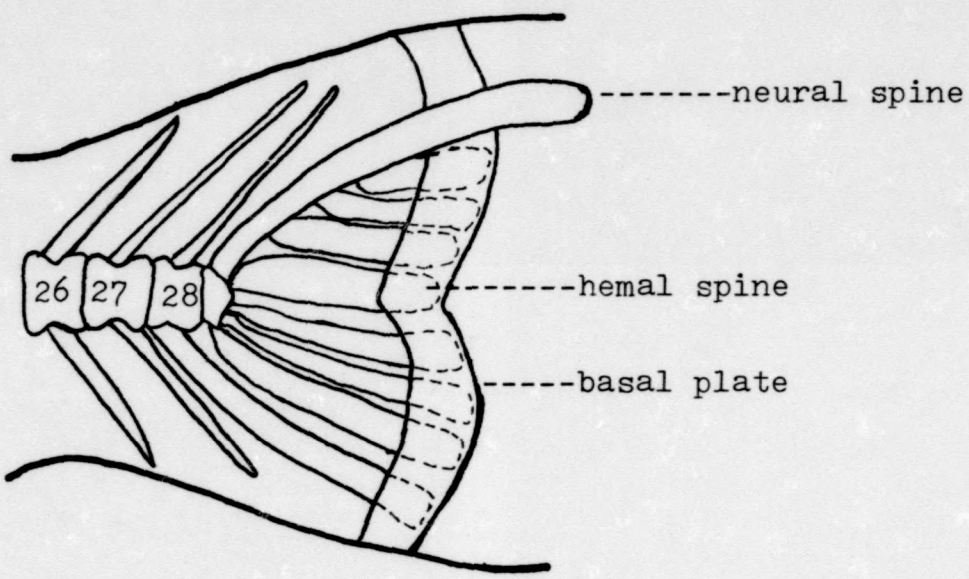
(Modified after Kozicki 1977)

The 6-hydroxydopamine treated fish were surgically treated in the same manner as the shams. Once the incision was completed, the forcep's tips were inserted just beneath the integument (midway along the incision) and relaxed (opening the cut and exposing the vertebral column). In those specimens in which bleeding occurred, blood and tissue fluids were drawn from the cut with the aspirator. A tuberculin syringe equipped with a 25 gauge needle was used to inject 0.25 cc of 6-hydroxydopamine (100 mg free base dissolved in 25 ml of 0.9% saline) into the spinal cord. The needle was directed anteriorly through the wall of the neural arch and into the spinal cord. The needle tip was slowly withdrawn as the injection was made to prevent the dopamine from leaking into the cut. Once injected, the fish were revived in holding tanks.

Denerved fish were treated as the shams above, but upon exposing the vertebral column, the neural arches of the 26th to 28th vertebrae were opened and the spinal cord in this region was severed. Approximately 5 mm of cord were destroyed with the drill (Figure 2). After drilling, the cord was flushed and aspirated and the concavity examined for spinal cord remains. The fish were revived in holding tanks.

Three days after surgery, the caudal fins of the fish in all four groups were cut. Six fish of each group received a proximal fin cut in which the fin was cut in close proximity to the fin base; six fish were given a distal cut in which the fin was cut just anterior to the tail fin notch (Figure 1).

Figure 2. Caudal fin vertebrae and site of surgical operation in the goldfish.



(Modified after Nabrit 1931)

Fins were placed upon a wooden block and severed with a single-edged razor blade. Upon amputation, each severed fin was injected either dorsally or ventrally with a biological stain and a corresponding mark (dorsal or ventral) was made in the stump of the remaining fin on the fish. This system of marks allowed for the recognition of individual fish and for comparison of regeneration percentages within each group at the conclusion of the experiment. Each amputated fin was measured with a set of calipers and metric rule. An average measurement (mm) of the dorsal and ventral lobes was determined and the fins placed in separate containers.

Following a ten week regeneration period, the fish were killed in an ice water bath. The amount of regeneration was determined immediately after death in the manner described earlier. Following the measurements, the caudal peduncle of the denerved fish was removed for histological examination in order to determine the effectiveness of the operation.

The histological method used was the 1957 Moliner modification of the Golgi Rapid Method (Humason 1972). Upon completion of fixing and staining, each block of tissue was quick frozen on the head of a clinical sliding microtome with a freezing attachment. Forty-micron sections were taken and examined for spinal cord presence.

The experimental data were analyzed using an analysis of variance, based on the procedure as outlined in Steele and Torrie (1960). Significant F values were analyzed using the Duncan's Multiple Range Test as outlined by Steele and Torrie (1960).

RESULTS

No mortalities occurred among the four groups during the ten week experimental period. The fish remained in good physical condition with no indication of infection or loss of color.

The denerved fish showed the least amount of fin regeneration among all groups with an average of 11.8 and 8.3 mm for the proximal and distal amputations, respectively (Figure 3). The average percentage fin regeneration for both types of cuts was also lowest, 75.4 in the proximal and 73.0 percent in the distal (Tables 1 and 2, Figures 4 and 5). There was a significant difference (0.05 % level) in proximal cut fin regeneration between the denerved group and all other groups. There was likewise a highly significant difference (0.01 % level) between the distal regeneration of the denerved group and the other groups.

Histological examination of the site of denervation showed no regeneration of the spinal cord and complete destruction of the neural arch on one side. The cavity where the spinal cord was previously located was infiltrated with connective tissue.

Six-hydroxydopamine treated fish averaged 14.2 and 11.7 mm regeneration for the proximal and distal cuts respectively (Tables 1 and 2, Figure 3). These regeneration values were

Table 1. Comparison of total length (mm) of fish before amputation, amount of fin regenerated (mm) and percentage regeneration in proximal amputations. Average values are represented as the mean ± 1 standard deviation.

	Denerved	6-H-dopamine	Sham	Control
Total length of fish before amputation (mm)	117.0	117.0	93.0	105.0
	94.0	108.0	118.0	116.0
	106.0	90.0	105.0	91.0
	117.0	115.0	110.0	115.0
	107.0	105.0	101.0	106.0
	107.0	114.0	117.0	110.0
Average	108.0 ± 7.7	108.2 ± 9.1	107.3 ± 9.6	107.0 ± 8.2
Amount of fin regenerated (mm)	14.0	15.0	13.0	14.0
	7.0	11.0	13.0	14.0
	10.0	11.0	13.0	12.0
	13.0	16.0	14.0	14.0
	14.0	16.0	13.0	13.0
	13.0	16.0	12.0	14.0
Average	11.8 ± 2.5	14.2 ± 2.3	13.0 ± .58	13.5 ± .76
Percentage regeneration	82.3	88.2	86.7	87.5
	53.8	78.6	100.0	87.5
	66.7	84.6	92.8	85.7
	81.2	94.1	82.3	87.5
	87.5	100.0	86.7	100.0
	81.2	84.1	85.7	87.5
Average	75.4 ± 11.6	89.9 ± 7.2	89.0 ± 5.8	89.3 ± 4.8

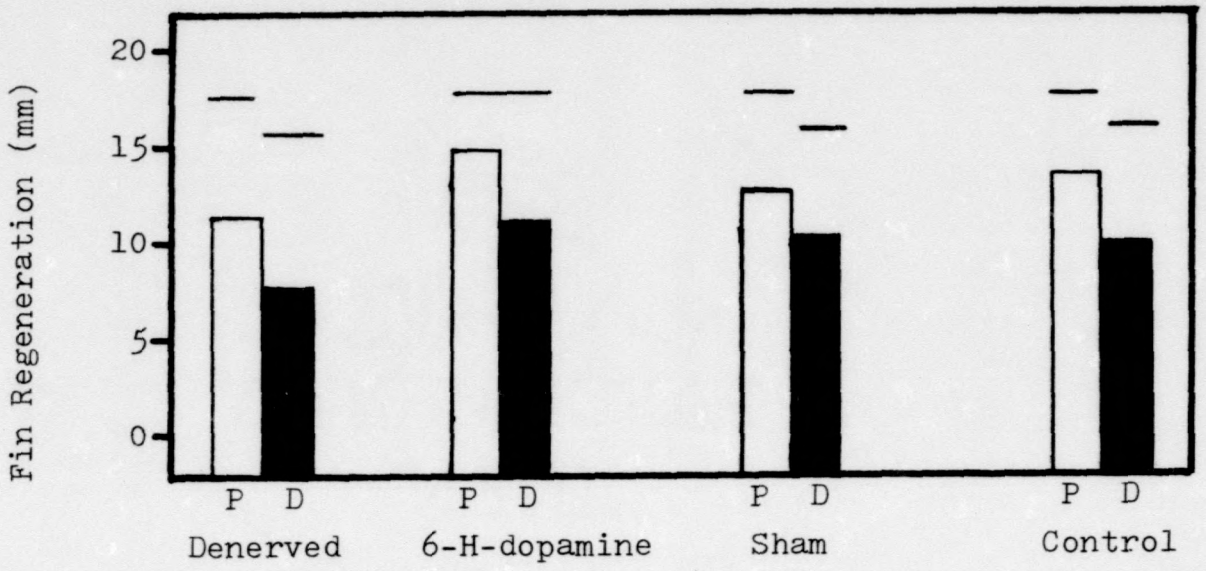
Table 2. Comparison of total length (mm) of fish before amputation, amount of fin regenerated (mm) and percentage regeneration in distal amputations. Average values are represented as the mean ± 1 standard deviation.

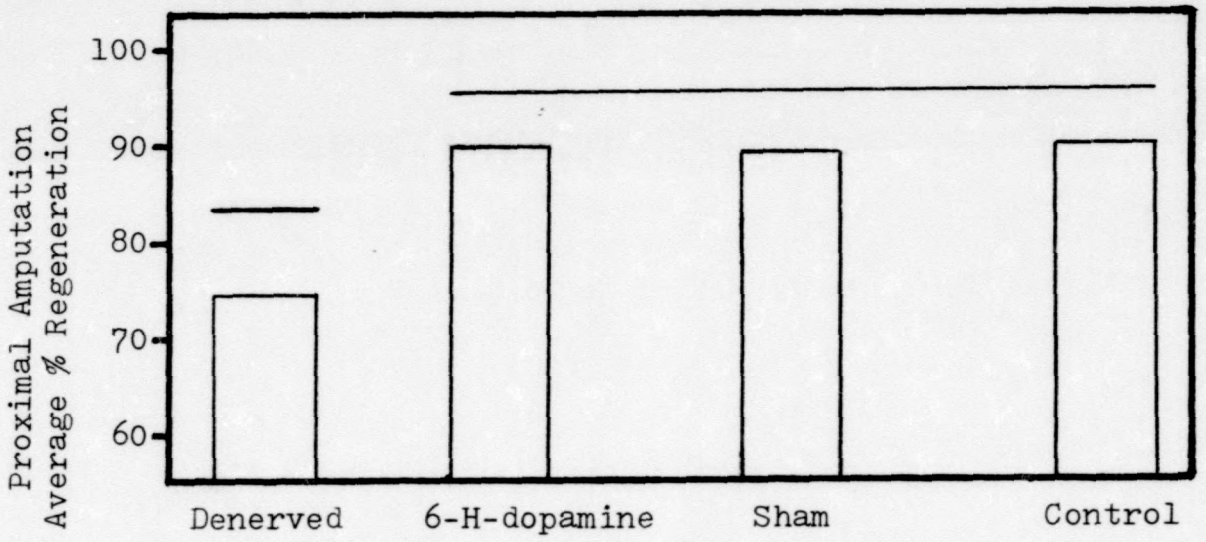
	Denerved	6-H-dopamine	Sham	Control
Total length of fish before amputation	101.0	114.0	108.0	116.0
	106.0	105.0	115.0	105.0
	103.0	110.0	105.0	107.0
	113.0	111.0	90.0	110.0
	91.0	104.0	115.0	90.0
	112.0	90.0	101.0	102.0
Average	104.3 ± 7.4	105.6 ± 10.2	105.7 ± 8.6	105.0 ± 8.0
Amount of fin regenerated (mm)	9.0	12.0	9.0	11.0
	7.0	11.0	11.0	9.0
	7.0	11.0	11.0	11.0
	9.0	13.0	8.0	11.0
	9.0	13.0	14.0	8.0
	9.0	10.0	10.0	10.0
Average	8.3 ± .94	11.7 ± 1.1	10.5 ± 1.9	10.0 ± 1.2
Percentage regeneration	81.8	92.3	90.0	91.7
	63.6	91.7	100.0	90.0
	53.8	91.7	91.7	91.7
	75.0	92.8	80.0	84.6
	81.8	92.8	93.3	80.0
	81.8	83.3	83.3	100.0
Average	73.0 ± 10.7	90.8 ± 3.4	89.7 ± 6.6	89.7 ± 6.2

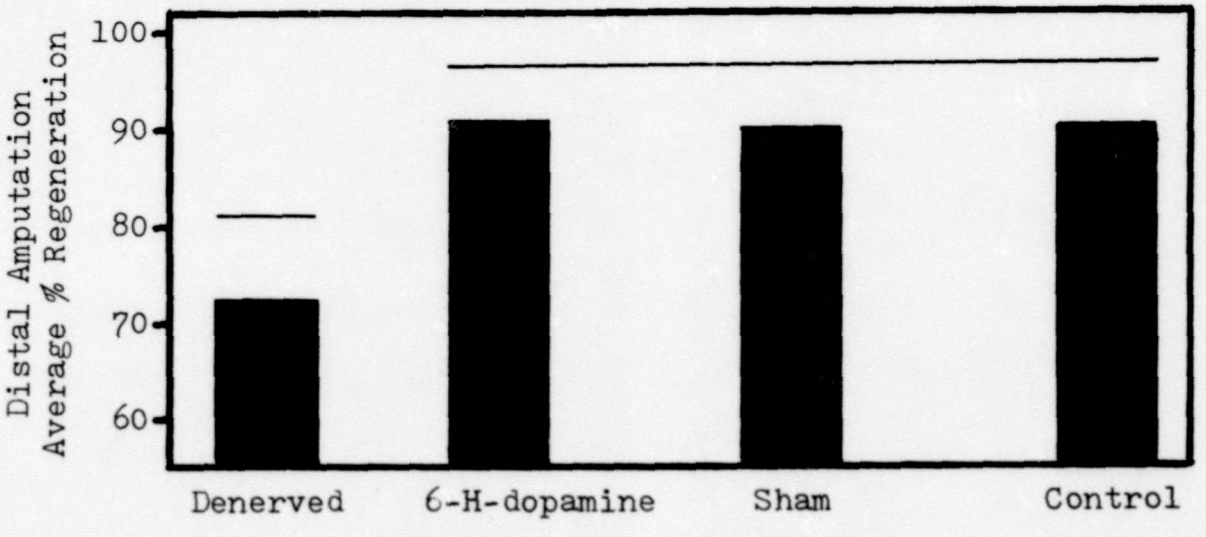
Figure 3. Comparison of average fin regeneration (mm) in the experimental and control groups. Bars under the same solid line are not significantly different at the 0.05 percent level.

Figure 4. Comparison of the average percent of regeneration of proximal amputations in the experimental and control groups. Bars under the same solid line are not significantly different at the 0.05 percent level.

Figure 5. Comparison of the average percent of regeneration of distal amputations in the experimental and control groups. Bars under the same solid line are not significantly different at the 0.05 percent level.


Fin Regeneration (mm)
20
15
10
5
0
P D
P D
P D
P D
Denerved
6-H-dopamine
Sham
Control


Proximal Amputation
Average % Regeneration
100
90
80
70
60
Denerved
6-H-dopamine
Sham
Control


Distal Amputation
Average % Regeneration
100
90
80
70
60
Denerved
6-H-dopamine
Sham
Control

the greatest among all groups. The average percentage fin regeneration was also the greatest for both types of cuts, (89.9 and 90.8%). However, there was no significant difference in the percentage of fin regeneration between the hydroxydopamine, control, and sham groups.

In the sham group an average of 13.0 and 10.5 mm regenerated fin was observed for the proximal and distal amputations. The average percentage fin regeneration for the proximal and distal cuts was 89.0 and 89.7 percent, respectively (Tables 1 and 2, Figures 4 and 5).

In the control group an average of 13.5 and 10.0 mm fin regenerated for the proximal and distal cuts (Table 1 and 2, Figure 3). Average percentage fin regeneration for the proximal and distal cuts was 89.3 and 89.7 percent, respectively (Figures 4 and 5).

DISCUSSION AND CONCLUSIONS

It was evident from the results of this study that denervation did not prevent regeneration in caudal fin amputations in the goldfish. Denervation did significantly reduce the overall average amount of regeneration in both proximal and distal fin amputations. These results are inconsistent with the findings of some researchers. Kamrin and Singer (1955) found that when a portion of the spinal cord of Anolis carolinensis was removed and the tail subsequently amputated, no regeneration occurred. In a few specimens (3 of 12) a partial regeneration of the destroyed spinal cord occurred, and there was some re-innervation of the wound area. In these cases a small tail resulted. The authors concluded that extremity and tail regeneration of reptiles, amphibians, and possibly of fishes was dependent upon the nervous system. The reason for the lack of regeneration was not due to the absence of the spinal cord, but a reduction in the number of neurons below the threshold needed for regeneration.

Additional evidence that denervation prevented regeneration was presented by Goss and Stagg (1957). Their work on the fin rays in the pectoral fins of Fundulus heteroclitus showed that denervation significantly affected regeneration. They found that the initial healing of the

wound occurred in the absence of nerves but there was no subsequent growth of the fin rays. Regressive changes also occurred in the soft tissues of the fin which were followed by erosion of terminal ray stumps by osteoclasts.

The idea that a certain threshold level is necessary for regeneration to occur has been proposed by Geraudie and Singer (1977). Their work on the pectoral fin of F. heteroclitus revealed that the number of nerve fibers necessary for regenerating fins varied between 16 and 25 and went as high as 35. This threshold level was much higher than that necessary to compensate for a lower efficacy of the fibers as neurotrophic agents. They found that fish tissue was less responsive to the neurotrophic agent.

The results of this study did not agree with the above findings, but none of the above works dealt specifically with the caudal fin. Weiss (1972) studied the effect of the nerve growth factor (NGF) on fin regeneration in the goldfish (C. auratus), specifically the caudal fin. However, she too noted that innervation was necessary for fin regeneration. The nerve growth factor, which is a protein, is known to have a stimulatory effect on regeneration by increasing the amount of innervation in the fin. The acceleration reaches a plateau as regeneration proceeds. Without injection of the NGF regeneration would not have occurred.

The fact that the denerved group regenerated an average of only 75.4 percent for the proximal and 73.0 percent for

the distal cuts was significant. This decrease in fin regeneration may have resulted from a reduced neuronal threshold level.

Denerving pectoral fins, as performed by Goss and Stagg (1957) and Geraudie and Singer (1977, 1979), was more successful than denerving caudal fins because of the close proximity of the brachial plexus of the pectoral fins. It seemed logical that the branches of nerves necessary for regeneration of the caudal fin would be in the area of the basal plate or the last few segments of the vertebral column, but such may not have been the case. Regeneration occurred despite the fact that the spinal cord had been removed. Histological examination of the site of denervation showed absence of the spinal cord and suggested innervation must have occurred anterior to the site.

A possible explanation for regeneration in the denerved individuals was that the nerves necessary for regeneration of the caudal fin branched anterior to the area of the spinal cord which was destroyed, thus providing branches of nerves to the area of amputation. As noted earlier, there must have been a decrease in the threshold of the nerves or more regeneration would have occurred. Studies have shown that the spinal cord of teleosts does have the potential to regenerate (Fridberg, et al. 1966). They found that removal of the caudal neurosecretory system reactivated the ability to differentiate in this area in adult organisms (Tilapia mossambica). This differentiation occurred in both a

cytogenetic and an organogenetic sense. Another possible explanation of why regeneration did not occur was presented by Singer and Mutterperl (1963). They found that the lack of regeneration was due to the tissues not being competent to respond to a low number of fibers even though the available nerve fibers were active. They concluded that the wound tissue was important in establishing the threshold nerve requirements and contributing substances necessary for regeneration.

The use of 6-hydroxydopamine as a possible agent for denervation was not effective in this study, in fact it appeared to have stimulated regeneration instead of preventing it. Little literature is available on the effects of 6-hydroxydopamine. Work by Johnson et al. (1979) showed that 6-hydroxydopamine destroyed sympathetic neurons and prevented the accumulation of the NGF in neonatal rats. Injection of 6-hydroxydopamine in adult rats did not completely prevent the transport of the NGF but produced an alteration in its accumulation. A possible explanation of why this drug had no effect in denerving goldfish is based on the non-uniform response of different species to it.

The sham operation had little effect on the regeneration of the caudal fin since the spinal cord was not damaged. The slight reduction in regeneration in this group was attributed to the initial trauma the fish endured in exposing the vertebral column.

The findings of this study agreed with the findings of Tassava and Goss (1966), who determined that the amount of regeneration was proportional to the amount of amputation. The greater the amount of fin amputated, the greater the rate and proportion of regeneration. The proximal fin cut regeneration values for the denerved group were greater than those in the distal cuts, which supported the idea that the more fin removed, the greater the percentage of regeneration. The other treatments resulted in similar percentages of regeneration for both types of cuts.

The fact that denervation did not prevent regeneration in goldfish suggested that the process of regeneration might be regulated by a combination of several factors. The combined effects of the nervous system and endocrine system seem likely based upon the results of this study and those of Fortner (1979). The results of this study do suggest that further research is required.

In conclusion, it is noted that denervation did not prevent regeneration in the goldfish but did significantly affect the amount of regeneration. The proximal fin cuts supported the hypothesis that the more fin removed the faster the rate and percentage of regeneration.

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