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An Investigation of Barbel Regeneration in the Catfish, *Ameiurus nebulosus*

by

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Submitted in partial fulfillment of the requirements for the Senior Scholars Program

Colby College

1973
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Introduction:

Sensory barbels of the catfish, *Ameiurus nebulosus*, regenerate readily following excision. The first histological studies of barbel regeneration were conducted by Biegel (1912), who described their structure and regeneration. She observed that nerve fibers lie dorsal and ventral to the central cartilaginous axis and that regeneration is complete except in some older specimens (barbel length 10-12 cm.), where regrowth may fail to occur. Bifurcated barbel regeneration has been demonstrated by Biegel (1912), Roth (1904), and Goss (1954b).

The dependence of regeneration on innervation has been studied by Olmstead (1920a, 1920b), Olivo (1928), Kamrin (1953), Kamrin and Singer (1953), and Goss (1954a, 1954b, 1956). Olmstead (1920b) claimed that the barbels are innervated solely by the seventh cranial nerve, while Olivo maintained, based on observations of histological cross-sections, that nerve fibers run only dorsal and ventral to the cartilaginous rod. Innervation at the base of the barbel by the V/VII cranial nerve complex is necessary not only to maintain the integrity of the barbel, but also to prevent dedifferentiation of the taste buds followed by atrophy of the barbel itself in a disto-proximal direction (Kamrin and Singer, 1953; Olmstead, 1920a, 1920b). However,
regeneration of the severed nerve causes reappearance of
taste buds, stimulated by nerve fibers penetrating the epi­
dermis, and subsequent regeneration of the barbel. Kamrin
and Singer have further demonstrated that normal regenera­
tion occurs even if the central root of the V/VII cranial
nerve complex is destroyed, as long as the fibers are intact
where they innervate the barbel. Therefore, neural control
for regeneration lies within the neuron itself and not with­
in the central nervous system. Goss (1954b) has shown that
barbel amputation following destruction of nerve fibers which
innervate the barbel results in wound healing by epithelial
cells, but not in regeneration.

Goss (1954a, 1954b) has further demonstrated that barbel
excision immediately following extirpation of the cartilag­
inous rod is followed by abnormal, inhibited regeneration.
In the abnormal case, nerves, the central artery, and connec­
tive tissue reappear beneath the skin after the wound is
healed by epidermal cells, which are subsequently inter­
spersed with taste buds. Both Goss and Biegel (1912) have
observed that regeneration of the cartilaginous axis pro­
ceeds from differentiation of blastema cells, which they
hypothesize are derived from the perichondrium. Biegel
conjectures that perichondrial cells proliferate to form
the blastema, while Goss claims that they deplete the peri­
chondrium by migrating to the blastema, where they undergo
mitotic divisions exclusively to form new cartilage.

The data presented in this paper are divided into five
sections. The first describes brain dissection of an adult catfish; the second is an histological study of normal barbel structure; the third cites observations of the histological details of normal regeneration; the fourth is a study of the temperature-dependence of regeneration; and the fifth includes several methods of investigation designed to substantiate the conclusions drawn by observations of normal regenerating barbels.

Materials and Methods:

The 8-15 cm. horned pout employed in these experiments were obtained in two batches, one from Oakland, Maine, and the other from the Essex Marine Laboratory, Essex, Connecticut. Due primarily to lethal fungal infections (Saprolegnia sp.), the supply of fish obtained in September 1971 was virtually depleted by December 1971. The first experimental group was kept in constantly aerated freshwater aquaria at 21-23°C. It proved necessary to keep them in pond water because of apparent toxicity of tap water. The second experimental group was also kept in constantly aerated aquaria, but at colder temperatures (15-17°C) to help control the fungus. The fungal infections were also curbed by Fungus Stop, a product of the Jungle Laboratory, composed of sodium chloride, acriflavine, sodium sulfathiazole, methylene blue, and nitromersol. The aquaria were filled partly with brackish water from the Connecticut River and partly with distilled water. One 25-gallon tank was equipped
with a handmade filter system, including a false bottom, which was covered with mud and gravel. The fish were fed bi-weekly on freeze-dried worms and cracker crumbs.

Ethyl-m-amino benzoate methane sulfonic acid (150 mg./liter H₂O) was used as the anaesthetic during operations involving removal of the cartilaginous rod, but all other operations were performed without anaesthesia. All experimentation has been executed on the two lateral barbels because they are larger than the four mandibular or two dorsal barbels (Plate I).

A careful brain dissection of a 3.5-foot catfish, obtained from Texas, was performed to ascertain the origin and arrangement of cranial nerves in *Ameiurus*. To determine the structure of normal barbels, lateral barbels were removed using iridectomy scissors and were prepared for histological observation. Information regarding normal barbel regeneration was collected by histological observation of regenerating tips, which were amputated at various time intervals (days) after the original excision. In most cases the entire barbel was removed, leaving a stub of only 1.0-2.0 mm.; however, in some cases only the distal half of the barbel was excised. Temperature-dependence of regeneration was studied by comparing barbel regeneration rates of fish kept at 20°C, 15°C, and 12°C.

Experiments designed to verify the conclusions drawn by histological observation of normal regenerating barbels
involved several treatments: (1) removal of the cartilagi¬
nous rod prior to amputation of the barbel; (2) injection
of the stub of an excised barbel with mitomycin-C or aza-
guanine to inhibit DNA synthesis and thereby inhibit mito-
sis; (3) injection of the fish with the vital dye trypan
blue to trace the destiny of phagocytic cells colored by
the dye; and (4) removal of barbel epidermis to observe the
origin of epidermal wound-healing cells.

The operative technique for extirpation of the carti-
laginous rod involved making a small lateral incision in a
barbel of an anaesthetized fish, and cutting through the
cartilage, taking care not to destroy the two large nerve
bundles or the main artery in the barbel. Using watch-
maker's forceps, about two-thirds of the rod was removed
disto-proximally. However, because of its distal attenuation,
the rod eventually broke, leaving a small cartilage segment
intact. In most cases the rodless barbel was then excised
in the excised region, leaving a flexible barbel composed
of skin, nerves, artery, and loose connective tissue. In
a few cases the rodless barbel was amputated at the proxi-
mal end of the region containing the intact cartilage,
leaving about 0.4 mm. cartilage from which a regenerate
could develop.

The stubs of several removed barbels were injected
three times a week with a few drops of mitomycin-C (0.04 mg./
ml.) or aza-guanine (0.05 mg./ml.) dissolved in Frog Ringer's
Solution to inhibit regeneration by interfering with DNA
replication and thereby inhibiting cell division in the blastema. Mitomycin-C inhibits DNA synthesis without interfering with RNA or protein synthesis. It inhibits DNA replication by establishing covalent cross-linkages between complementary strands of DNA. Because of its resemblance to guanine, aza-guanine interferes with nucleic acid metabolism by substituting for guanine during replication.

![Mitomycin-C](image1.png)  
![Aza-guanine](image2.png)

Mitomycin-C inhibits DNA synthesis without interfering with RNA or protein synthesis. It inhibits DNA replication by establishing covalent cross-linkages between complementary strands of DNA. Because of its resemblance to guanine, aza-guanine interferes with nucleic acid metabolism by substituting for guanine during replication.

An experiment employing the specificity of the vital dye trypan blue for phagocytic cells was designed to help determine the cells responsible for blastema formation. Several fish were injected intraperitoneally with trypan blue suspended in Frog Ringer's Solution (10 mg./20 g. body weight) at two-day intervals, while several other fish were given a single intraventricular injection of 0.2-0.5 ml. of 1% trypan blue. Following excision of the barbels, the location of the cells colored by the dye was observed.

The final method of investigation was used to examine the role, if any, of preexisting epidermis in producing a new epithelium. Using iridectomy scissors and a fine scalpel, sections of epithelium were removed from barbels, taking care not to injure the nerves, artery, or cartilaginous rod. Barbels were removed at various intervals...
after the operation and studied histologically to ascertain the origin of the wound-healing cells.

All of the barbels were prepared for histological observations by fixing in Bouin’s Solution or in a thionin and formalin solution, which is a combined fixative and stain, specific for nerve tissue. The barbels fixed in Bouin’s Solution were subsequently stained with a variation of Mayer’s hematoxylin and eosin, Mallory’s triple connective tissue stain, Heidenhain’s Iron Hematoxylin, or Romanes’ (1950) silver nitrate technique for nerve fibers. Both cross and longitudinal semi-serial sections were prepared, and observations were recorded by ink drawings and photomicrographs.

Results:

That innervation is solely by the seventh cranial nerve (Olmstead, 1920) and that nerve fibers exist only dorsal and ventral to the cartilaginous rod (Olivo, 1928; Biegel, 1912) are conclusions not substantiated by careful dissection. Although innervation appears to be primarily by fibers of cranial nerve VII, there are also fibers from cranial nerve V in the region of the barbels (Plates II and III). The fibers of both cranial nerves are extensions of the common V/VII cranial nerve root complex. Dissection also demonstrates that fibers are not restricted to the dorsal and ventral sides of the cartilaginous axis, but rather are distributed non-uniformly around the entire periphery of the barbel, with concentration on the dorsal and ventral
Histological observations of dorsal, mandibular, and lateral barbels show no significant structural differences. The barbel is supported by a central cartilaginous matrix, surrounded by a thin layer of perichondrial cells (Plates IV, V, and VII). The fibroblasts of the perichondrium differentiate to form the chondrocytes of the cartilage, within which there are no apparent mitoses. The layer of fibroblastic connective tissue which surrounds the perichondrium contains both nerve tissue and an artery. Tiny branches of the main artery may be seen, particularly in cross-sections. Although nerve fibers are concentrated in two bundles, dorsal and ventral to the cartilaginous rod, there are also fibers which penetrate the epidermis, innervating the taste buds. Separating the epidermis from connective tissue is a fine fibrous membrane.

The stratified epithelium contains numerous pigment cells, containing tiny granules of melanin, which are responsible for *Ameiurus'* ability to change hue depending on the intensity of its background. Pigment cells are most dense near the boundary between the epidermis and dermis, and around the periphery of taste buds. However, chromatophores are conspicuously absent within the taste buds, which are concentrated on the anterior and ventral sides of the barbel. The taste buds appeared to be of two distinct shapes, indicating the possibility that there may be two kinds of "taste" buds, one with a taste, and the other with
a touch, function. If subsequent investigations demonstrate that there are indeed two types of taste buds, it may be found that barbels function not only as chemoreceptors, but also as mechanoreceptors. Nerves are present in the taste buds, penetrating among, but not within individual sensory cells.

Within 24 hours after barbel amputation, the wounded stub is healed by a thin layer of squamous epithelial cells, which continues to proliferate, forming an abnormally thick wound epithelium, up to 0.2 mm., compared with about 0.1 mm. in normal areas (Plate IX). Beneath the epithelium and at the tip of the severed cartilaginous rod, a blastema begins to form 7-9 days after excision (Plate VI). Once the blastema has been established, rapid regeneration begins 8-10 days after amputation and continues at a rate of 1.0-1.5 mm. per week at 20°C. until the original barbel length has been regained.

Concomitant with blastema formation, there is a depletion of cells in the perichondrium, but few, if any, mitoses are visible in the perichondrial cells (Plate X). It also appears that severing the cartilaginous matrix releases a few chondrocytes, which are incorporated into the developing blastema, but no dividing chondrocytes are visible anywhere in the cartilage. Biegel denies any role of chondrocytes in blastema formation, but Goss allows for this possibility. Connective tissue, apparently composed primarily of dermis, appears to penetrate between the wound epithelium and blastema,
but does not seem to contribute directly to blastema formation. Fibroblasts of the connective tissue do, however, give rise to the fibroblasts of the perichondrium.

As regeneration proceeds, the blastema elongates, differentiating proximo-distally to form new cartilage (Plate IX). It appears that the blastema gives rise only to new cartilage and not to regenerating nerves, connective tissue, blood vessels, taste buds, or pigment cells. Thionin staining indicates that nerves penetrate the epidermis early in the regeneration process and that in areas where nerve concentration is greatest, incipient taste buds appear. Taste buds form from epidermal cells at the dermis-epidermis boundary which, apparently stimulated by penetrating nerves, push into the epidermis, where they develop into a characteristic papilla. As a new taste bud develops from the papilla, it pushes toward the surface of the epidermis. Pigment cells also reappear in the epidermis during the early blastemic phase. Although blood vessels begin to regenerate early, they do not reach their original dimensions until regeneration is nearly complete. Perichondrium is absent around the transition zone between undifferentiated blastema cells and new cartilage, and seems to be laid down later by fibroblastic connective tissue cells. Therefore, it is logical that the dimensions of the regenerating barbel are somewhat less than those of the original until regeneration is nearly complete, at which time the original diameter is regained. The rate of regeneration, but not the sequence
in which regenerating tissues differentiate, is dependent upon the proportion of barbel excised. Once the blastema is formed, regeneration occurs more quickly when virtually the entire barbel is amputated than when only the distal portion is removed.

The dependence of regeneration on temperature is clearly demonstrated by comparing regeneration rates of fish kept at 20°C, 15°C, and 12°C. Once the blastema has been formed, regeneration at 20°C proceeds at a rate of 1.0-1.5 mm. per week until the original barbel length has been regained. Regeneration at 15°C progresses at a rate of 0.3-0.5 mm. per week, while all regeneration is halted at 12°C.

To substantiate the conclusions drawn by histological observations of normal regenerating barbels, the barbels of many fish were subjected to various abnormal treatments. Extirpation of the cartilaginous rod prior to amputation in the affected region inhibits normal regeneration of the barbels of Ameiurus. In accordance with Goss’ observation, following removal of the cartilaginous axis, epithelial cells heal the wound, but no blastema is formed and no regenerated cartilaginous rod is visible at the point of excision (Plate XI). Histological observations of both extirpated cartilaginous rods and the barbels from which they were removed, show that virtually all of the perichondrium is removed with the cartilage. Following wound healing by formation of a thick epithelium, connective tissue invades the
area internal to the epidermis. Pigment cells reappear in
the regenerated epithelium, and nerve fibers extend into
the epidermis giving rise to incipient taste buds. However,
only inhibited and abnormal regeneration occurs, as elonga­
tion even after two months never exceeded 0.5 mm. In no
case did a blastema appear and, with one exception, no car­
tilage was regenerated in the absence of the cartilaginous
rod. In one case a small piece of regenerated cartilage
was visible 21 days after extirpation and excision.

Amputation of a rodless barbel at the proximal end of
the region containing the intact cartilage, leaving about
0.4 mm. of cartilage from which a regenerate could develop,
results in normal regeneration (Plate X).

Injection of the stubs of amputated barbels with mito­
mycin-C or aza-guanine had similar effects. It proved dif­
ficult to determine an antibiotic dosage which had an in­
hibitive effect on DNA synthesis, but yet was not lethal to
the fish. In order not to kill the fish, it was never pos­
sible to totally arrest cell proliferation in the barbel
stub. However, DNA replication and therefore cell division
was inhibited in several cases. In all fish injected with
an antibiotic immediately following barbel amputation, the
wound was healed by a layer of epithelial cells, and a layer
of connective tissue appeared between the cartilage tip and
wound epithelium (Plates XII and XIII). Unlike the situa­
tion in normally regenerating barbels, the wound epithelium
in the treated animals was not abnormally thick. The con­
nective tissue formed a smooth layer juxtaposed to the am-
putated cartilage tip and the wound epithelium. Even 47
days following amputation, the wound had healed, but a blas­
tema had not been formed. There were a very few connective
tissue cells and a few chondrocytes, released by the severed
cartilage, visible at the rod tip. The perichondrial cells
surrounding the rod had not proliferated and no barbel elon-
gation had occurred. As much as one and a half months after
amputation there were no incipient taste buds and abnormally
few pigment cells in the epithelium.

As with the antibiotics, it was difficult to determine
suitable dosages of trypan blue. Intraventricular injection
was unsuccessful because the fish died shortly after the in-
jection, while intraperitoneal injection was also somewhat
unsuccessful because only very small quantities of the dye
reached even the base of the barbels, and almost none ex-
tended significantly into the barbels. Therefore, the des-
tiny of the colored cells following amputation could not be
traced because there were no colored cells as far distal as
the excisions. However, it was observed that trypan blue
is taken up primarily by fibroblastic connective tissue
cells, to a lesser extent by perichondrial cells, and very
little by epidermal cells.

Removal of epidermis from a barbel results in rapid
wound healing by mitotically-dividing epidermal cells. As
an adaptive reaction to frequent natural abrasions in the
epidermis, epidermal cells are capable of rapid proliferation
resulting in wound healing, by spreading over or into a wound area from its margins.

**Discussion:**

From observations of cross-sectional slides of catfish barbels, Olivo concluded that all nerve fibers innervating the barbels are concentrated in two bundles, dorsal and ventral to the cartilaginous rod. Although two distinct nerve bundles are prominent in cross-section preparations, careful observation of both cross and longitudinal sections stained with thionin indicates that there are additional smaller nerve groups around the periphery of the cartilaginous axis. Anatomical dissection also clearly shows that although nerves are concentrated in two bundles, they are distributed non-uniformly around the entire rod. Dissection substantiates the conclusion that barbels are innervated by fibers both of the fifth and seventh cranial nerves, an observation made also by Atema (1971).

With respect to the amputated catfish barbel, regeneration at $20^\circ$C is complete in about two months. Following excision, nearby epithelial cells proliferate to form a wound epithelium. Because the wound-healing cells have the same dimensions and density as normal epithelial cells, it appears that wound healing results from locally dividing, rather than from migrating cells. It appears that the blastema, formed at the severed cartilaginous tip, is derived primarily from migrating perichondrial cells, although a few chondrocytes released by severing the cartilaginous matrix, are also in-
corporated into the undifferentiated mass of cells. Although fibroblastic connective tissue cells differentiate to form fibroblastic perichondrial cells, histological observations do not indicate that connective tissue cells are incorporated into the blastema. Rather, it appears that a layer of connective tissue, which forms from preexisting connective tissue, penetrates between the new epidermis and developing blastema, giving rise to the dermis of the regenerate.

Simultaneous depletion of perichondrial cells near the severed tip and accumulation of blastema cells clearly indicates that the blastema is formed by migrating, rather than proliferating perichondrial cells. Although cell division is conspicuously absent among the perichondrial cells, rapid division and differentiation characterize the blastema (Plate VIII,D). Proliferation causes elongation of the regenerate as the blastema cells adjacent to the old cartilage differentiate into new chondrocytes. Prior to chondrification, nerves, incipient taste buds, and pigment cells reappear in the regenerating tip, indicating that their regeneration is not dependent upon new cartilage formation.

The results of experiments involving extirpation of the cartilaginous rod prior to barbel amputation have provided support for Gosa’s hypothesis that the blastema is derived from perichondrial cells. Easton (1949, unpub.) had cautiously hypothesized that the blastema may be derived from epithelial cells. Although there is an inhibited regeneration of rodless barbels, no blastema forms and, in all but
one case, no cartilage regenerated. Without elongation, apparently caused by proliferating and chondrifying blastema cells, regeneration is halted. However, it is significant that the only structures which do not reappear in the absence of the cartilaginous rod and its perichondrium are new cartilage and new perichondrium.

In the one exceptional case mentioned above, after 21 days a small piece of regenerated cartilage was visible in cross-section. However, because new cartilage regenerated in the absence of old cartilage in only one case, it is felt that either a small fragment of old cartilage must inadvertently have remained following extirpation or that the new cartilage was laid down by differentiation of a few remaining perichondrial cells. Despite the appearance of a small fragment of cartilage, regeneration did not occur and no blastema was formed.

That amputation of a rodless barbel at the proximal end of the region containing the intact cartilage results in normal regeneration indicates that the blastema is derived only from cells adjacent to the severed tip. Therefore, extirpation experiments indicate that the blastema is derived from the perichondrial cells directly proximal to the point of amputation.

Injection with mitomycin-C (0.04 mg./ml.) or aza- guanine (0.05 mg./ml.) inhibited, but did not totally halt, mitosis. A wound epithelium comparable in thickness to normal epithelium, but not to normal wound epithelium, formed,
indicating that mitosis had been inhibited, but not completely halted. Although a sparse layer of connective tissue formed between the wound epithelium and cartilage, there were no signs of blastema formation even 46 days after excision. The perichondrium showed neither depletion nor proliferation of cells, demonstrating that no mitosis had occurred and that cells had not migrated to form a blastema. Inhibition of epithelial mitosis prevented blastema formation apparently by steric hindrance of perichondrial cell migration to the cartilage tip. Although a few connective tissue cells appeared at the severed tip, a blastema was not formed.

The greater density and smaller size of connective tissue cells near the severed tip as compared to those proximal to the injury indicate that some proliferation of connective tissue had occurred, but that the newly-formed cells were hindered from migrating to the region around the severed tip. No accumulation of perichondrial cells similar to that of connective tissue cells, was evident (Plates XII and XIII). A smaller dosage of mitomycin-C (0.035 mg./ml.) at 3-day intervals resulted in formation of wound epithelium and connective tissue between the cartilage tip and epithelium, relieving the dense accumulation of connective tissue cells, but still preventing blastema formation (Plate XIV).

Normal regeneration results in accumulation of epithelial and connective tissue cells around the excised tip, in addition to blastema cells of perichondrial origin juxta-
posed to the old cartilage. Although incipient taste buds are visible in the wound epithelium formed after amputation of both normal and redless barbels, none are visible in the epithelium formed after amputation of barbels treated with an antibiotic. Therefore, inhibition of epidermal mitosis inhibits formation of new taste buds.

Although many fish were injected with trypan blue, the results of the injections were meager. However, it is felt that with improved injection technique, which allowed a greater dosage of the dye to get into barbel tissues, the destiny of the colored cells could be traced. Although the results probably would not yield unequivocal conclusions regarding blastema origin, it should be possible to conclusively eliminate those cells not stained as precursors to the blastema.

Removal of epidermis followed by rapid wound healing by epithelial cells demonstrates that epidermal cells proliferate rapidly and profusely to heal abrasions. It appears from the experiments involving injection with antibiotics that it is more difficult to inhibit proliferation of epidermal cells than connective tissue cells, a result in accordance with the abrasion-healing function of the epidermis.

Conclusion:

These studies demonstrate that formation of a blastema is a requisite for regeneration of the catfish sensory barbel. From observations of barbels treated with mitomycin-C
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or aza-guanine and from experiments involving the extirpation of the cartilaginous rod prior to amputation, it is concluded that the blastema forms by accumulation of migrating perichondrial cells. A few chondrocytes, released by severing the cartilaginous matrix, also contribute secondarily to blastema formation. Space for the accumulation of perichondrial cells is provided by proliferating epidermal and connective tissue cells. It is further concluded that inhibition of cell proliferation by antibiotic treatment eliminates the formation of sufficient space for the perichondrial cells to accumulate, thereby inhibiting blastema formation, and halting regeneration.

Normal regeneration results from extirpation of the cartilaginous rod up to 0.4 mm proximal to the level of amputation, demonstrating that blastema cells are derived from the area immediately adjacent to the excision point. The accumulated blastema cells proliferate, causing barbel elongation as they undergo chondrification proximo-distally. Only abnormal, inhibited regeneration results from amputation in the affected region of a rodless barbel. Such treatment results in no blastema formation and little barbel elongation, although epidermis and connective tissue proliferate as usual, pigment cells reappear in the epidermis, and taste buds are reformed. Therefore, the hypothesis that the blastema is derived from the epithelium is invalidated, and the hypothesis that it is derived almost solely from perichondrial cells is further strengthened.
Summary:

The source and arrangement of nerves in the catfish sensory barbel have been carefully determined through dissection, and normal barbel structure ascertained through histological observation. It has been clearly demonstrated several times that *Ameiurus* barbels are capable of complete regeneration following excision. Normal regeneration is dependent upon formation of a blastema, which undergoes rapid proliferation and proximo-distal chondrification, causing elongation of the barbel at a rate of 1.0-1.5 mm per week until the original dimensions have been regained. During the period of elongation, connective tissue also proliferates, and pigment cells and taste buds become visible in the epithelium.

The presence of the cartilaginous rod is necessary for normal regeneration, but is not required for wound healing by epithelial cells, reappearance of taste buds and chromatophores in the epithelium, or connective tissue proliferation. However, no blastema forms in the absence of the cartilaginous matrix.

Further evidence to support Goss' hypothesis that the blastema is derived from perichondrial cells, accumulated from around the cartilaginous rod itself, has been gained through experiments involving injection with mitomycin-C or aza-guanine. Both antibiotics inhibit regeneration by inhibiting DNA synthesis, and thereby interfering with physical elongation of the healed cap of epithelium by re-
stricting the formation of available space at the end of the cartilaginous rod. Therefore, the migration of the perichondrial cells to the tip of the severed rod is sterically hindered.

Although new epithelium is derived from preexisting epithelial cells, the hypothesis that the epithelium may be a source of blastema cells has been invalidated.

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Key:  Plates I-III

Lat. . . . . Lateral barbel
Dor. . . . . Dorsal barbel
Lat. Man . . Lateral mandibular barbel
Med. Man . . Medial mandibular barbel
Super. ophth . Superficial ophthalmic nerve trunk
Supra. orb . Supraorbital nerve
Fr. B. N . Frontal barbel nerves
Max. . . . Maxillary nerve trunk
Pre-max. . Pre-maxillary nerve branch
Man. . . . Mandibular nerve trunk
Lat. B. N . Lateral barbel nerves
Man. B. N . Mandibular barbel nerves
Musc . . . . Muscular branch
Vag. . . . . Vagus
Ant. . . . . Anterior nerve branch
Post. sup. . Postero-superior branch
Post. inf. . Postero-inferior branch
Lat. br. . . Lateral nerve branch
Int. br. . . Intermediate nerve branch
Med. br. . . Medial nerve branch
Nar. pit . . . Narial pit
Key: Plates IV-VI

Ep ........ Epithelium
TB ........ Taste bud
Pig C. ...... Pigment cell
N. .......... Nerve
BV .......... Blood vessel
Car. ........ Cartilage
FM .......... Fibrous membrane
Per. .......... Perichondrium
CT ........ Connective tissue
NCT .......... New connective tissue
B. .......... Blastema
D. per ...... Depleted perichondrium
OC .......... Old cartilage
D. .......... Dorsal
V. .......... Ventral
Plate I: Head-on sketch of *Ameiurus nebulosus* showing barbel arrangement.

Amputations were made on the lateral barbels primarily at the level indicated by the arrow.
Plate II: Drawing showing innervation of the anterior portion of *Ameiurus* by branches of the V/VII cranial nerve complex.

Super. ophth.: Superficial ophthalmic trunk (V/VII).

Supra. orb.: Supraorbital nerve branches off superficial ophthalmic to run just beneath the skin on the lateral side of the barbel.

Fr. B. N.: Frontal barbel nerves consist of three branches: lateral, intermediate, and medial. See Plate III for greater detail.

Max.: There are several branches of the maxillary trunk, (V), one of which bifurcates to form the pre-maxillary nerve.

Pre-max.: Pre-maxillary nerve branch innervates the most anterior portion of the head, and the base of the narial pit.

Man.: Mandibular trunk (V) innervates the lower jaw region and the mandibular barbels.

Lat. B. N.: Lateral barbel nerve complex consists of three branches: anterior, postero-superior, and postero-inferior. See Plate III for greater detail.

Musc. Br.: Muscular branch (V) runs deep under the orbit.

Vag.: Vagus (X).
Plate III: Dorsal view of right lateral and frontal barbel nerves.

Lateral barbel: The three nerves which enter the base of the lateral barbel subdivide into fine fibers within the barbel. Although the endings could not be clearly distinguished, it appears that all the nerve endings are cutaneous.

Frontal barbel: Similar to the case of the lateral barbel, three nerve bundles enter the base of the frontal barbels. The lateral branch appears to be single, but the intermediate branch separates into three parts, the central portion being larger than the two medial sections. The medial branch consists of two cords, one of which is slightly larger than the other.
Plate IV: Diagram of a longitudinal section showing normal structure of the horned pout barbel. See also Plate VII.

TB: Note the two shapes of taste buds, both with cells concentrated in the interior portion.

PigC: Pigment cells are numerous in the epithelium, particularly around the periphery of the taste buds and at the junction between the columnar epithelial cells and dermal connective tissue.

N: A prominent nerve stained with thionin is visible in the connective tissue.

BV: Section of an artery visible in connective tissue.

FM: Fibrous membrane underlies the epithelium.

Per: Layer of fibroblastic perichondrium is difficult to distinguish from the fibroblastic connective tissue which surrounds it.
Plate V: Cross-section showing normal structure of *Ameiurus* barbel.

**N:** Two large nerve bundles are concentrated primarily dorsal and ventral to the cartilaginous rod.

**Ep:** Note the squamous epithelial cells on the surface and the columnar epithelial cells at the base of the epithelium.

**Per:** A layer of perichondrial cells, apparently laid down by surrounding connective tissue, encloses the cartilage.

**BV:** A single large artery penetrates the barbel next to the ventral nerve bundle. Several smaller arteries are also visible in this section.
Plate VI: Portion of the tip of a regenerating barbel, illustrating the blastema 17 days after amputation.

Pig C: Pigment cells containing melanin granules have regenerated in the region distal to the level of excision.

NCT: New connective tissue, derived from pre-existing connective tissue, lies between the blastema and the wound epithelium.

D. per: Note the depletion of cells in the perichondrium just proximal to the level of amputation.

B: The blastema, composed of tiny undifferentiated cells, appears to be derived from migrating perichondrial cells, which subsequently undergo mitotic divisions to cause elongation.
Plate VII: Longitudinal section through a normal barbel.

A: Note the taste buds and numerous pigment cells interspersed throughout the epithelium. Beneath the epithelium is a layer of dermal connective tissue which contains prominent aggregations of chromatophores. Between the dermal connective tissue and cartilage lie layers of loose sub-dermal connective tissue and perichondrium.

B: Enlargement showing taste bud structure. Note the concentration of cells on the internal portion of the bud.
Plate VIII: Sequence showing normally regenerating barbels.

C: Regenerating barbel 5 days after amputation. The wound epithelium has begun forming at the severed tip of cartilage, although blastema formation has not yet commenced.

D: Barbel with a well-defined blastema 10 days after excision. The tightly-packed undifferentiated blastema cells are undergoing rapid mitosis as barbel elongation begins.
PLATE VIII
Plate IX: Sequence of normally regenerating barbels cont.

E: Regenerating barbel 12 days after amputation.

F: Advanced stage of regeneration 41 days after amputation. New cartilage has begun to chondrify in the regenerate (arrows), which has deviated to the side due to lack of cartilage support in the early stages of regeneration.
PLATE IX
Plate X:

G: Distal portion of amputated barbel showing depletion of perichondrium during blastema formation.

H: Same regenerating barbel proximal to the point of excision showing the normal thickness of the perichondrium.

I: Barbel regenerating normally 18 days after extirpation of the cartilaginous rod and simultaneous barbel amputation at the proximal end of the intact cartilage. The arrow indicates the proximal level of intact cartilage.
Plate XII: Barbel 30 days after removal of the cartilaginous rod followed by immediate amputation.

J: Although normal regeneration has not occurred and a blastema has not formed, a small extension has developed consisting of connective tissue, nerve fibers, and epidermis.

K: Cartilage and surrounding perichondrium which was removed from the barbel in "J".
Plate XII: Non-regenerating barbel treated with mitomycin-C.

L: Healed barbel stub 47 days after excision and first injection with mitomycin-C. Although a wound epithelium has formed, elongation has not followed.

M: Note the accumulation of connective tissue cells, whose motion has been hindered by the tightly-packed mass of wound epithelial cells.

N: Note that perichondrial cells appear to be hindered from migrating to the end of the cartilage. A few connective tissue cells and released chondrocytes are visible at the cartilage tip. The arrow indicates perichondrial cells.
Plate XIII: Non-regenerating barbel 20 days after excision and first injection with aza-guanine.

O: Even 20 days after excision there are no signs of blastema formation. A wound epithelium interspersed with pigment cells has covered the severed tip and a few connective tissue cells and chondrocytes are visible under the epithelium. Perichondrial cells appear to be sterically hindered from migrating to the cartilage tip by the mass of connective tissue cells whose motion has been impeded by the lack of space under the epithelium.

P: Perichondrial cell migration impeded by subdermal connective tissue cells, some of which have undergone mitotic divisions.

Q: Note blockage of perichondrial cells by tightly-packed connective tissue cells.
Plate XIV: Non-regenerating barbel 30 days after amputation and first injection with mitomycin-C. A smaller antibiotic dosage was used here than in the barbels shown in Plates XII and XIII (0.035 mg./ml. as compared with 0.04 mg./ml. at 3-day intervals.

R: Although no blastema has formed, the smaller antibiotic dosage allowed more prolific mitotic divisions in the epithelium, creating space for formation of a layer of connective tissue cells between the wound epithelium and severed cartilage tip.

S: No dense accumulation of connective tissue cells proximal to the amputation level is evident here (cf. Plates XII and XIII). Despite more prolific epidermal mitoses, resulting in relief of the accumulation of connective tissue cells, perichondrial cells are still restricted from migrating to the severed cartilage tip.
PLATE XIV
LITERATURE CITED


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An Abstract on
An Investigation of Barbel Regeneration
in the Catfish, *Ameiurus nebulosus*

It has been clearly demonstrated that the barbels of *Ameiurus nebulosus* regenerate completely following amputation. The current investigations involve experimentation dealing primarily with determination of the mechanisms by which regeneration occurs. However, prior to conducting a series of experiments designed to elucidate the processes which initiate regeneration, several preliminary investigations to determine normal barbel structure and regeneration were made.

Brain dissection of an adult catfish confirmed that innervation of the barbels is by both the fifth and seventh cranial nerves, and not solely by the seventh cranial nerve as suggested by Olmstead (1920). Further information regarding normal barbel innervation and structure was obtained from an histological examination of longitudinal and cross sections. The barbel is supported by a central cartilaginous axis, which is surrounded by a perichondrium. Enclosing the perichondrium is a layer of looser connective tissue, containing nerve fibers concentrated chiefly dorsal and ventral to the cartilage, and an artery. Interspersed among the squamous and cuboidal epithelial cells which cover the barbel are numerous taste buds and pigment cells.
Because taste buds of two distinct shapes were observed, the possibility that there may be two types of sensory buds in the barbel cannot be overlooked. One undoubtedly serves the function of taste, while the other may be used for touch.

Observation of the histological details of normal regeneration indicates that the wounded stub of an amputated barbel is healed within one day by a layer of squamous epithelial cells. Despite rapid healing, elongation of the regenerate does not commence until a blastema is formed, apparently from migrating perichondrial cells which deplete the perichondrium proximal to the amputation level. As the blastema cells undergo rapid proximo-distal proliferation and the epidermal cells continue mitotic divisions, the barbel enters a period of rapid elongation. At 20°C regeneration proceeds at a rate of 1.0-1.5 mm. per week until the original dimensions have been regained. Regeneration is inhibited by colder temperatures, and is totally halted at 12°C. Because nerves, blood vessels, and connective tissue are not fully regenerated until the original length has been approximated, the regenerating barbel diameter is somewhat smaller than that of the original.

To substantiate the conclusions drawn by histological observation of normal regenerating barbels, the barbels of many fish were subjected to abnormal treatments. The first treatment involved extirpation of the cartilaginous rod prior to amputation of the barbel in the affected region.
The subsequent regeneration is abnormal and inhibited. In the absence of the cartilaginous rod and its surrounding perichondrium, no blastema forms and therefore little elongation occurs due to lack of physical pressure apparently exerted on the wound-healing epidermal and connective tissue cells by the proliferating and chondrifying blastema cells. Even without blastema formation, nerves, connective tissue, blood vessels, and epidermis, with its pigment cells and taste buds, regenerate. Sub-dermal and dermal connective tissue rapidly grow beneath the healed epidermis, but do not form a zone similar to, or part of, the blastema. Because the blastema does not develop without the presence of the cartilage and perichondrium, it appears that the blastema is derived primarily from perichondrial cells and to a limited extent from chondrocytes released from the severed cartilage. It further appears from observation of normally regenerating barbels that the blastema is initially derived from migrating rather than proliferating perichondrial cells.

To determine if the blastema originates from migrating or dividing perichondrial cells, the fish were subjected to a second abnormal treatment. Regenerating barbels were injected with either mitomycin-C or aza-guanine to inhibit DNA synthesis, and thereby also to inhibit mitotic cell division. Although it was not possible to totally inhibit cell division without killing the fish, mitosis was significantly inhibited in many cases. Following antibiotic injection, the wound was healed as usual, but by an abnor-
mally thin layer of epithelial cells. Although a few subdermal connective tissue cells penetrated between the cartilaginous tip and the wound epithelium, most of the cells were prevented from penetrating beneath the wound epithelium, and therefore accumulated proximal to the amputation point. Because the antibiotics apparently inhibited mitosis sufficiently to prevent formation of the space necessary to allow perichondrial cells to accumulate at the severed tip, the perichondrial cells also were sterically hindered from penetrating between the epidermis and the cartilage. However, unlike the case of the connective tissue cells where there was an abnormally large accumulation of cells immediately proximal to the level of amputation, there was no abnormal cell accumulation in the case of the perichondrial cells, indicating that they had not undergone even inhibited mitosis. It therefore appears that the perichondrial cells form the blastema by migration to the severed cartilaginous tip, rather than by proliferation.

Further evidence in support of the hypothesis that the blastema is derived from migrating perichondrial cells was sought by a third treatment. Many fish were injected with various dosages of the vital dye trypan blue, which is specific for phagocytic cells. Intraventricular injection was unsuccessful because the fish died shortly after the injection, while intraperitoneal injection was also somewhat unsuccessful because only very small quantities of the dye reached even the base of the barbels, and almost none ex-
tended significantly into the barbels. Therefore, the destiny of the colored cells following amputation could not be traced because there were no colored cells as far distal as the excisions. Because it is felt that the basis for the trypan blue experiments is sound and that with improved injection technique, the experiment would be successful, the author intends to pursue the problem further.

A fourth treatment, removal of barbel epidermis, was used to determine the origin of new epithelial cells. As an adaptive reaction to frequent natural abrasions in the epidermis, epidermal cells are capable of rapid proliferation, resulting in wound healing, by spreading over or into a wound area from its margins.