MELANOPHORE BANDS AND AREAS DUE TO NERVE CUTTING,
IN RELATION TO THE PROTRACTED ACTIVITY OF NERVES

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PLATES 1 AND 2
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1. INTRODUCTION

Brücke (1852), in his study of the color changes of the African chameleon, was the first to record the fact that the cutting of chromatic nerve fibers was followed by a darkening of the area of skin thus denervated. He ascribed this condition to paralysis. Pouchet (1876) also recorded such a darkening in fishes, particularly in turbots, and accepted Brücke’s interpretation of it. Von Frisch (1911) added to the list of fishes that showed these peculiarities and noted incidentally that 8 days after the initiating operation the dark area thus formed on the fish began to show signs of blanching and that in 13 days it was strikingly pale. The blanching of such dark areas was confirmed on the minnow Phoxinus by Smith (1931) who pointed out that the ultimate loss of color in these areas was possible only when the fish was maintained in a relatively pale state. Meanwhile Wyman (1924) had used denervated bands on tails, now generally known as caudal bands, for an extended study of chromatic physiology in fishes, and the technique of the caudal band came into common use among numerous workers (Abolin, 1925; Smith, 1928; Fries, 1931; Mills, 1932; Matthews, 1933; Parker and Porter, 1933; Abramowitz, 1935; Kleinholz, 1935; Foster, 1937; Dalton and Goodrich, 1937; Kamada, 1937; Odiorne, 1937; Wykes, 1938; Osborn, 1938; Matsushita, 1938; Tomita, 1938; Vilter, 1938; and others).

A little over half a decade ago it was pointed out that when in a fully blanched caudal band on such a fish as Fundulus a new cut was made strictly within the limits of the original band and slightly distal to the first cut, the dark tint of the original band would reappear though not so fully as at first (Parker, 1934 a). This condition was of prime importance in two respects. It showed, first, that the chromatic nervous mechanism of the band was not paralyzed, as had been assumed since the days of Brücke, but that it was merely quiescent and could easily be reactivated. And it showed further that the darkening of the band was not the result of checking
certain assumed central influences whereby the melanophores had been held contracted (Zoond and Eyre, 1934; Zoond and Bokenham, 1935; Sand, 1935; Wykes, 1938), but that this darkening resulted from an unusual stimulation of the dispersing nerve fibers at the site of the wound. This new interpretation has been called the superactivity hypothesis as contrasted with the older one which was designated as the paralysis hypothesis (Parker, 1936). Since a caudal band in Fundulus may remain visible for hours or even a day or more after its first formation it was suggested that the dispersing nerve fibers in such a band are active over a correspondingly long period, a view that has met with very little approval from other workers. The possibility of long continued activity in such nerve fibers will be considered in the following pages and this question will be discussed not only from the standpoint of caudal bands, but from that of the larger areas of skin darkened by denervation.

The technique employed in these studies calls for no special description. Where operations of considerable extent were performed on the fishes the creatures were anesthetized by cold in water and cracked ice (Parker, 1939), a method which proved to be in every way satisfactory.

2. Revival of Bands and Other Areas

Faded caudal bands in Fundulus are readily revived by recutting. In Fig. 1 is shown the tail of a moderately pale Fundulus on which 3 days previously a caudal band had been formed by cutting a ray near the root of the tail (lower band in the figure). After this band had fully blanched it was recut distal to the position of the initial cut with the result that the band was revived though with less intensity of shade than that of the original band. At the same time that the second cut was made a ray slightly above the middle of the tail was also cut and a strong caudal band was thus produced as shown in Fig. 1. This new band agreed in intensity with that of the first band when it was originally cut and could be used as a check on this band. The condition of the melanophores as seen in the two bands and the normal tail in Fig. 1 are shown in Figs. 2, 3, and 4. In the normal tail (Fig. 2) the melanophore pigment is nearly though not completely concentrated, a condition characteristic of a fairly pale fish. In the newly formed band (Fig. 3) the melanophore pigment is almost fully dispersed and in the recut band (Fig. 4) this dispersion is pronounced though not so extensive as in the newly formed band. Thus the gross appearance of the bands in the tail as well as the condition of the pigment in the three states of the melanophores agree in showing that a caudal band in Fundulus may be revived by recutting though the band thus reactivated is never so intense in its revived state as in its initial one.
The revival of a band in Fundulus is not only thus possible but this process may be repeated in this fish at least twice. One example will suffice. In a pale Fundulus in which a caudal band had blanched in about 11 hours the band was revived by recutting and was again blanched in about a day after which on a renewed cutting a third darkening took place. This third response was by no means so pronounced as the second, but it was visible beyond a doubt. A fourth attempt failed to elicit an unquestionable darkening. This failure was probably due to the beginning of degeneration in the chromatic nerve fibers which makes itself manifest usually in about 5 days after the initial cut (Parker and Porter, 1933). A simple reactivation of bands such as occurs in the melanophore system of Fundulus has also been observed in the erythrophore system of the dorsal fins in the squirrel-fish Holocentrus (Parker, 1937).

The revival of blanched caudal bands in the catfish Ameiurus as described by me some years ago (Parker, 1934 b) has been questioned by Wykes (1938) and by Osborn (1938). Osborn in particular stated that although he recut faded bands in this fish repeatedly he never was able to produce a second darkening. I reinvestigated this matter (Parker, 1940) and found that faded bands in both normal and hypophysectomized catfishes could be readily revived by recutting provided the fishes tested were kept in water at ordinary summer temperatures, about 20°C. I attributed Osborn's failure in this respect to the low temperature at which he had worked, 12°C. In a recent retesting of catfishes in water at this degree of cold I too was unable to obtain reactivation of bands. This is in line with Wykes' statement (1938) that at the winter temperature of 6°C the color activities of Ameiurus are in almost full abeyance.1 The revival of blanched caudal bands, though not without exception, has been reported for the Japanese catfish Parasilurus by Matsushita (1938). Catfishes are less satisfactory for testing color revival than Fundulus probably because of the slowness with which their bands blanch. This step in Ameiurus often takes some days and before the bands are pale enough to be recut their nerves have probably begun to degenerate and are thereby rendered incapable of response. This I suspect may be the reason Vilter (1938, 1939 a, 1939 b) was unable to reactivate the bands in the dorsal fins of Gobius, though here too temperature may play a part. Unfortunately this worker makes no statements as to the details of his procedure. Certainly the overlapping

1 The effect of differences in temperature on the chromatophore changes in Macropodus has recently been recorded by Dalton and Goodrich (1937) who observed that the blanching of dark caudal bands in this fish required more time (10-18 hours) at 20°C. than at 29°C. (5-8 hours).
of the areas of distribution of the chromatic nerve bundles which he has observed in the dorsal fins of Gobius can offer no explanation for the revival of caudal bands in Ameiurus, for no such overlapping occurs in the tail of this fish (Parker, 1934 b). This in fact is one of the advantages of Ameiurus for this type of work.

Although caudal bands and other like areas appear to be open to reactivation wherever they are properly tested, it is quite unknown, so far as I am aware, what occurs in the larger areas of skin darkened by denervation. Such areas as these, however, often covering a considerable part of a fish, were what called the attention of the earlier investigators to this subject. By cutting an appropriate bundle of nerves much of the head of a Phoxinus can be darkened (Smith, 1931), or a quadrant of the body of a Fundulus (Parker, 1936), or even the posterior half of a Macropodus (Kamada, 1937). Will these large areas blanch and are they open to the kind of revival that has been shown to occur in caudal bands?

For convenience in this kind of experimentation the head regions of such fishes as Fundulus and Ameiurus are very satisfactory and the cranial nerve best adapted for cutting in this part of the fish is the ophthalmic. When in a pale Fundulus the orbit is opened dorsally and the ophthalmic nerve which lies on the roof of this cavity is severed, the corresponding half of the head from the snout to the eye and from the lateral wall to the mid-dorsal line darkens within about a minute. This darkening increases for half an hour after which it gradually blanches until in a day or so it has largely disappeared. It vanishes not by fading throughout its whole expanse, but by shrinking on its edges till the last part that is visible is a small dark patch dorsal to the eye. When this experiment is carried out on fully dark killifishes, no change in the tint of their heads is to be noticed, for the heads of such fishes like the rest of their bodies are from the beginning very dark. If in a pale Fundulus the ophthalmic nerves of both sides of the head are cut simultaneously, the head as a whole darkens after which it slowly and completely blanches. These observations on Fundulus substantiate fully those of Smith (1931) on Phoxinus.

Similar tests on Ameiurus can be carried out with greater convenience because of the large size of its head. The responses of Ameiurus, however, are relatively slow. If the ophthalmic nerve in a pale Ameiurus is cut by penetrating at an appropriate position the dorsal bony wall of the orbit with a narrow knife-blade, the half of the head concerned will begin to darken in about 10 minutes and will be fully dark in from 4 to 5 hours (Text-fig. 1). After this the dark area will commence to lose its deep tint and in 30 to 50 hours will have become about as pale as the rest of the fish. The blanching of this large cephalic area in Ameiurus shows details which
are not visible in Fundulus. The large cephalic area in Ameiurus shrinks toward the eye as it does in Fundulus, but with greater irregularity of outline. Before, however, the area in Ameiurus has diminished greatly one or two pale spots appear within its contour (Text-fig. 2). These eventually coalesce and sooner or later unite with the shrinking irregular outline and thus contribute to the gradual disintegration of the area as a whole. In this respect the disappearance of the area in Ameiurus is unlike that in Fundulus and very unlike the disappearance of caudal bands in either fish where the operation is strictly a shrinkage from the periphery.

Text-Figs. 1, 2, and 3. Diagrams of the dorsal aspect of the head of a pale catfish, Ameiurus nebulosus, showing darkened areas resulting from the cutting of the ophthalmic nerve.

Text-Fig. 1. A catfish head showing the extent of the darkened area (stippled) that resulted from the severance of the ophthalmic nerve through the initiating aperture I.

Text-Fig. 2. The same catfish head as shown in Text-fig. 1 a day or two after the initiating cut had been made and showing the shrinkage of the original dark area by lateral invasion and by internal disintegration.

Text-Fig. 3. A catfish head like that shown in Text-fig. 1 whose dark area having been completely blanched was partly revived by a second and more distal cutting (R) of the ophthalmic nerve.

In Ameiurus as in Fundulus the simultaneous cutting of both ophthalmic nerves is followed by a darkening of the whole dorsal aspect of the head. Such very large areas in Ameiurus disappear also by irregular internal disintegration as has been described for the darkened half-cephalic area in this fish. From these observations on the killifish and the catfish I conclude that large areas darkened by nerve cutting in pale fishes can blanch as fully as caudal bands can.

Are these large areas open to revival of coloration as are caudal bands? To test this catfishes about 15 cm. long were much more favorable than killifishes which at a maximum were about half that length. By a transverse, vertical cut in the head of a pale catfish close to the region of the internal ear and on the exterior of the cranium, it was possible to sever the strand of autonomic nerve fibers supplied from the posterior part of the
system to the anterior cranial nerves. As a result of such a cut the head soon darkened by melanophore expansion and then in a few days blanched. If, when this stage had been reached, a second cut was made anterior to the first one and so that the ophthalmic nerve in the posterior part of the orbit was severed, the chromatic fibers for the top of the head could thus be cut for a second time. As a result of this the half of the head in front of the new cut began to darken in about 30 minutes after which the darkening continued for an hour or more till this somewhat smaller area was again decidedly dark (Text-fig. 3). This test was carried out on five catfishes all of which gave essentially the same result. In some the blanching of the first area came more quickly than in others and in some the second darkening was more pronounced than in others, but in all the initial dark area blanched in course of time and a final second darkening was always induced. I therefore conclude that large areas darkened by the severance of chromatic nerves such as the cephalic areas of the catfish not only blanch but may be revived by the recutting of their nerves as is true of caudal bands.

Other parts of catfishes on which somewhat similar tests can be made are the pelvic fins. The advantage of these fins for such work was first pointed out by Wykes (1938). These fins, which are situated on the ventral aspect of the fish immediately anterior to the cloaca, consist of a very transparent membrane supported by some eight fin rays (Fig. 5). They are provided with a sparse, rather uniformly scattered supply of well defined melanophores (Fig. 6) and are convenient for study in that the fin of one side of the fish may serve as a convenient control for that on the other side. When a ray in the pelvic fin of a pale fish is cut, a dark band forms (Fig. 7) as in the case of the tail. The pigment in the melanophores of such a band is fully dispersed (Fig. 9) as contrasted with that of the color cells in the rest of the fin (Fig. 8).

Each pelvic fin is innervated by some six spinal nerves which can be easily traced from near the vertebral column over the inner face of the body wall and into the fin. By a single longitudinal cut through the body wall to the body cavity slightly dorsal to the root of the fin all these nerves can be severed. Such a cut, about a centimeter long, can be easily closed by a few stitches. Fishes thus operated upon live well, remain active, and have been kept in aquaria for 10 days or more.

When one pelvic fin of a catfish is thus denervated, it soon darkens and remains dark for some days. This circumstance led Wykes to remark that it is difficult to ascribe this persistent melanophore expansion to an injury discharge in the severed fibers since it lasts unchanged for so long a time. She was therefore led to assume another explanation, namely, that the cut
acted not by excitation but by the elimination of some central activity such as inhibition. A simple experiment would have shown the error of this view.

In my tests on *Ameiurus* a denervated pelvic fin was found to begin darkening in from 5 to 10 minutes. In 2 hours or so it was fully dark and in 3 to 4 days it was blanched almost to the same degree of paleness as that of the fin of the opposite side. It was now comparatively easy to cut in the blanched denervated fin a single ray with its nerve and to ascertain thereby whether reactivation was possible. In all five fishes thus tested dark bands developed about the newly severed rays in strong contrast with the paleness of the fin as a whole (Fig. 10). The pigment in the melanophores of these bands was obviously though not extremely dispersed (Fig. 12) as compared with that in the melanophores of the other parts of the fin (Fig. 11).

Such darkened bands, which were observed in fishes kept at about 20°C, could have depended in no sense upon the elimination of central influences, for, if there were such, they had already been excluded by the first cut. The darkening as induced by the second cut must have originated in the cut itself. In this respect the pelvic fins of *Ameiurus* respond to chromatic tests in the same way as the caudal fins of this fish do. Thus all the evidence from nerve cutting including that from the pelvic fins, from the cephalic areas, and from the caudal bands, leads to one conclusion, namely, that when nerves with dispersing chromatic fibers in them are cut these fibers are not at once paralyzed but are especially activated whereby the melanophores associated with them are induced to disperse their pigment and thus to darken the denervated area.

3. Is the Activity of Cut Dispersing Nerve Fibers Protracted?

It was my original opinion (Parker, 1934 a) based upon a study of the dispersing chromatic nerves in the tail of *Fundulus* that after severance, these nerves were excited from the region of the cut distally for the approximate period during which the dark caudal band was clearly visible. This interval varied from a dozen or more hours to several days. In the Japanese catfish, as pointed out by Matsushita (1938), a caudal band may be present for more than 2 weeks, a very long time over which to assume the continuous activity of a nerve. Matsushita was therefore disposed to regard my suggested explanation as premature, and it is true that since this question was first discussed a number of new and significant observations have been made on the mechanism of color changes in fishes.

It is now generally admitted that at least three elements are involved in the chromatic responses of catfishes. These are the pituitary gland, and
two sets of autonomic nerve fibers one dispersing and the other concentrating. These three are very probably not the only elements concerned, but they are unquestionably the chief ones. From the pituitary gland is derived the blood-borne, dispersing neurohumor intermedin, from the concentrating nerves adrenalin, and from the dispersing nerves acetylcholine (Chin, 1939; Chang, Hsieh, and Lu, 1939; Parker, 1940). Osborn (1938) has shown that intermedin can be identified in the blood of a catfish in physiologically significant amounts some 70 hours after the loss of the pituitary gland. So far as color changes are concerned this substance disappears from the blood of hypophysectomized catfishes within 5 days after the operation. These observations show that intermedin is a reasonably stable and persistent agent. Adrenalin is known to remain active in the blood of the catfish for some hours after injection. On the other hand acetylcholine unprotected by such substances as eserine is destroyed almost at once in the circulation of this fish. Both acetylcholine and adrenalin, however, if carried in olive oil, may be introduced subcutaneously into catfishes in the form of coarse emulsions and under such circumstances these agents will remain effective as color activators for several days. In oil acetylcholine and adrenalin are evidently protected from destruction, and their action is thus prolonged (Parker, 1940). Under natural conditions they probably enjoy a similar protection and extension of activity by residence in the lipoid materials of the skin. Is it possible that the experimental extension of the color phase after nerve cutting attributed originally by me to continued nerve action is due to this protection and persistence of the activating substances in fatty materials? An answer to this question might settle not only this particular problem but other related ones in the general field of color change.

One means of attack on this question would be to ascertain by oscillograph methods whether chromatic nerve fibers after having been cut would continue to show action potentials. In making tests of this kind I am under great obligations to Dr. C. L. Prosser, then at the Marine Biological Laboratory, Woods Hole, and to Dr. Hallowell Davis of the Harvard Medical School. Caudal rays from four different fishes were examined for action potentials. Each ray consisted of a flattened, imperfect, cartilaginous tube through the cavity of which extended a bundle of nerve fibers containing chromatic elements. The nerves in some of these rays before tests were made had been activated about 18 hours earlier by cutting, others were freshly cut, and still others were uncut and used as controls. In a group of eight rays four showed some electric activity, physiological or physical, beyond what could be identified as amplifier noises. One of
these was a ray cut the previous night, two were freshly cut, and one was an uncut control. The other four rays three of which had been cut and were in excellent state for possible action potentials showed no trace of such activities. The sensitivity of this apparatus was about 2 microvolts. Strong evidence that the nerve disturbances, where they occurred, were physical and not action potentials is their persistence for at least 3 minutes in a totally excised ray which had been liberally stroked with chloroform. Dr. Davis's conclusion was that no activity that could be identified as physiological was to be observed in any of the rays either activated or quiescent. This inability to observe such activity may be attributed to the large amount of shunting tissue and the insulating sheath of the ray itself for the removal of which no satisfactory technique could be found.

As contrasted with the caudal nerves in the catfish the ophthalmic nerve in this animal is readily accessible for oscillograph tests. When electrodes are applied to this nerve in the dorsal part of the orbit and the snout of the fish is gently stroked a burst of sensory impulses may be observed. Results of this kind were noted in six of nine preparations. Care was taken to distinguish between true responses and the artifacts due to bodily movements of the fishes, the movements of the nerve on the electrodes, and other like disturbances. These observations showed the ready excitability of the ophthalmic nerve. The cutting of this nerve near its central end was followed by an abundant volley of spikes. Attempts were then made in the region external to the ear capsule to cut the autonomic tracts to the exclusion of other nerve bundles and thus to excite only this particular component in the ophthalmic nerve. But no such favorable region could be found, for at all places on the side of the head of the fish these tracts were accompanied by nerve fibers, probably lateral-line components from the vagus complex, and it was impossible to cut one set of fibers without doing the same to the other. Moreover the greatest length of the ophthalmic nerve available for oscillograph tests in the catfish, about 1 centimeter, was too short for satisfactory work. Hence I was forced to abandon this type of test on the ophthalmic nerves as well as on the caudal nerves and I turned to other methods of attack on this question. The most promising of these appeared to be some form of nerve interference whereby the chromatic impulses could be locally and temporarily blocked with the possibility of a subsequent return.

To this end one naturally reverts to anesthetics and other like substances, and for fishes to such agents as magnesium sulfate.

In preparing catfishes for tests with this salt they were enveloped in wet cheese-cloth, fastened sidewise on an inclined pine bench, and provided with a continuous current of...
fresh water over their gills. Their caudal fins were then spread out and rendered im-
mobile by being held in place on the top of the wooden bench by push-pins. Two short,
parallel cuts were then made through the fin membrane and on opposite sides of a given
fin ray. To these cuts a drop of saturated, aqueous solution of magnesium sulfate was
next applied on the assumption that this material would enter the cuts and soon reach
the nerves. A crystal of magnesium sulfate was also placed on the cuts to reinforce the
solution. After 40 minutes, to take a particular example, the given ray which had
shown no darkening at all, was cut transversely at a point proximal to that at which the
salt had been applied. A neighboring ray the sixth from the anesthetized one was also
severed at the same time as a control. About 20 minutes later the control ray showed a
darkened band, but the anesthetized one was only slightly darkened between the cut and
the point at which the magnesium sulfate had been applied. The fact that this ray was
fully pale distal to the spot treated with magnesium sulfate showed that this salt had been
an effective nerve block. When this fish about an hour later was freed and allowed to
swim in a white-walled illuminated tank, the distal part of the anesthetized ray became
in the course of an hour or so moderately dark.

Thus for a period of about an hour the magnesium sulfate blocked some
influence which, after the presumable loss of the salt, exerted upon the
melanophores the same dispersing effect that cutting the nerve of the
control ray had done. Responses of this kind though of constant occurrence
were sluggish and far from regular in their time relations. They nevertheless
were of such a nature as to make it difficult to understand them except
on the basis of a protracted activity of dispersing nerve fibers.

An interesting feature in the tests with magnesium sulfate that should
not be lost sight of was the irresponsiveness of anesthetized nerves to
cutting. When a nerve was anesthetized as described and after about half
an hour was severed in the anesthetized region, no darkening appeared on
any part of its length though a cut made distal to the region of anesthetiza-
tion was soon followed by a darkening of the distal innervated area. This
condition points to the inexcitability of the dispersing nerve fibers when
under the influence of magnesium sulfate in addition to their inability to
conduct impulses, a state that is after all not surprising.

Although the responses of the dispersing nerve fibers when treated with
magnesium sulfate are significant, the uncertainty in their timing led me to
try other forms of block. Of these I found cold the most effective.

For the application of a cold-block a simple piece of apparatus was devised (Text-
fig. 4). This consisted of a glass reservoir (Text-fig. 4, R) made from a bottomless,
large bottle held inverted and containing about 1½ liters of 50 per cent alcohol. Into
this liquid was lowered a long beaker weighted with lead and containing a supply of dry
ice (Text-fig. 4, D). By controlling the amount of ice the temperature of the alcohol
mixture could be held at any desired point from -15°C. upwards. The aperture in the
bottom of the reservoir, the neck of the bottle, was tightly closed with a rubber stopper
from which a glass tube led to a short rubber tube which carried the blunt cold-point (Text-fig. 4, II). This was a piece of glass tubing the lower part of which was of capillary fineness and rather sharply bent upon itself at an acute angle. The remainder of the capillary glass tube was turned to one side and directed in such a way as to serve as a convenient outlet (E). The rubber tube connecting the glass tube from the reservoir with that carrying the blunt cold-point was provided with a metal screw-clamp (C) by which the flow of fluid in the apparatus could be controlled. When the cold alcohol mixture was flowing through the tube it ran from the capillary exit at a temperature of
2 or 3° above that in the reservoir. The temperature at the cold-point must have been between these two extremes. With the temperature of the alcohol mixture in the reservoir at about −10°C. that at the capillary exit was some −7°C. and a drop of water put on the cold-point, though exposed to the air, would soon freeze.

Below the cold-point was a small, movable, inclined, pine bench (Text-fig. 4, B) provided with hooks and a cord by which a live catfish (F) enveloped in cheese-cloth could be firmly bound on its side to the top of the bench. By means of glass pushpins the tail of the fish (T) was spread out immovably on the bench and, by shifting the bench slightly any desired part of the fish's tail could be brought into contact with the cold-point. Thus the cold-point, whose diameter was a little over a millimeter, could be applied to any spot on the length of a caudal ray. Fresh water from a rubber tube (W) was led into the mouth of the fish for respiration and flowed out over the gills to escape down the inclined bench. Pale fishes thus attached to the bench could be tested over periods of a number of hours. For some reason not wholly clear they gradually darkened on the bench till their condition was such that they were not very favorable for the inspection of their caudal bands. However fishes that had been bound to the bench 7 to 8 hours were still serviceable and when liberated were fully active and showed no signs of having suffered from their confinement.

When a pale catfish was put in the cold-point apparatus and the fluid was allowed to flow through the point in position on the fish's tail at room temperature, 22°C., no change was observed in the fish except the gradual darkening already noted. When after 15 minutes of flow the ray in contact with the point was cut near the base of the tail a complete, dark, caudal band was formed from the cut to the edge of the tail. The same was true when the temperature of the escaping fluid was about 10°C. When it was 5°C. the caudal band resulting from the cut was almost invariably incomplete in that it could be seen only from the cut to the cold-block and not beyond that point distally. At 0°C. the block was always perfect and no dark band was ever observed distal to the block so long as this outlet temperature was maintained. With the temperature of the fluid at the outlet at 0°C. that in the reservoir was usually 3 to 4° below zero. When the outlet temperature was −10°C. a small cake of ice formed between the cold-point and the fish's tail. This ice spread over a larger area of the tail than the area of the cold-point and as a rule covered about three rays. On releasing a fish thus treated it was found that the spot on the tail immediately below where the ice had been was very pale and that the three rays covered by the ice were dark from the region of the ice almost to the edge of the tail; in other words the very low temperature of the ice cake acted upon the nerves of the rays as cutting them would have done. It was evident from these preliminary tests that the satisfactory temperature for the cold-block was about 0°C., for lower temperature (−10°C.), like nerve severance, induced the formation of dark bands and higher temperatures...
were insufficient to restrain the nerve impulses. In operating with
the cold-block I therefore subjected the given ray of the fish to a tempera-
ture of about 0°C. for some 15 minutes and then proceeded to further
experimentation. I have seen nothing in my tests that would lead to the
suspicion that this treatment was not wholly satisfactory.

The special experimentation carried out upon catfishes with the cold-
block had to do with the interval of time between the cutting of the blocked
ray and the extension of the caudal band beyond the block itself after it had
been removed and the fish liberated. When a pale catfish was put in the
cold-block apparatus and the block was applied at about 0°C. for a quarter
of an hour, a severance of the chilled ray proximal to the block was followed
by the formation of a dark caudal band from the cut to the block but not
beyond it. This was so invariably the case that it may be looked upon
without question. If 15 minutes later the block was then removed and the
fish allowed to swim in a white-walled aquarium, the caudal band became
extended in that it could be seen in about 10 minutes to cover the course
of the ray from the cut to near the edge of the tail. It thus came to occupy
that part of the ray which was distal to the block and which before the fish
was freed was fully pale. It is difficult to understand this extension of the
band except by assuming that that nervous activity which when the cut
was made induced the formation of the proximal part of the band was also
present to excite the production of its distal part.

When tests similar to the one just described were carried out but with
longer intervals of time between the cutting of the ray and the removal of
the cold-block, the results were precisely like the 15 minute test. The
extension of bands distal from the block occurred after intervals of 30
minutes, 1, 2, 3, 5, and even 6½ hours. The longest of these periods was
tried twice after which the released fishes showed no signs of exhaustion
and developed dark distal bands obviously visible even against the fish's
own darkening background.

It has already been shown (Parker, 1940) that in the catfish, caudal bands
may be formed, though not at full intensity, in hypophysectomized indi-
viduals. It is therefore probable that the extension of these bands in pale
fishes of long standing is not necessarily dependent upon the pituitary
neurohumor intermedin. To be reasonably secure of this, however, the
block test was repeated on hypophysectomized catfishes. Five fishes were
deprived of their pituitary glands and were allowed a little less than a week
in which to recover. During this interval one died. Of the four remaining
fishes two were subjected to tests with the cold-block over periods of about
5 hours each. In both instances on freeing the fish a fairly dark extension
of the caudal band took place showing that this activity was dependent upon nerves. The results from the tests on these two fishes then agree with those on fishes whose pituitary glands were intact and justify the general conclusion that the activity of severed, dispersing nerve-fibers shows a real protraction.

What the means of exciting such severed nerves are is not known. I have elsewhere suggested (Parker, 1934 a) that excitation may be due to a chemical activation of the severed ends of the nerves by substances liberated from the adjacent tissues in the cut itself. Such an injury effect chemical in nature might well be compared with the kind of chemical stimulation of the cut ends of nerves as reported on recently by Fessard (1936), Brink and Bronk (1937), and others.

The fact that not only caudal bands but relatively large integumentary areas in such fishes as Fundulus and Ameirus may be darkened by nerve cutting, then blanched, and finally again darkened by the recutting of their nerves affords a potent argument against the views that such types of darkening are due to either paralysis (Brücke, 1852) or the exclusion of certain central nervous influences (Zoond and Eyre, 1934). Furthermore revived darkening demonstrates that vasomotor readjustments or other circulatory disturbances suggested as possible causes for integumentary color changes both pale and dark (Hogben, 1924; Lundstrom and Bard, 1932; Young, 1933; Wykes, 1938; Waring, 1938) are not essential to these changes, for the influence on the circulation of cutting a nerve must be fully expended with the first cut and yet the characteristic color changes reappear with the second one. I am therefore of opinion that none of these factors are responsible for the occurrence of melanophore expansion when a given chromatic nerve is severed.

As tests with the cold-block show the kind of activation here seen may persist for at least some hours. Such an extension of nerve activity is repugnant to most neurophysiologists. Accustomed as they are to the momentary activity of a cut nerve as seen in the single twitch of its muscle or in the very brief volley of spikes exhibited in an oscillograph record of its responses they are averse to accepting the idea of an extended period of activity. It must be remembered, however, that Adrian (1930) has shown that the cut nerves in cats and rabbits exhibit fluctuations of electric potential that may last for an hour or more and that when these fluctuations subside, they may be revived by recutting the nerves. Barnes (1930) has also recorded that after the motor nerves of the walking legs in the crab, Cancer, are severed nervous discharges of long duration follow. Hoagland (1933) in his study of the lateral-line nerves of fishes has shown that when
these nerves are cut centrally and freed peripherally from their terminal organs, neuromasts, they will exhibit injury discharges which may keep up for from 10 to 15 minutes. More recently Prosser (1934) has demonstrated that the cut nerve to the chela of the crayfish will emit high frequency discharges that ordinarily may be observed for 5 minutes after the preparation has been made. These instances support the idea that after certain nerves have been cut the activity thereby excited may continue for a relatively long period of time. In the case of the dispersing autonomic nerve fibers in *Ameiurus* this period may be as long as 6½ hours.

4. DISCUSSION

In an earlier part of this paper it was pointed out that large skin areas which had been darkened by the cutting of their nerves blanched as completely as caudal bands did. In some of these, such as the cephalic areas in *Fundulus*, the region became pale by lateral encroachment precisely as do the caudal bands in fishes generally. The dark cephalic areas in *Ameiurus*, however, blanched not only by lateral encroachment but also by internal disintegration. Such lateral encroachments have been ascribed to the passage of a blanching neurohumor from the adjacent pale field into the dark area by a cell-to-cell transmission (Parker, 1933) through the lipoid components of the cells (Parker, 1934 a, 1935). A neurohumor of this kind would be carried in oily material, a lipohumor, as contrasted with a water-soluble agent or hydrohumor. That this operation is due to an invasion of the dark area by a blanching humor and not to the loss of an opposing humor from the darkened region into the neighboring pale area was shown by Matsushita (1938) who demonstrated a blanching of a dark caudal band on its side next a pale innervated field and the absence of such blanching on its opposite side next a denervated pale field. This explanation of loss of tint by positive invasion applies well to blanching in dark caudal bands or in cephalic areas such as those of *Fundulus* where the whole area disappears by lateral encroachment, but it fails to make clear the way in which internal disintegration of dark areas takes place. Here a pale spot appears inside a dark region and enlarges quite independently of the periphery of that region. This must be due to some other operation than lateral invasion.

2 Waring (1938) states that O'Shaughnessy and Slome (1935) in their study of experimentally produced traumatic shock, have established the persistent effect of injury currents in nerves. However, the view expressed by these two authors, though in my opinion very probably correct, is advanced by them not as a demonstration, as is implied by Waring, but as a working hypothesis.
What that operation may be is not easy to surmise. It is very probable from the work of the last year or two that in *Amiurus* the nervous dispersing neurohumor is acetylcholine and the concentrating one adrenalin. The blanching activities that have been discussed in the preceding paragraph must then be due to adrenalin. This substance, as already stated, is moderately stable in the blood and lymph of the catfish where it may remain active for some hours after it has been injected. It partakes of the nature both of a hydrohumor and of a lipohumor, for it may be carried both in water and in oil. In my opinion the two steps in the blanching of such a dark region as a cephalic area in the catfish represent these two conditions of adrenalin. The lateral encroachment on such a dark region is due, I believe, to the action of adrenalin as carried in the lipoids of the adjacent tissues, and the internal disintegration results from the action of this substance as carried in blood and lymph directly under the melanophore layer. Thus lateral encroachment is an invasion by way of the skin and internal disintegration a subdermal invasion. This at least is an explanation of the total blanching of catfish cephalic areas to which I have not been able to find serious objection. According to this view the peripheral blanching of caudal bands and dark areas is due to adrenalin as a lipohumor and the internal blanching of dark areas to the same agent as a hydrohumor. This leads to a suggestive interpretation of the blanching of the pelvic fins in *Amiurus*. In my study of this phenomenon I have never seen any evidence of a progressive peripheral loss of shade in these fins such as is characteristic of caudal bands and other dark areas. When the pelvic fin blanches it blanches in toto and with great uniformity. Such an operation is what would occur if the blanching resulted from the action of adrenalin as a hydrohumor; *i.e.*, adrenalin dissolved in watery lymph. This I believe to be the case and in truth when a dark catfish is injected with the appropriate amount of adrenalin its pelvic fins blanch in precisely this way. This general view of the double action of adrenalin depending upon the way in which it is carried is obviously hypothetical, but as an hypothesis it appears to meet all the requirements of the situation.

From what has been presented in the preceding pages it is evident that the darkening of *Amiurus* is dependent upon two neurohumors, acetylcholine and intermedin. Although these two substances in general act in the same way on catfish melanophores, they are not precisely similar from an operational standpoint. It is now well established contrary to my original opinion (Parker, 1934 b; Osborn, 1938; Parker, 1940), that of these two materials intermedin is the more effective as a chromatic activator.
At full dispersion the pigment of a macromelanophore in *Ameiurus* may cover an area whose diameter is about 145 microns. This degree of dispersion can be accomplished often with intermedin alone, as for instance on completely denervated melanophores in a caudal band, but in a hypophysectomized fish where acetylcholine from nerves is the exclusive dispersing agent only from a quarter to a half of this amount of pigment dispersion is possible. Hence acetylcholine is much less effective than intermedin as a means of darkening catfishes.

Although acetylcholine is second to intermedin as a melanophore activator in this fish it is probably a somewhat earlier agent in initiating darkening. A hypophysectomized palish catfish fully devoid of any functionally significant intermedin and in consequence dependent upon dispersing nerves and their acetylcholine for darkening will begin this process in half an hour after the fish has been put in a black-walled, illuminated aquarium. As contrasted with this a denervated, pale, caudal band in a pale catfish will start darkening only a number of hours or even a day or more after the fish has been placed in a similar aquarium. Thus the nervous agent, acetylcholine, probably really initiates the darkening process in the catfish and is followed and supported only after a considerable interval by the pituitary secretion intermedin. Hence in a second respect the two darkening agents, intermedin and acetylcholine, are noticeably different.

So far as the main question of this research is concerned—the protracted activity of cut chromatic nerves—only an incomplete answer can be given. The evidence herein presented strongly favors the view that dispersing autonomic nerves when cut pass into a state of activity that is greatly protracted in comparison with that of other cut nerves. This extension of activity may last for at least 6½ hours and during that time there is good evidence for a continued discharge of acetylcholine. That this activity may last for days or weeks cannot be asserted though there is no evidence, so far as I am aware, to the contrary. During the life of a dark caudal band which in *Fundulus* may be a day or more, in *Ameiurus* about a week, and in *Parasilurus* some 2 weeks, the earliest part of this period in *Ameiurus* at least is marked by continued nerve activity with the steady discharge of acetylcholine. This state may possibly continue with gradual abatement throughout the rest of the life of the band or it may be gradually replaced by a darkening operation dependent upon excess acetylcholine stored in the neighboring lipoids or it may involve both processes. Of course during the greater part of this whole period the caudal band is accentuated by the presence of the hydorhumin intermedin.
500 MELANOPHORE BANDS AND AREAS DUE TO NERVE CUTTING

5. SUMMARY

1. When appropriate chromatic nerves are cut caudal bands, cephalic areas, and the pelvic fins of the catfish _Ameiurus_ darken. In pale fishes all these areas will sooner or later blanch. By recutting their nerves all such blanched areas will darken again.

2. These observations show that the darkening of caudal bands, areas, and fins on cutting their nerves is not due to paralysis (Brücke), to the obstruction of central influences such as inhibition (Zoond and Eyre), nor to vasomotor disturbances (Hogben), but to activities emanating from the cut itself.

3. The chief agents concerned with the color changes in _Ameiurus_ are three: intermedin from the pituitary gland, acetylcholine from the dispersing nerves (cholinergic fibers), and adrenalin from the concentrating nerves (adrenergic fibers). The first two darken the fish; the third blanches it. In darkening the dispersing nerves appear to initiate the process and to be followed and substantially supplemented by intermedin.

4. Caudal bands blanch by lateral invasion, cephalic areas by lateral invasion and internal disintegration, and pelvic fins by a uniform process of general loss of tint equivalent to internal disintegration.

5. Adrenalin may be carried in such an oil as olive oil and may therefore act as a lipohumor; it is soluble in water and hence may act as a hydrohumor. In lateral invasion (caudal bands, cephalic areas) it probably acts as a lipohumor and in internal disintegration (cephalic areas, pelvic fins) it probably plays the part of a hydrohumor.

6. The duration of the activity of dispersing nerves after they had been cut was tested by means of the oscillograph, by anesthetizing blocks, and by cold-blocks. The nerves of _Ameiurus_ proved to be unsatisfactory for oscillograph tests. An anesthetizing block, magnesium sulfate, is only partly satisfactory. A cold-block, 0°C., is successful to a limited degree.

7. By means of a cold-block it can be shown that dispersing autonomic nerve fibers in _Ameiurus_ can continue in activity for at least 6½ hours. It is not known how much longer they may remain active. So far as the duration of their activity is concerned dispersing nerve fibers in this fish are unlike other types of nerve fibers usually studied.

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EXPLANATION OF PLATES

The photomicrographs on Plates 1 and 2 were taken by Miss Jane Bridgman.

PLATE 1

Fig. 1. Tail of a moderately pale killifish, Fundulus heteroclitus, showing, above, a newly excited caudal band fully dark and, below, a re-excited caudal band moderately dark. The re-excited band when first cut was as dark as the newly excited one seen in this tail. It was then allowed to blanch fully after which it was re-cut. It then darkened considerably but not completely.

Figs. 2, 3, and 4. Photomicrographs illustrating the conditions of the melanophores in different bands in the tail shown in Fig. 1.

Fig. 2. Melanophores from a pale band with almost fully concentrated pigment.

Fig. 3. Melanophores from the newly cut band; pigment almost fully dispersed.

Fig. 4. Melanophores from the re-excited band; pigment fairly dispersed, but not as much so as in the newly excited band (Fig. 3).
(Parker: Melanophore bands and areas due to nerve cutting)
PLATE 2

Fig. 5. Photomicrograph of the pelvic fin of a pale catfish.
Fig. 6. Melanophores from the fin shown in Fig. 5; pigment fully concentrated.
Fig. 7. Pelvic fin of a pale catfish showing near the middle a dark band produced by cutting a ray.
Fig. 8. Melanophores from an uncut ray in the fin shown in Fig. 7; pigment fully concentrated.
Fig. 9. Melanophores from the dark band in Fig. 7; pigment fully dispersed.
Fig. 10. Pelvic fin of a pale catfish fully denervated by having had all the nerves which entered the fin severed in the body of the fish before they reached the fin. After this fin had blanched one ray was cut whereupon this ray darkened somewhat.
Fig. 11. Melanophores from a blanched ray in the fin shown in Fig. 10; pigment almost fully concentrated.
Fig. 12. Melanophores from the cut ray in the fin shown in Fig. 10; pigment partly but not fully dispersed. Compare with Fig. 9.
(Parker: Melanophore bands and areas due to nerve cutting)