AN OIL-SOLUBLE NEUROHUMOUR IN THE CATFISH *AMEIURUS*

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(With One Plate.)

I. INTRODUCTION.

The idea that neurohumours rather than direct nervous activities are responsible for the pigment migration in chromatophores appears to be gaining ground. In 1932 Mills pointed out that in the normal fading of a caudal band in *Fundulus* this process does not take place throughout the band simultaneously, but begins on its edges and progresses slowly toward its axis. If, however, the band is made to disappear by an injection of adrenalin, the whole band blanches uniformly and rapidly. These two methods of disappearance led me to the view that there are two kinds of neurohumours, one soluble in water (and consequently also in blood) and quick and general in action, and the other soluble in oil and slow in action in that it passes sluggishly from cell to cell probably by means of the lipoid components of these bodies (Parker, 1934*a*). These two classes of neurohumours I tentatively designated as hydroneurohumours and liponeurohumours respectively (Parker, 1935). Hydroneurohumours, or more briefly hydrohumours, are well seen in such substances as adrenalin and pituitrin. Lipohumours can scarcely be said to have been identified. In fact the only example of this class seems to be one noted this past year in the smooth dogfish, *Mustelus canis* (Parker, 1935). The dark phase of this fish has been shown by Lundstrom and Bard (1932) to be dependent upon a water-soluble pituitary humour carried in the blood. The light phase was found by Parker and Porter (1934) to result from what would ordinarily be called direct nervous action, but this type of action on further investigation was shown to be quite otherwise (Parker, 1935). If an oil extract of the fins of a light dogfish is injected with proper precautions under the skin of a dark one, it produces a temporary local blanching of the skin. The light area thus formed is not caused by the death of the skin, but is due to a concentration of pigment in the melanophores from which the animal eventually recovers. Such a light area cannot be induced by an injection of a mixture of pure olive oil and Ringer's solution and it was, therefore, concluded that the oil extract of the light fins contained some material derived from these fins and capable of inducing the concentration of the melanophore pigment. What this material is has not yet been ascertained, but it is hoped in time to isolate it or at least to learn something of its nature. Meanwhile other fishes have been examined with the view of dis-

covering similar materials. The most promising form thus far studied is the common catfish, *Ameiurus nebulosus* Le Sueur, with which the present paper is concerned.

II. OBSERVATIONS.

The melanophore system of *Ameiurus* has already been somewhat worked out (Parker, 1934*b*). The dark phase of this fish is due in part to a pituitary hydrohumour carried in the blood and in part to what is generally regarded as direct, local, nervous action. The light phase of the fish is under direct, local, nerve control. When a bundle of nerve fibres in the tail of a catfish is cut, a dark radiating band results. This is the same as in *Fundulus* but the reverse of what occurs in *Mustelus* where the band resulting from a cut is light-tinted. Since the melanophores of any part of the integument of *Ameiurus* show a dispersion of pigment whenever their nerves are severed, it follows that all triturated preparations of the skin and fins of this fish take on a dark coloration even though they may have been obtained from a light fish. Consequently it is impossible in the catfish to triturate integumentary tissues and keep them in the light condition. All oil extraction in this fish is limited, therefore, to the dark phase. In preparations of this kind it is of course necessary to avoid the presence of the dispersing pituitary neurohumour. Hence the following procedure has been employed.

Catfishes about 20 cm. long were thoroughly lightened in tint by being kept several days in a white-walled aquarium illuminated continuously from above by an electric light and provided with a supply of fresh water not far from 20° C. Under such circumstances the fishes became extremely light-tinted. It was found to be very important that they be kept in relatively warm water, for in cold water, irrespective of the other environmental conditions, they became very dark and remained so. After the fishes had persisted in the light phase for several days, they were decapitated quickly and their bodies were allowed to stand half an hour or so in a moist chamber. Decapitation had the double effect of removing at once the whole pituitary system before any of its secretions could reach the rest of the body and of exciting to the extreme the nervous components for the dark coloration of the skin. The body of such a decapitated fish then was that of a light fish whose skin was devoid of pituitary neurohumour and whose ultimate darkening must have depended wholly upon local nerve action. It is from such a preparation as this that the dispersing nervous humour might be extracted provided such a humour exists.

The fins and skins of dark fishes prepared in the way just described were stripped off the animal and ground for about an hour with pure Italian olive oil in a rough porcelain mortar. To obtain a sufficient quantity of extract it was found best to take the fins and skins of at least five fishes and to triturate this amount of tissue with 2 c.c. of olive oil. The resulting pasty mass was then allowed to stand in a cool place for about a day for extraction. It was then mixed with enough Ringer's solution to yield a total volume of approximately 100 c.c. After this mixture had been standing an hour or two in a beaker most of the oil in the form of droplets collected as a thick layer on the top of the fluid. This oil together with some of the associated water

extract was then skimmed off, freed from much of the organic detritus by being passed through fine cheese cloth, and thoroughly shaken so as to form a coarse emulsion. This was then quickly taken up in a hypodermic syringe and injected under the lateral skin of a light-tinted catfish. As a control a similar injection of an emulsion of pure olive oil and Ringer's solution was made generally on the opposite side of the same fish. The amount of fluid introduced under the skin of the fish at each injection was 0·1 c.c.

In all, twenty-one catfishes were treated in the way just described. One of these, whose history will be given in some detail, is shown in Pl. I, figs. 1 and 2. The oil extract with which this fish was injected was made from five large catfishes by the process already described. After this fish had been thoroughly lightened, an injection of 0·1 c.c. of this extract was made on its right side below the adipose fin. The point at which the needle was inserted is indicated in Pl. I, fig. 2 by a black dot on the side of the fish midway between the adipose fin and the anal fin. After the needle was inserted it was run anteriorly under the skin for about a centimetre. There the oil extract was discharged. As a control a corresponding injection of an emulsion of pure olive oil and Ringer's solution was made on the left side of the fish (Pl. I, fig. 1) and the fish was then placed in a white-walled, illuminated aquarium for observation.

On the right side of this catfish, that on which the oil extract had been injected, no change was noted for three-quarters of an hour after which a vertical area of dark splotches began to appear. These gradually increased in size and distinctness till they formed a conspicuous marking (Pl. I, fig. 2). This corresponded exactly to that portion of the skin under which the extractive material had been discharged. The splotches forming this dark area remained almost unchanged for about 40 hours after which the fish was killed and the preparation shown in Pl. I, fig. 2 was made. In other fishes in which these dark areas had been excited, the areas were as a rule readily visible for some 2 days after the injection. By the end of the third day they had mostly disappeared.

The control injection of a mixture of pure olive oil and Ringer's solution, which was made on the left side of the catfish under special consideration (Pl. I, fig. 1), was not followed by any change of coloration except around the point of insertion for the needle. Here a small dark area developed, a condition also to be observed on the right side of the fish and obviously a traumatic effect. Pure oil and Ringer's solution plainly induce no change in the coloration of *Ameiurus*.

Of the twenty-one catfishes that were treated in essentially the same way as the one just described fifteen reacted as the fish shown in Pl. I, figs. 1 and 2 did. In the remaining six, however, no change of colour occurred on the side into which the oil extract had been introduced. This side remained of the same light tint as the opposite side. At first sight this state of affairs might seem to militate against the validity of any general conclusion to be drawn from these tests, but, in my opinion, such is not the case. In one or two of the early tests on these fishes the oil extract was made from the fins and skins of only two or three catfishes instead of five and these extracts failed in most instances to excite colour changes. I believe this failure was due to an

insufficient amount of the dispersing humour. This insufficiency may have recurred in some of the later tests, though, after the preliminary trials, five fishes were regularly used in the preparation of the extract. It is also possible that in making the injections the discharge of the extractive may in some instances have been into the deeper tissues of the fish's flank rather than directly under the skin and thus the introduced fluid may have failed to reach the region intended. An occasional failure on the part of an injection of extractive material to excite a dark area in the skin does not seem to me to throw doubt on the numerous instances in which this occurred. It indicates in my opinion possible instances of insufficiency in technique. Admitting the correctness of this view, it may be stated that in the twenty-one catfishes subjected to the extractive and the control injections all showed no colour change in the controls and fifteen of the twenty-one showed dark areas from the oil extracts. It seems clear from these figures that the oil extract of dark fins and skins contains some material whereby the skin of a light *Ameiurus* can be induced to darken.

The nature of the dark areas thus produced can be determined under the microscope. Even in a living catfish under a low power it can be seen that the dark splotches are regions in which the pigment in the melanophores is dispersed as contrasted with its concentration in the light portion of the skin. These conditions can be more clearly made out in pieces of skin cut from a preserved catfish and prepared for microscopical examination. In *Ameiurus* there are two sets of integumentary melanophores, epidermal elements small in size and dermal ones of much larger dimensions (Parker, 1934*b*). In the light skin of an injected *Ameiurus* the epidermal melanophores have well concentrated pigment and appear as numerous clearly defined dots (Pl. I, fig. 3). In a dark splotch induced by an injection of oil extract the pigment in these cells is more or less dispersed in consequence of which their outlines are often very irregular (Pl. I, fig. 4). The same in general is true of the dermal melanophores; in the light skin the pigment of these cells is concentrated (Pl. I, fig. 5) and in the dark splotches it is much spread out (Pl. I, fig. 6). Thus both sets of melanophores in *Ameiurus* respond to injections of oil extracts by changes of a normal character. When in the course of days an induced dark area gradually disappears from the fish, it does so by the slow concentration of the pigment in its melanophores, an operation again entirely normal.

Not only do the melanophores in the induced splotches on the catfish behave normally as these markings gradually appear and disappear under the influence of the extractive, but these colour cells also continue to respond normally to other reagents. Thus the dark markings disappear temporarily under the influence of adrenalin. A single instance will serve to illustrate this point. A dose of 0·15 c.c. of adrenalin, one part in a thousand of water, was given to a light-tinted catfish on which a four-parted, dark splotch had previously been produced by an injection of oil extract. Three minutes after the introduction of the adrenalin the splotch had begun to fade and six minutes later it had entirely disappeared. Nineteen hours after the injection of the adrenalin the splotch began to return. It remained visible for about one day after which it disappeared spontaneously. Such tests were several times repeated and always with confirmatory results. These several operations show

clearly that though melanophores are profoundly affected by oil extracts they are not killed by these extracts, but they remain as living cells essentially normal in their responses.

Aqueous extracts of the skin and fins of dark catfishes were several times made and injected into light-tinted individuals, but in no instance was there a darkening of the skin. The only means by which darkening was induced was the olive-oil extract of dark fins and skin. Apparently the olive oil dissolves from those tissues that were subjected to it a material that excites pigment dispersion. Such material I believe to be a dispersing liponeurohumour. The exact source of this material cannot be stated though its origin from dark fins and skin seems to be established. What other tissues may produce it remains to be ascertained, nor can much be said of its nature.

As an additional means of extraction, the dark skin and fins from five catfishes were ground up in their own fluids and agitated in a chemical shaker for 24 hours with about 100 c.c. of pure ether. This treatment yielded a lemon yellow filtrate which on full evaporation left behind it a small amount of yellow oily residue. This was mixed with about its own volume of Ringer's solution and injected into a light-tinted catfish. Three such independent extractions were made, two of which had no effect upon a light-tinted catfish while the third was followed by a faint but unmistakable darkening of the skin.

In another attempt the skin and fins of five catfishes were dried for a day in a chemical oven at temperatures between 95 and 110° C. The dried tissue was then pulverised and extracted in a Soxhlet apparatus for 24 hours. The resulting ether extract was of a deep cherry brown colour. On evaporation this yielded from the five fishes about two grams of reddish brown oily residue. This too was agitated with an equal volume of Ringer's solution and the rough emulsion was injected hypodermically into light catfishes. Here one of the four trials gave a slight local darkening to the skin. The other three gave no certain reactions at all. Ether extraction, both cold and hot, yields, therefore, very little substance that could effect a change in the tint of the catfish. In this respect the extractions were very unlike those carried out on *Mustelus* from the fins of which a relatively efficient ether extractive could be made (Parker, 1935). It is to be noted further that while the oil extract from *Mustelus* induced a concentration of melanophore pigment with a consequent blanching of the animal, that from *Ameiurus* called forth a dispersion of pigment and in consequence a darkening of the fish.

I also attempted to extract by means of Italian olive oil a dispersing neurohumour from the skin and fins of *Fundulus heteroclitus* in the dark phase, but these efforts were entirely without avail. I am inclined to attribute my failure in this respect to the fact that much of the melanophore system of this fish is contained within scales which because of their compactness and toughness are very difficult to triturate and probably do not admit the oil particles with sufficient freedom to the region where this agent might come into contact with the neurohumour to be dissolved. At all events this fish, even when reasonably large amounts of its skin and fins were used, gave no positive results.

III. SUMMARY.

1. The skin and fins of *Ameiurus*, irrespective of their original state, take on the dark coloration (dispersion of melanophore pigment) whenever they are triturated. Hence oil extracts can be made from these parts only in the dark condition.

2. Olive oil extracts from the skin and fins of dark *Ameiurus* when injected under the skin of light-tinted individuals induce the formation of temporary dark splotches in an otherwise light skin.

3. These splotches result from the dispersion of pigment in the melanophores, both epidermal and dermal, in the region of the injection.

4. On the spontaneous disappearance of these splotches, which ordinarily occurs a few days after their formation, the melanophore pigment reassumes its concentrated state.

5. Newly formed dark splotches in *Ameiurus* may be obliterated by an injection of adrenalin and will return spontaneously after the adrenalin has disappeared.

6. From these several tests it is concluded that the nervous factor in the dispersion of the melanophore pigment of *Ameiurus* is an oil-soluble neurohumour of local activity.

7. Ether extractions, hot and cold, yielded residues that were only slightly active in darkening the skin of the catfish.

8. No effective oil extracts could be obtained from the dark skins and fins of *Fundulus heteroclitus* probably because of the inaccessibility of the tissues to the oil.

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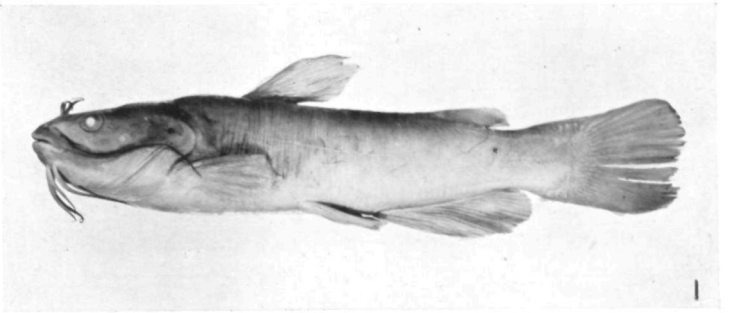

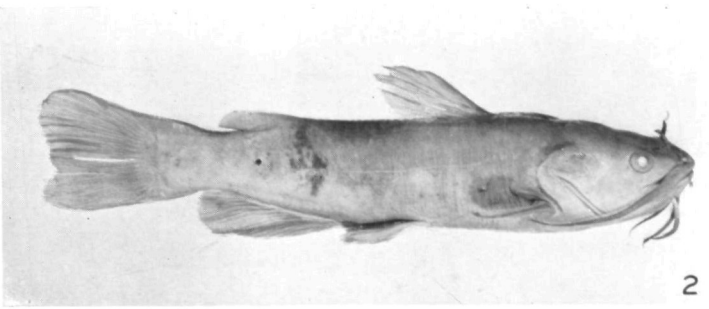

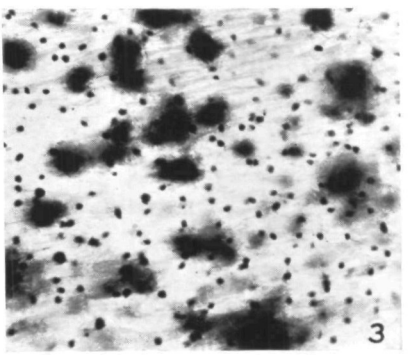

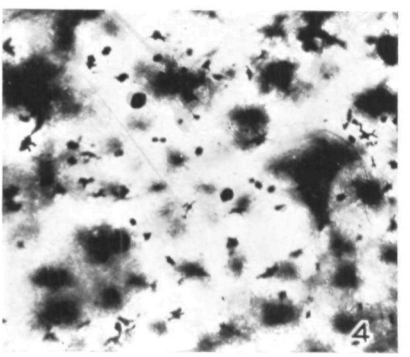

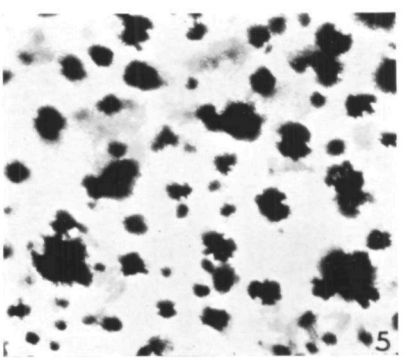

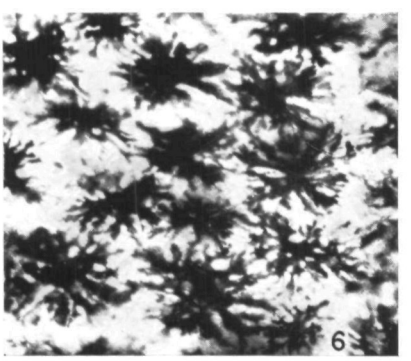

PARKER—AN OIL-SOLUBLE NEUROHUMOUR IN THE CATFISH *AMEIURUS* (pp. 239—245).

EXPLANATION OF PLATE I.

All figures are from the catfish, *Ameiurus nebulosus* Le Sueur. I am under obligations to Dr F. M. Carpenter for the preparation of the photographs.

Fig. 1. Left side of a light-tinted catfish into which at the point marked by a black dot between the adipose fin and the anal fin, an anterior injection of 0·1 c.c. of a mixture of pure Italian olive oil and Ringer's solution had been made. The fish showed no change in tint as a result of this injection.

Fig. 2. Right side of the same catfish as that shown in Fig. 1. Here an injection of an olive oil extract from the dark fins and skins of five other catfishes had been made anteriorly from the spot indicated by a black dot between the adipose fin and the anal fin. There resulted from this injection a darkening of the skin of the fish. This darkening which is made up of irregular dark splotches is anterior to the point at which the needle of the syringe was inserted, superficial to the region where the extract was discharged, and extends from the dorsal line well down toward the ventral line.

Fig. 3. Epidermal melanophores with concentrated pigment as seen in the light-tinted skin of *Ameiurus*. The large deep-lying dermal melanophores are out of focus and appear each as a large indistinct dark area.

Fig. 4. Epidermal melanophores with more or less dispersed pigment as seen in a dark splotch induced by an oil extract from the dark fins and skin of *Ameiurus*.

Fig. 5. Dermal melanophores with concentrated pigment as seen in the light-tinted skin of a catfish.

Fig. 6. Dermal melanophores with dispersed pigment as seen in a dark splotch induced by an oil extract from the fins and skin of dark catfishes.