THE CHROMATOPHORAL NEUROHUMORS OF THE DOGFISH

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(Accepted for publication, November 3, 1934)

I

INTRODUCTION

The common smooth dogfish, *Mustelus canis*, has two extreme tints of skin, a light one due to the concentration of its melanophore pigment and a dark one resulting from a dispersion of this coloring matter. The dispersion of the pigment is excited by a neurohumor produced in the pituitary gland and carried from that gland to the melanophores by the blood (Lundstrom and Bard, 1932). The concentration of this pigment is the result of specific, local, nerve action by nerve fibers in all probability from the autonomic system and sympathetic in origin (Parker and Porter, 1934). It is well known that the defibrinated blood of a dark dogfish when injected into a light one will call forth a temporary dark spot in the skin of the recipient (Table I, 16), but it will have no effect upon the skin of another dark fish. Defibrinated blood from a light dogfish, however, is without effect upon the skin of either light or dark individuals (Table I, 15). If a neurohumor is produced by the concentrating nervous mechanism of the dogfish, it is not water-soluble, for, unlike the dispersing humor, it does not occur in the blood. From work done on *Fundulus* and on *Ameiurus* (Parker, 1934a, 1934b) such a concentrating neurohumor has been demonstrated to exist and since this neurohumor is known to spread from place to place and yet is not carried in the blood (Matthews, 1933), it has been suggested that it is oil-soluble and that its means of transfer is through the lipoid constituents of the integumentary cells (Parker, 1934a).

Is there any evidence that such an oil-soluble neurohumor exists in the dogfish or is the action of the concentrating nerve fibers in this animal of an entirely different nature? This question will be discussed in the following pages.
Methods

In the smooth dogfish the parts of the integument that show the most pronounced changes in tint are the dorsal skin and especially the fins. It was soon found that of these two parts the fins were the more effective (Table I, 2, 4); hence in the majority of cases the fins only were used. They were removed from a dogfish that had been blanched to an extreme degree by having been kept for some 4 days or so in a white-walled tank illuminated from above by a strong electric light. They were then reduced to a pulp by being put through a kitchen pulverizer after which they were ground in a rough porcelain mortar for an hour or more with about 1 cc. of pure Italian olive oil. The pasty mixture that resulted was then allowed to stand some 12 or more hours in a refrigerator for extraction and after agitation with a small amount of sea water, it was set aside to separate. The oil rose to the surface, was decanted or skimmed off, and shaken thoroughly with the small amount of watery fluid that accompanied it. The emulsion thus produced was injected by means of a fine hypodermic syringe in known volume into the subcutaneous spaces of an appropriately tinted dogfish. The fish was then returned to a sea water tank and kept under observation.

This general procedure was followed throughout most of these investigations. In the early part of the work a number of the injections showed after some days clear signs of infection. Considering the way in which the emulsions were made and used, this was not surprising. In the latter part of the work sterilization was employed and under these circumstances little or no infection occurred. The procedure being such as it was (hypodermic injection through the wet skin of a dogfish with subsequent return of the fish to sea water) there was no possibility of excluding infection completely, but such disturbances were so greatly reduced in the later part of the work that they ceased to have any possible significance in the results. Extracted juices, olive oil, Ringer's solution, sea water and the like were sterilized by subjecting them to the temperature of boiling water for at least a quarter of an hour. Instruments were sterilized either by boiling them or by steeping them in alcohol.

As a rule, four injections were made on each dogfish, two on either side of the anterior and of the posterior dorsal fins, each about midway on the flank between the dorsal line and the lateral line. The volume of fluid injected in each test was very regularly 0.5 cc. In this way a technique was developed that was believed to meet the requirements of the research.

Observations

In all, twenty-five injections of non-sterilized oil extracts from light dogfish fins were made into over a dozen dark dogfishes (Table I, 1).
Many of these injections were followed almost immediately by one or more light spots in the region concerned. These spots were always temporary. Thus in one dogfish two non-sterilized injections were made, one to the right and the other to the left of the posterior dorsal fin. 5 minutes after this had been done a large, faint light spot over a centimeter in diameter began to appear in the region of the right hand injection. 5 minutes after this three light spots, each a few millimeters in diameter, had made their appearance over the left hand injection. 10 minutes later the large faint spot had mostly disappeared and 25 minutes after that, or three-quarters of an hour after the injections had been made, all three smaller spots had faded out. These initial temporary light spots, which may be called primary spots, were to be noticed in the great majority of injections. They never lasted over an hour or so and their disappearance was invariable. In my opinion these spots are purely operative in their origin, for they may occur with injections of sterilized oil and sea water containing no extractives as well as with other fluids. They are due, I believe, to the mechanical action of the injected fluid itself on the invaded tissues. The injected fluid when it first enters the tissue is in such concentrated volume as to strain and thereby stimulate small nerves or possibly to press upon blood vessels to such an extent that small areas in the skin are temporarily cut off from the necessary blood supply. Both these means are known to produce concentration of melanophore pigment and consequently to blanch the skin. As the injected fluid slowly seeps out from the region of entrance into the adjacent tissues, the pressure on the containing tissues must be relieved with the result that nerves and blood vessels return more nearly to their normal states and the spots thus tend to disappear.

If fishes of the kind that have been described are kept till after such primary spots have vanished, new, larger, and relatively permanent light spots may arise in them (Fig. 1A). These spots, which may be called the secondary spots, are first visible in from 1 to 2 days after the injection has been made and remain commonly for several days, after which they may gradually fade out. Of the twenty-five injections of non-sterilized oil extracts, fourteen yielded secondary spots and eleven failed to show them (Table I, 1). The failures in these tests are attributed to unsuccessful injection technique, in inserting

A. Left side of the fish showing a secondary light spot due to an injection of 0.5 cc. of an emulsion of olive oil extract of blanched fins and sea water made a little over a day previously. The injection was directed anteriorly from a point about 1.5 cm. posterior to the posterior edge of the light spot. The preparation was made 26 hours after the first appearance of the light spot.

B. Right side of the same fish showing no change in color after the injection of 0.5 cc. of an emulsion of olive oil and sea water made in the same way as the injection on the left side.
the needle of the syringe the point of which was probably thrust, in
the unsuccessful cases, too deeply into the muscle and was not slipped
closely enough under the skin. In consequence the emulsion was
discharged into deep-seated spaces rather than directly next the inner
surface of the integument and hence it failed to act effectively on the
color cells. It is also possible that some of the earlier extractions were
made with too much oil in relation to the amount of fin substance and

**TABLE I**

A summary of the collective reactions of the integumentary melanophores in
the smooth dogfish, *Mustelus canis*, to a variety of liquids introduced subcuta-
neously. The table includes records of the occurrence of dark-spots, D.S. (dis-
persed melanophore pigment), of secondary light spots, L.S. (concentrated
melanophore pigment), of failure to react, N.R., and of infection, Inf. The
volume of liquid injected in each instance was 0.5 cc.

<table>
<thead>
<tr>
<th></th>
<th>D.S.</th>
<th>L.S.</th>
<th>N.R.</th>
<th>Inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Light fins, oil extraction, not sterilized</td>
<td>0</td>
<td>14</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>2. Light fins, oil extraction, sterilized</td>
<td>0</td>
<td>8</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>3. Light fins, sea water, not sterilized</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>4. Light skin, oil extraction, not sterilized</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5. Light skin, sea water, not sterilized</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6. Dark fins, oil extraction, not sterilized</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7. Muscle, oil extraction, not sterilized</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>8. Oil and sea water, &quot; &quot;</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>9. Oil and sea water, sterilized</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>10. Mercuri chloride 0.5 per cent</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11. Formaldehyde 5 per cent</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12. Light fins, ether extraction, oil</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>13. Light skin, ether extraction, oil</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>14. Light fins, Soxhlet extract, oil, sterilized</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15. Light blood defibrinated</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>16. Dark blood defibrinated</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

were in consequence too weak. Six of the twenty-five injections
showed obvious infections in that they developed large, swollen, red
centers which sooner or later sloughed away.

The injection of sterilized oil extract of light fins into dark dogfishes
was made in fourteen cases of which eight showed secondary light spots
and six no reactions (Table I, 2). As might have been expected none
of these fourteen cases showed infection.
The question now to be considered is the nature of the secondary light spots. It is well known that in death the skin of a dogfish finally blanches. Is it possible that the methods of injection used lightened the skin of the experimental dogfish by producing local death? In other words, are not the secondary light spots dead areas in the integument of the fish? Such dead areas can readily be produced in dogfish skin by injecting solutions of mercuric chloride, 0.5 per cent, or formaldehyde, 5 per cent, immediately under the skin of the living fish. Such a solution of mercuric chloride produced a large white spot in a dogfish in an hour and a half after injection and this spot remained permanently on the fish for 18 hours when death intervened (Table I, 10). Formaldehyde, 5 per cent, began whitening the skin of a fish some 2 hours after its injection and produced a pronounced light spot 3 hours later. This also was a permanent spot (Table I, 11). When 1 cc. of obstetrical pituitrin (Parke, Davis and Co.) is injected into a light dogfish the fish turns quickly but temporarily dark. When such an injection was made into a dogfish with a light spot from either mercuric chloride or formaldehyde, the whole fish turned dark except the light spot which remained unaltered. Spots induced by these two reagents were not changeable in any way and gave every evidence of being composed of dead tissue. When obstetrical pituitrin was injected into a dogfish with a light spot resulting from the injection of an oil extract of light dogfish fins, the spot became fully dark (like the rest of the integument of the fish) in three-quarters of an hour to return to a light tint 2½ hours later. I concluded from these tests that the oil extract of the fins does not kill the integumentary melanophores of the dogfish, but induces them to concentrate their pigment and leaves them in a fully active and responsive condition. Hence the blanching of the skin in the dogfish by oil extract of light fins is not the result of the death of the melanophores.

This blanching is also not due to the injected oil and sea water either unsterilized (Table I, 8; Fig. 1 B) or sterilized (Table I, 9) nor is it produced by a non-sterilized oil extract of muscle (Table I, 7). It is to be regretted that there was no opportunity to extract other tissues, but certain aspects of the work took so much time that when this topic was reached the dogfish season was so far advanced that sufficient material was no longer available.
Other methods of extraction than by olive oil were likewise used. Plain sea water extract of light fins (Table I, 3) and of light skin (Table I, 5) failed to produce light spots as might have been expected from the fact that the blood of a light dogfish when injected into a dark one was without effect on the melanophores. Ether was also used as a means of extraction. To avoid the complicating factor of heat, fresh, wet fin-paste from a light dogfish was mixed directly with about 100 cc. of pure ether and was agitated in a closed bottle from time to time over a period of some 15 days. The ether was then decanted and filtered and the light straw-colored liquid was allowed to evaporate. On full evaporation there was left in the small beaker that contained the fluid a translucent, thick deposit. In amount this was roughly 1 cc. It was mixed with an equal volume of sterilized olive oil and this mixture was emulsified with 1 cc. of sterile sea water. Four injections were made with this emulsion. One of them was followed by a secondary light spot and three showed no reaction (Table I, 12). A similar injection of ether extract of light skin excited no response at all (Table I, 13). Apparently ether is a means of extracting what blanching substance there may be in the fins. As a source of this material the body skin appeared to be entirely unfavorable.

As a result of these tests ether extraction was attempted with a Soxhlet apparatus. I am indebted to Professor R. Höber for many suggestions and much kindly help at this stage of the work. The fins of two light dogfishes were dried in an oven at 110° C. for 12 hours. They were then ground to a powder and this powder was extracted with ether in a Soxhlet apparatus for 28 hours. The apparatus ran with a turnover of about once in every 10 minutes. The final result was some 50 cc. of a straw-colored ether deeper in tint than that which had been obtained by direct ether extraction. This was fully evaporated after which a thick transparent deposit of material was left in the beaker, in all about 1 cc. in volume. This was mixed with 1 cc. of sterilized olive oil after which the mixture was emulsified with 1 cc. of sterilized sea water. Four injections of this emulsion in two places each in two dark dogfishes were then made. These fishes had been tested previously by having had cuts made in their fins to show that they could lighten locally. Primary light spots appeared in all four places in about 15 minutes. In 3 hours these had completely disap-
peared. 21 hours after the injections each of the four areas showed a secondary light spot. All four spots were faint but easily noticeable. 5 hours after their first appearance all the spots were well marked. 1 cc. of obstetrical pituitrin was then injected into one fish and 12 minutes after the injection the light spot nearer the region of introduction had lost much of its conspicuousness; half an hour later it had entirely disappeared. 6 hours after the injection it again regained visibility. Therefore, the conclusion was drawn that the fin substance that induces a concentration of the melanophore pigment is open to extraction by ether with a Soxhlet apparatus and that it is a material that is stable even after having been heated to 110°C. The single Soxhlet extraction that was completed came unfortunately at the end of the dogfish season, and in consequence it was not possible to repeat it. Such a test should be gone over many times and the extracts obtained should be subjected to fractionation with a view of isolating the substance or substances concerned in melanophore activation. Because of the lack of dogfishes these steps in the work must be deferred till some time in the future.

As the summary in Table I shows, true secondary light spots in the skin of dark dogfishes have been induced only by oil extracts (1, 2) or by ether extracts (12, 14) of light fins. They have never been excited by oil extracts of dark fins (6) nor by water extracts of light fins (3). Extracts of light skin with oil (4), with ether (13), or with sea water (5) have never yielded secondary light spots. Light spots are not produced by emulsions of oil and sea water (8, 9), by oil extract of muscle (7), nor by defibrinated blood from light or dark fishes (15, 16). Apparently some substance in the fins of the light dogfish, soluble in olive oil or in ether, but not in water, is capable of exciting the concentration of melanophore pigment. This substance is believed to be a neurohumor produced by the concentrating nerve fibers of the melanophores, but it must be admitted that its origin is certain only in that it is from some part of the fin. So far as my observations go, it may come from any part of that structure. Nevertheless it is strongly suspected that it is of nervous origin. Quantitative studies may eventually help in deciding the exact source. Till some such step is taken this source must remain unknown. It is, however, of no small interest from the standpoint of the neurohumoral hypothesis.
(Parker, 1932) that in the dogfish the concentrating response which has all the appearances of a purely nervous one, as contrasted with the hormonal dispersing reaction, should be open to excitation by extractives of the kind described. The whole situation seems to point to a system of neurohumors in which two well defined categories can be distinguished, one composed of substances like the pituitary extract which is soluble in water and hence open to quick carriage by the blood, and the other made up of materials that are soluble in oil and that pass only slowly from place to place probably through the lipid components of the cells. Such materials might be called liponeurohumors as contrasted with those soluble in water, hydroneurohumors. In Mustelus, if this analysis is correct, darkening is due to a pituitary hydroneurohumor and blanching to a liponeurohumor from integumentary nerves. In Fundulus, whose blood carries no neurohumor, both concentration and dispersion of melanophore pigment must be due to liponeurohumors whereas in the frog, whose blood is generally conceded to be the all important element, only hydroneurohumors appear to be present. These results give some insight into the complexity of the neurohumoral system, a complexity which, however, is not beyond analysis.

IV

SUMMARY

1. The common dogfish, Mustelus canis, as is well known, exhibits two temporary extremes of tint, one dark, the other light. The dark phase is induced by a secretion from the pituitary gland which is carried in the blood, hence a substance soluble in water (a hydroneurohumor). The light phase is under the control of nerves and cannot be excited by blood from a light fish.

2. When an olive oil or ether extract is made from the fins of a light dogfish and this extract is injected into a dark fish, large light spots may appear in from 1 to 2 days and persist for several days. These light spots, which may be called secondary spots, are not to be confused with certain small and very temporary light spots, the primary spots, which occur soon after the injection and which are believed to be purely operative in origin.

3. The secondary light spots are not due to the death of the integu-
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mentary tissue, for, after their formation, they can be made to dis-
appear by the action of obstetrical pituitrin and will subsequently
reappear.

4. They are produced by some substance extracted from the light
fins by ether or by olive oil. They are not produced by sea water,
ether, or olive oil alone.

5. The extracted substance, which can resist dry heat up to at
least 110°C., owes its limited range of action in the dogfish to its in-
ability to dissolve in water. It is soluble in oil (a liponeurohumor).

6. This liponeurohumor is believed to emanate from the nerve ter-
inals concerned with the concentration of melanophore pigment and
to spread through the fatty components of the integumentary cells.

The work herein reported was done in part at the Marine Biological
Laboratory and in part at the Woods Hole Oceanographic Institution;
to the directors and assistants of these two laboratories I am under
great obligations for their generous cooperation in enabling me to carry
out this investigation. I am also greatly indebted to Mrs. Helen
Porter Brower who, as laboratory assistant, gave me great aid in this
undertaking.

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