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INTEGUMENTARY COLOR CHANGES IN THE NEWLY-BORN DOGFISH, MUSTELUS CANIS

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Introduction

It is well-known that the integumentary melanophores of the lower vertebrates are functionally active at a very early stage. These color-cells become responsive at about the time young fishes hatch from their eggs. This is true of *Fundulus*, according to Bancroft (1912), Spaeth (1913), and Gilson (1926) and of *Coregonus* and of two species of *Salmo* according to Becher (1929). At this stage the melanophores begin to disperse or concentrate their pigment, thus giving to the young fish a dark or a light tint. Although the beginnings of larval life may be said thus to mark the period of the first color changes, Becher noted that the embryos of fishes on which he worked when artificially removed from their egg-shells would often show color changes. This demonstrated that melanophore activity is possible at least under experimental conditions earlier than the time of hatching. Such a view was also taken by Duspiva (1931), who worked upon two fishes, *Salmo salvelinus* and *Perca fluviatilis*. In *Perca* the first integumentary melanin appears five days after the eggs have been fertilized. Duspiva saw melanophore responses three days later or about eight days after fertilization. He believed, however, that responses may have occurred still earlier. As the young larvæ of *Perca* hatch from the eggs about ten days after fertilization, the first melanophore responses in this fish must occur in what is obviously its embryonic period. From all his observations Duspiva was led to conclude that melanophores probably become active as soon as their processes are developed and their melanin formed. Such a view, which appears to be fairly well supported, places the initiation of melanophore activity in fishes at a very early stage, not later than about the time of hatching and probably in some species somewhat earlier.

The first responses of melanophores in lower vertebrates were shown by Babák (1910) to be quite unlike those of later life. In the very young larvæ of the Mexican axolotl about 1.5 cm. long, when the first color changes had begun, the following conditions were found by Babák. In complete darkness the young animals were pale and in bright light they were dark. When they had attained a length of about 5 cm. their reactions in these respects were almost reversed. At this later stage they were in complete darkness dark and in bright light either pale or dark depending upon their surroundings. This second stage of their melanophore responses persisted until maturity when they became dark and remained permanently so, having lost their capacity to change. The early larval condition may be called the primary phase, the succeeding one the secondary phase. If a larva in the secondary phase is blinded, it was found by Babák to revert to the primary phase in that it would be pale in darkness and dark in bright light. A young larva in the primary phase when deprived of its eyes remained unchanged and was responsive in the same way as it had been before the operation. From these tests Babák concluded that what has been called here the primary phase was dependent upon the direct stimulation of the larval melanophores by light or its absence and that the secondary phase resulted from their indirect stimulation through the eyes of the larva. These conclusions were supported in one way or another by Pernitzsch's work (1913) on axolotl, by Hooker's work (1914) on *Rana pipiens*, and especially by an extended series of contributions from Laurens (1914, 1915, 1916, 1917) on the larvæ of several species of *Amblystoma*. Babák's views also received confirmation from the work of Fischel (1920) on various amphibians and especially from that of Duspiva (1931) on the two fishes already mentioned, *Salmo salvelinus* and *Perca fluviatilis*.

The references thus far cited pertain exclusively to oviparous fishes and amphibians. I know of only one writer who has recorded notes on the melanophore system of ovoviviparous vertebrates. In his paper on the genesis of chromatophores in fishes Eigenmann (1891) refers very briefly to the condition in the young of the ovoviviparous rockfish, *Sebastodes*, an embryo of which is figured by him. This small fish, when about to escape from the ovarian apparatus of the mother, is said by this author to have a very fully formed melanophore system whose functional possibilities, however, are not reported. Aside from this very meagre reference I know of no other on the melanophores in ovoviviparous vertebrates. It is therefore of interest to look into the condition of the newly-born pups of the smooth dogfish, *Mustelus canis* (Mitchill). This fish with its young can be had from time to time in the early summer at the Marine Biological Laboratory, Woods Hole, and I am under obligations to this Laboratory for the

material on which the following observations were made. I am also indebted to the Woods Hole Oceanographic Institution for the use of their equipment in working on these fishes. My studies were made under a grant given me from the Milton Fund of Harvard University for the investigation of color changes in marine animals. To the administrators of this Fund I wish to express my sincere thanks for the aid generously extended to me.

Observations

The smooth dogfish of the New England coast releases its young, four to a dozen or more at a time, in early summer. The following observations were made either on young dogfishes born in the laboratory tanks or on individuals removed from the females by what may be called a Cæsarean operation. After the envelopes and umbilical vessels of these young fishes had been severed they could be removed and carried gently to the sea-water tanks. During this procedure they were as a rule perfectly passive. After they had been immersed in the sea water of the tank for a fraction of a minute or so, normal respiratory gill movements began and at about the same time swimming was initiated. Such newly-born fishes swam for a short time with somewhat unsteady equilibrium, but they soon gained in steadiness and mingled with others of their kind in the tank. At the time of these tests in June the young fishes were about 25 cm. in length, the largest measuring 33 cm.

A young dogfish when first taken from the uterus of the female, a dark situation, is slightly dark in tint. This indicates that the young fish is in what has been called in this paper the secondary phase of melanophore activity, that is, the phase in which the fish is dark in darkness and pale or dark in the light according to the environment. Whether the dogfish passes through an earlier primary phase while it is still in the uterus of the mother is unknown. More likely this stage has been omitted in this fish as it appears to have been in the amphibians *Bombinator* and *Hyla* (Babák, 1910) and in the fish *Fundulus* (Bancroft, 1912; Spaeth, 1913; Wyman, 1924; Gilson, 1926).

Newly-born dogfishes respond in tint very quickly to that of their environment. On June 4 seven dogfishes were born in one of the experimental tanks. Five hours after their birth four were put in a white-walled, illuminated tank and three in a similar black-walled one. Two hours later those in the black-walled tank were extremely dark and those in the white-walled one decidedly light (Fig. 2). Preparations were made of the skin from an individual in each of these sets and microscopic views of the two preparations are shown in Figs. 3 and

4. As might be expected, the melanin in the color-cells from the pale dogfish is concentrated (Fig. 3), that from the dark one is dispersed (Fig. 4). On the following day the pale fishes were put into the black-walled tank and the dark ones into the white-walled one. Within two hours the fishes had reversed their tints, the pale ones having become dark and the dark ones pale.

In a second litter of dogfishes obtained by Cæsarean operation three were put immediately after their removal from the uterus into a black-walled illuminated tank and three into a white-walled one. The three fishes destined for the black tank were put into it at 9:17 in the morning. By 9:55 they had increased their dark tint. At 10:30 they were dark but not fully so, and at 10:50 they were fully dark. The three dogfishes intended for the white tank were put into it at 9:20 in the morning. By 9:55 they had lightened considerably but were still somewhat dark. By 10:30 they were fully blanched as compared with other young dogfishes used as checks. From these two sets of tests it is fair to conclude that specimens of *Mustelus* immediately after birth are capable of responding to the tint of their environment by appropriate melanophore reactions. In these responses they were quite like adults of their own species.

In another respect the young dogfishes also resembled adults. It has already been shown (Parker and Porter, 1934) that when a small transverse cut is made in the fin of a relatively dark *Mustelus* a pale band is soon formed extending from the cut toward the free edge of the fin. This band is believed to result from an excessive stimulation of the concentrating melanophoric nerve-fibers cut by the operation. Similar conditions obtain in the newly-born dogfishes. The pectoral fins of two such fishes were cut as described and 25 minutes after the operation both fishes had well-developed pectoral bands. One of these fishes was killed and a preparation of its pectoral fin was made. A photograph of this fin is reproduced in Fig. 1 and shows the typical pectoral bands.

Explanation of Plate

Fig. 1. Dorsal view of the left pectoral fin of a newly-born *Mustelus canis*. Two small cuts transverse to the fin rays have been made, the anterior one nearer the root of the fin, the posterior one nearer its lateral edge. A pale band extends from near each cut to the light edge of the fin.

Fig. 2. Dorsal views of two newly-born *Mustelus canis*, the upper one in the dark condition, the lower one in the light condition. Preparations preserved in formaldehyde-alcohol.

Fig. 3. Microscopic view of the melanophores on the dorsal surface of the pectoral fin of a *Mustelus canis* in the light condition, melanin concentrated.

Fig. 4. A view similar to that shown in Fig. 3 of the fin of *Mustelus canis* in the dark condition, melanin dispersed.

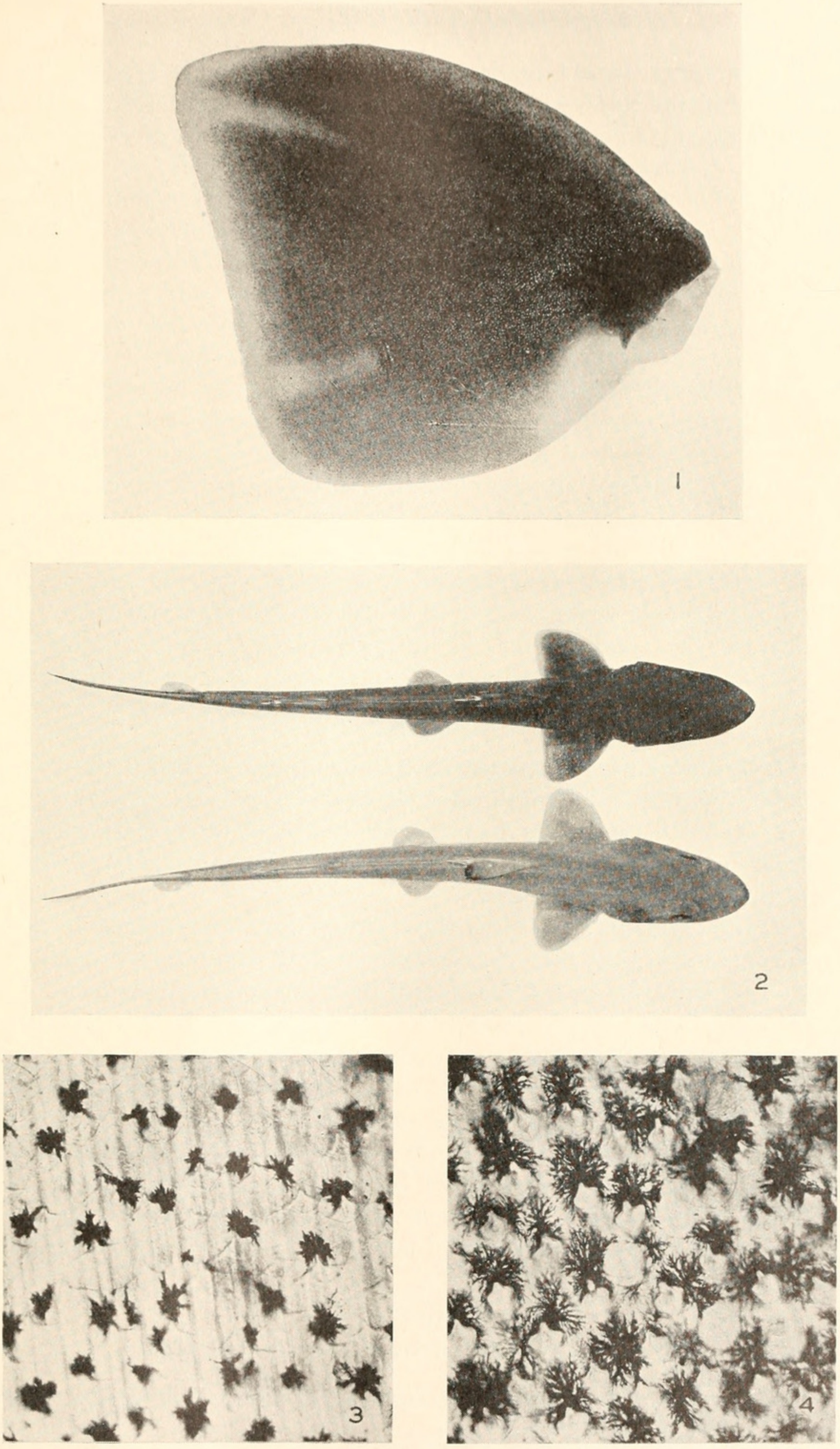

Plate I

In a third respect newly-born dogfishes resemble adults. They respond in the same way to adrenalin and to pituitrin (Parke, Davis and Company's preparations) as the mature dogfishes do (Lundstrom and Bard, 1932). If 0.2 cc. of a solution of adrenalin, one part in a thousand of water, is injected into a young dogfish of moderately dark tint, the animal will begin to blanch in about ten minutes and shortly after that it will assume a tint of extreme paleness. After two to three hours the young fish will reassume its darker tone. In a similar way an injection of 0.2 cc. of obstetrical pituitrin into a pale dogfish will induce its slow darkening. This change also passes away in about two hours after which the young dogfish will return to its former state. In both these respects the newly-born fishes resemble the adult.

These records show quite clearly that a young *Mustelus* immediately after birth possesses an active melanophore system whose reactions in a number of significant ways agree very fully with those of the adults. These ovoviviparous dogfishes then begin life in what has been called the secondary phase of melanophore activity. On the loss of their eyes they should lapse, according to the general theory of these relations, to the condition of the primary phase. Unfortunately my material was not sufficiently abundant to allow this test to be made.

Summary

1. *Mustelus canis* is an ovoviviparous dogfish in which the young are born with a body length up to 33 cm.

2. At birth the young dogfishes are of a moderately dark melanophoric tint. This is doubtless the influence of the maternal body within which they have been lodged.

3. Immediately after birth these young dogfishes respond to their environment in that they change light or dark, conditions brought on by a concentration or a dispersion of their melanophore pigment.

4. Pale bands can be produced on the fins of newly-born *Mustelus* by cutting their nerves, as can be done with the adults.

5. A young *Mustelus* responds to injections of adrenalin by blanching and to pituitrin by darkening as adults do.

6. A newly-born *Mustelus* shows no evidence of the primary phase of color change seen in some other fishes and in some amphibians. It appears to omit this phase in its ontogeny and is born with a melanophore system that responds in the same way as this system does in the adults.

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