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Studies on melanomas induced by treamatode infection on the skin of fresh-water teleosts

I. Physiological properties of the melanoma cells

by

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In winter, the individuals having dark spots on the skin have been observed in some fresh-water fishes (Sasaki 1935, Yamaguti 1939). These dark spots are attributable to infection of the encysted treamatode larvae, namely, the cysts are enveloped in these spots which are constituted with heavy aggregations of melanin containing cells. Yamaguti (1939) worked out experimentally the life history of this treamatode in *Carassius auratus* and identified it as *Metagonimus* sp.

These dark spots become clearer with increasing the cold, and disappear gradually as it becomes warmer again. However, little is known about the physiological properties of these melanoma cells and, in this connection, a peculiar interest attaches to their activity as compared to that of normal functional melanophores in the adjacent areas of the skin.

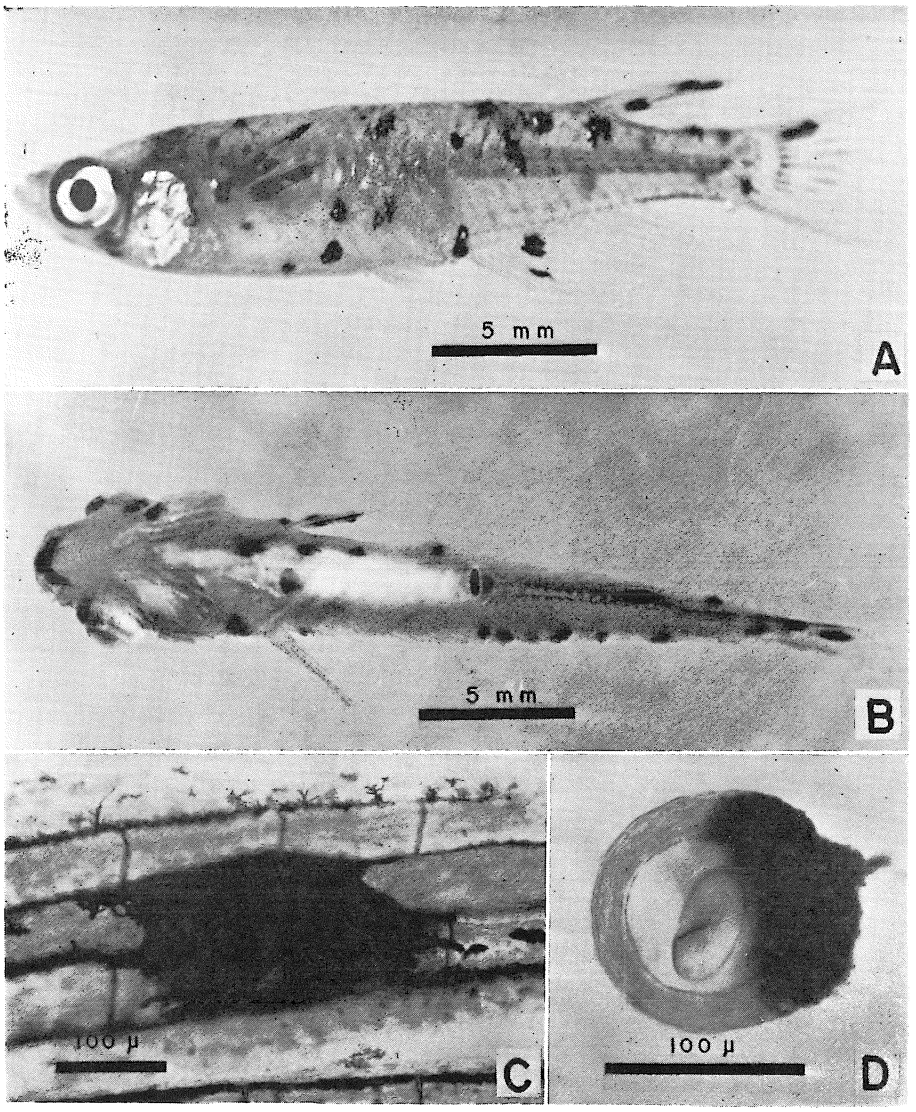
In the present study, the effects of pigment-concentrating stimulants for the melanophores, such as adrenaline and potassium chloride, and of electric current on the melanoma cells were investigated.

Experimental

In November, 1962, we found *Oryzias* having many dark spots on the skin from a stream in the vicinity of Shimane University, in Matsue (Fig. 1, A and B). Microscopic studies of the fresh and fixed specimens revealed that these dark spots were formed with aggregations of pigmemt cells which were produced in connection with encysted treamatode larvae (unidentified) which attacked the whole tegumentary system (Figs. 1 C and 1 D).

Oryzias latipes infected by the treamatode larvae was mainly used as materials and a crucian carp, *Carassius auratus*, with melanomas also was used.

On the experiments, a piece of the skin or slit strip of fin including a parasitized area was isolated and was immersed in physiological solution (M/7.5 NaCl, M/7.5 KCl and M/11 $CaCl_2$, mixed in a volume ratio of 100 : 2.0 : 2.1 respectively, pH adjusted to 7.2 by $NaHCO_3$). Test solutions were M/7.5 KCl and 10^{-4}—10^{-5} M adrenaline made up with physiological solution. For the electric stimulation, an alternating current (60 cps) was applied through a pair of Ag–AgCl electrodes (diameter : 0.3 mm, distance ; 1 mm) placed on a slit preparation of near the proximal extremity.



Figs. 1 A and 1 B. *Oryzias* having melanomas which were shown as black spots. Fig. 1 C. Melanotic portion of the tail fin. A black spot is constituted with heavy aggregation of melanin containing cells. Fig. 1 D. Encysted unidentified parasite from the melanotic skin of *Oryzias*.

I. Response to adrenaline

The preparation isolated was kept in physiological solution for times enough to cause the dispersion of the granules within the pigment cells* (Figs. 2 A and 2 D). When the external solution was changed to the test solution of adrenaline, the pigment

* The term "pigment cells" in this paper will be reffered to the melanin containing cells generally.

cells, being refferable to the dark spots, were concentrated fully in a few minutes, as dermal melanophores were done (Figs. 2 C and 2 F).

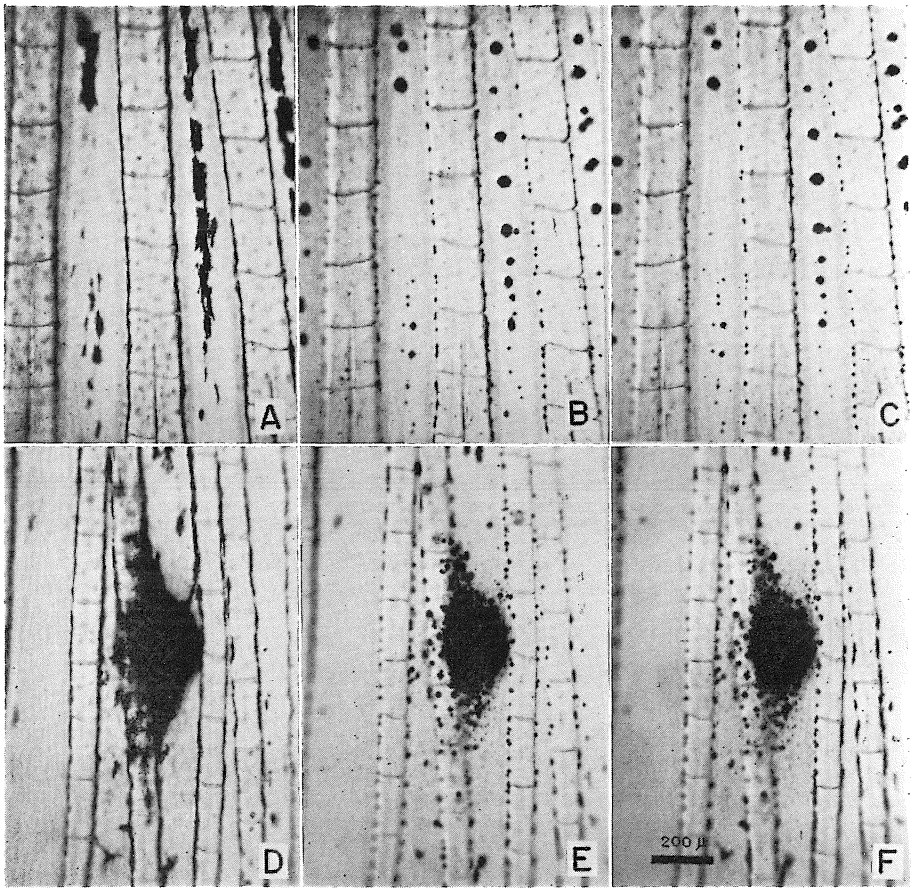


Fig. 2. Responses to KCl and adrenaline of the normal melanophores (A−C) and the melanoma cells (D−F) in the caudal fin of *Oryzias*. A and D, initial dispersion state in physiological solution. B and E, 15 minutes after KCl application. C and F, 5 minutes after the application of 10^{-4} M adrenaline. Room temp. 20.0° C.

Also, in *Carassius auratus*, adrenaline made to concentrate the pigment granules within the melanoma cells. On returning the immersing fluid from the test solution to physiological solution, the pigment cells began to disperse slowly.

Thus, melanoma cells respond to adrenaline in the same manner as do the melanophores.

II. Response to KCl

When an isolated preparation was immersed in isotonic (M/7.5) KCl solution, the melanophores on the margin of the isolated piece began to concentrate at first and in the course of time the concentrated area gradually spread towards the center of the preparation. In this case, melanoma cells also responded to the stimulant ion with a typical concentration of their pigments and there was no appreciable difference of aquired time

for the beginning of the concentration response in the melanophores and the melanoma cells (Figs. 2 B and 2 E). On changing the immersed fluid from the experimental solution to physiological solution, pigment cells assumed a fully dispersed state again.

In *Carassius auratus*, a scale possessing the parasite could be isolated because the scale was so large to the cyst. If such a scale was used, comparision of the responses of the melanoma cells and of the normal melanophores was clear (Fig. 3 B).

The fact that these melanoma cells responded to K ion so fast as melanophores did seems to imply that the melanoma cells recieve the terminals of the chromatic nerves, because it is generally believed to-day that K ion acts indirectly on the melanophores by exciting the nerve terminals (Fujii 1959).

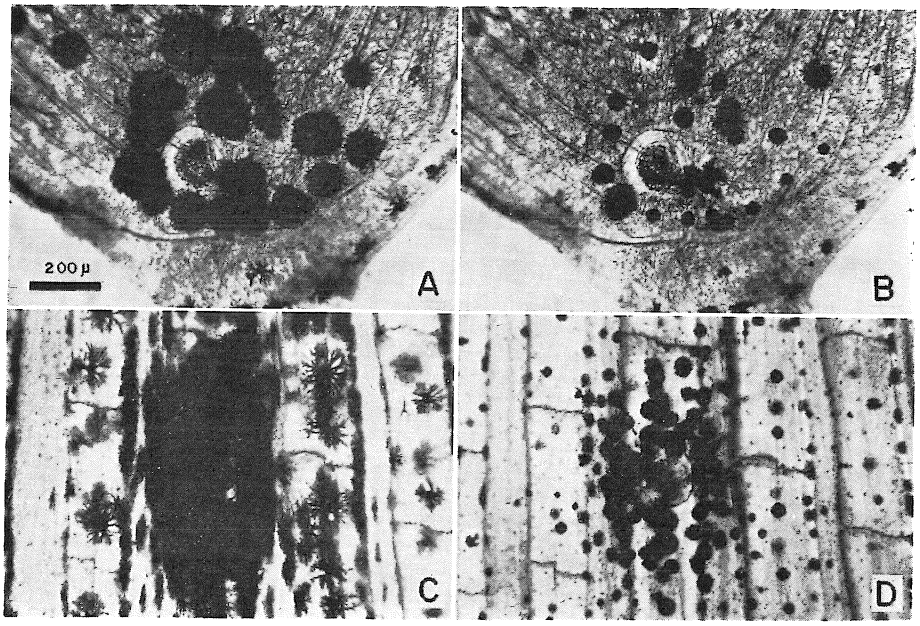


Fig. 3. Responses to KCl of the melanoma cells in the scale (A and B) and the caudal fin (C and D) of *Carassius auratus*. A and C, initial dispersion state in physiological solution. B, 5 minutes after KCl application. D, 15 minutes after KCl application. Room temp., 20.0° C.

III. Response to electric stimulation

If the preparation which had been equilibrated in physiological solution (Fig. 4 A) was stimulated by A. C. at its proximal extremity, melanoma cells began to concentrate within a few seconds (Fig. 4 B). If the intensity of the current was strong enough, pigment cells attained to the maximal concentration within one minute (Fig. 4 C). When the stimulus was removed, the pigment granules within the melanoma cells began to disperse and recovered their initial state. No essential differences in the response to alternating current were observable between the two kinds of pigment cells, the melanoma cells and normal melanophores.

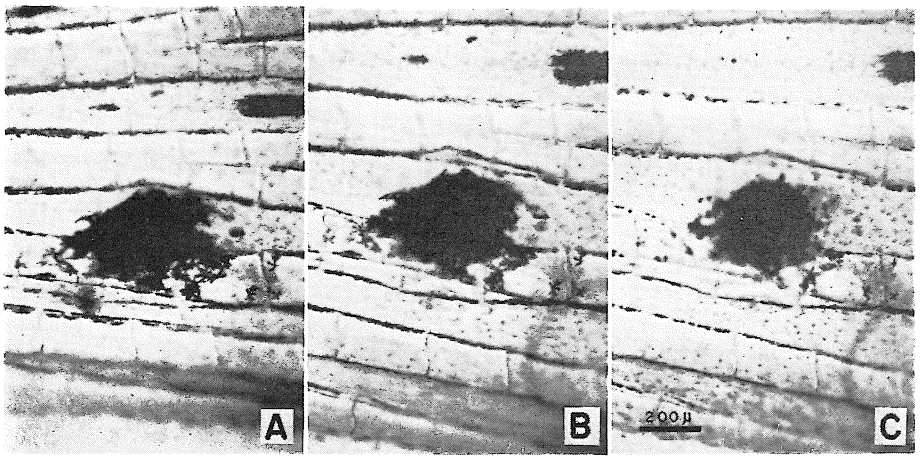


Fig. 4. Responses of melanoma cells to A. C. stimulation. Current was flowed with Ag-AgCl electrodes for 60 secnds, at 4 V. A, just before stimulation. B, 30 seconds after stimulation. C, 60 seconds after stimulation. Room temp., 22.5°C.

Discussion

Many cases of the individuals having the dark spots on the skin associated with treamatode larvae have been observed in fresh-water fishes. According to Nordmann, the parasite is a species of *Diplostomum* (*Holostomum*) *cuticola* in some European fresh-water fishes. Ishii (1915 a) has reported the melanoma associated with encysted treamatode larvae in the crucian carp, *Carassius auratus*. He named this fish disease Diplostomiasis, because he supposed the treamatode infected *Diplostomum* sp. He (1915 b) supplemented the fresh-water fishes suffering from this disease on the following species, *Cyprinus carpio*, *C. carpio* var. *spectaris*, *Leucogobis güntheri*, *Paracheilogunathus* sp., *Acheilognathus* sp. As stated in the introduction, Yamaguti (1939) made to clear experimentally that this parasite belonged to a species of *Metagonimus takahashii*, in some Japanese fresh-water fishes. There are also some cases of melanosis in marine fishes parasitized by treamatode larvae (Smith 1935, Hsiao 1941, Caldwell and Caldwell 1962). Reichenbach-Klinke (1954) reported that, in fishes, pigmentation on the tissue of the host was induced by Phycomycetes, *Ichtyosporydium hoferi*(Plehn et Mulsow), besides the treamatode larvae. Howewer, as far as the author is aware, there is no report on the melanosis in *Oryzias* due to parasitism. In *Oryzias* found here, the heaviness of infection and the development of excessive pigment cells were very remarkable. Furthermore, in *Carassius auratuus*, the dark spots induced by the infection of the parasite fade and become difficult to be distinguished as it is getting warmer, as has been already reported by some investigators (Sasaki 1935, Yamaguti 1939). In *Oryzias*, the dark spots on the skin seemed not to fade all the year round though observations were not so precise. Whether there is a fundamental difference between *Oryzias* and *Carassius* with regard to the pigment cell-parasite relation, must wait for future inquiry.

Osborn (1940) reported the development of melanin pigment cells on the lower surfaces of the animals which were exposed to light ventrally. Furthermore, he observed that these

new pigment cells showed a typical concentration to adrenaline. On the results of the present experiments, the pigment cells produced by the infection of the parasite responded with concentration of their pigment granules when the preparation bearing the melanoma was placed in adrenaline solution. These cells showed concentration of their pigment granules to KCl, and also to A. C. stimulation. These may imply that these cells recieve already supplies of the chromatic nerve fibers. Thus, it may be concluded that these melanotic cells are the same ones as "melanophores" morphologically and physiologically, differing with pigmented macrophages or leucocytes which phagocytized simply the melanin pigment.

The appearance of the pigment cells concerned with the infection of the parasite suggests that melanophores possess a defense role pathologically. Smith's experiments (1931, 1932) on the evoking of melanophores through experimental wounds and X-ray exposure may indicate that role in melanophores.

It may be interesting that the melanomas were found on the normally unpigmented areas of the fishes. The source of these new melanophores will be given elsewhere.

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Summary

1. Individuals possessing dark spots on the skin were found in *Oryzias latipes*. Microscopic observations revealed that, as has been described in some other fresh-water fishes, the dark spot, melanoma, comprises a mass of pigment cells of branched types, in which an encysted larva of a treamatode (unidentified) is enclosed.

2. The activity of the melanoma cells in the excised skin or a slit strip of fin was investigated. They show active concentration of the pigment in response to adrenaline ($10^{-4}-10^{-5}$ M) applied through the external fluid (physiological solution) of the isolated piece, just as do the usual melanophores in the adjacent areas.

3. The melanoma cells in the isolated piece are also caused to concentrate their pigment entirely in the same manner as do the neighboring melanophores, either by bathing the piece in isotnic (M/7.5) KCl solution or by flowing alternating current through the electrodes placed on the piece. This seems to imply that the melanoma cells have been supplied with pigment-concentrating nerve fibers for the melanophores.

4. Some experiments were done on the melanoma cells in *Carassius auratus*, infected by larvae of treamatode, *Metagonimus takahashii*, with similar results.

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