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The Action of Potassium Ions on the Xanthophores of the Teleost, *Oryzias latipes*

Tetsuro IGA

(Department of Biology, Shimane University, Matsue, Japan)

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The fresh water teleost, *Oryzias latipes* (the wild type) possesses in the dermis three kinds of chromatophores: the melanophores, the xanthophores and the guanophores. The melanophores are the most conspicuous type of the chromatophores in the teleosts and many studies on the fish chromatophores have been done in these cells. It has been well accepted that the melanophores in many of the teleosts are controlled under the autonomic nervous system. In excised preparations, melanophores and xanthophores are in expanded state and guanophores in contracted one in physiological solution. When the perfused solution is exchanged from physiological solution to isotonic KCl or adrenaline solution, the melanophores and the xanthophores contract and the guanophores expand within a few minutes. Namely, the melanophores and the xanthophores respond in the same manner to stimulant ions and drugs and the guanophores behave with a reversed response. Miyoshi (1952) studied the response of the guanophores of *Oryzias latipes* to some chlorides. However, much less attention has been paid to the physiology of the yellow cells, no doubt because their appeal as a subject for study suffers from their relatively poor visibility. In this paper, the responses to potassium ions of the xanthophores in an isolated scale were examined and the mode of action in this ion was discussed.

Materials and Methods

Scale xanthophores of *Oryzias latipes* (the wild type and the red type), 22–35 mm in body length, were mainly used. A scale isolated from the dorsal side of the fish was fixed in position under a cover glass, epidermis side down, on a glass trough for microscopic observation, which was filled with physiological solution. The physiological solution was a mixture of $M/7.5$ NaCl, $M/7.5$ KCl and $M/11$ $CaCl_2$ in volume ratio of 100 : 2.0 : 2.1, pH being adjusted to 7.2 by $NaHCO_3$ (Yamamoto,1949). KCl solution was isotonic as the physiological solution.

The response of the chromatophores was expressed exclusively as a magnitude

of pigment dispersion. That is, in the melanophores and the guanophores, the length of a given branch of a cell was measured by means of an occular micrometer during the experimental treatment at a given time interval. As a quantitative expression of the states of active xanthophores, a xanthophore index was used for difficulty of the measurment with the micrometer. The index consists of six division points and giveing to each of these six points a numerical disignation from 0 for punctate to 5 for full expansion. Thus the number of the index, 0, 1, 2, 3, 4 and 5 will be very close to 0, 20, 40, 60, 80 and 100 % expansion, respectively. The method for obtaining the denervated preparations was the same as that described previously for the melanophores (Iga, 1962, 1968).

Experimental Results

(I) *Innervation of the xanthophores*

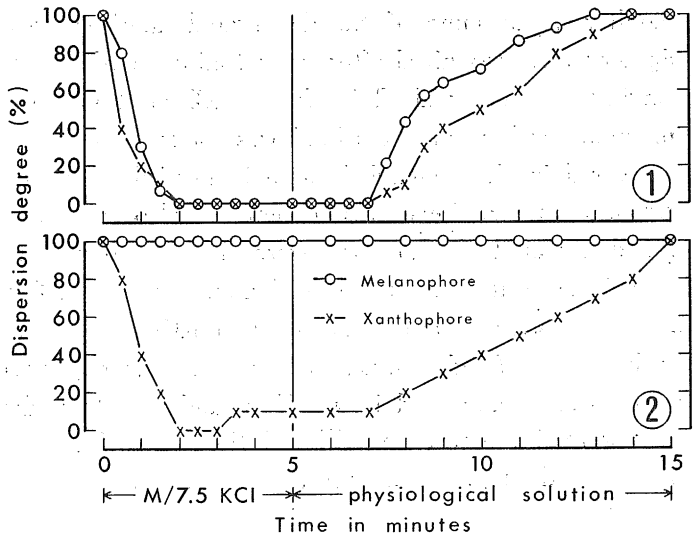
When the spinal cord which was transected at about the third vertebra was stimulated by AC, the animal blanched promptly with great regularity. Microscopic examination discloses that this blanching depends on mainly the contraction of the melanophores in the skin. In this case, the xanthophores did also contract rapidly just as the melanophores did so. When the stimulus was removed, the contracted xanthophores expanded with a similar rapidity as the melanophores did. If, previous to such a test, a nerve-cutting procedure is made in a part of the skin of the creature, the xanthophores, as well as the melanophores, in the scales of the area remained at the expanded state. From these results, it will be indicated that the xanthophores in *Oryzias latipes* also possess direct innervation, and that, moreover, such a incision brings not only severance of the melanophoral nerves, but also the cut of the nerve fibers reaching the xanthophores in the operated area.

When a microelectrode was put on the epidermis of an isolated scale and alternating current was applied, a rapid contraction of the xanthophores, as well as of the melanophores, was evoked. Such a characteristic response to electric stimulation may be induced by the mechanism that electric current activates the chromatic nerve fibers. On the other hand, two to five days after a nerve cutting operation, the scales situated in the operated area were removed from the body and the same stimulating test was made. When AC was applied through the microelectrode on the epidermis of the scale, the xanthophores did not respond except them around the electrode. The contracting response of the xanthophores adjacent to the electrode will be due to a direct stimulation of the electric current. A detailed report of this response will be described in another paper. Thus, it may be indicated that the nerve-cutting operation used here produces also the degeneration of the xanthophore nerves 2 days after the

operation.

(II) *Response to the innervated xanthophores*

When a scale was removed from the dorsal part of *Oryzias latipes* (the wild type) and was immersed in physiological solution, the xanthophores of the scale soon established the equilibrium in expanded state, as the melanophores did so. In the wild type of *Oryzias latipes*, the xanthophores are generally more numerous than the melanophores. In extreme expansion the yellow pigment is spread out so far that it is imposible to distingish boundaries between large numbers of adjacent xanthophores. On exchanging the perfused fluid from physiological solution to *M*/7.5 KCl solution, the xanthophores began to respond with central migration of the yellow pigment and attained the distinctly punctate state within one to two minutes after application of the fluid as illustrated in figure 1. Similar concentrating response by KCl was also induced



Figs. 1 and 2. Time graphs of concentrating responses of an innervated (Fig. 1) and denervated xanthophore (Fig. 2) to *M*/7.5 KCl and of dispersing processes in physiological solution, showing in relation of the melanophore responses. Room temp.: 23.0–24.0 °C.

in the xanthophores from the red type of *Oryzias latipes* which possessed no melanophores in the dermis. It was found that there was no observable difference in the reaction time required for pigment concentration between the xanthophores and the melanophores. The specific effect of K ion, thus, is the concentration of the pigment within the xanthophores.

On reimmersion in physiological solution, the concentrated pigment dispersed again through the cell. The time course of the pigment dispersion was similar or slow slightly as compared with that of the melanophore pigment. Figure 1

showed a typical example of the speed of transition in the pigment within a xanthophore from equilibrium in physiological solution to that in KCl solution, and the reverse, in comparison with the time course of the response of a melanophore in the same scale by the same treatment.

(III) *Response to the denervated xanthophores*

Two to five days after the operation of the nerve-cutting in the scales, the scales were isolated from the area of the operation and immersed in physiological solution for 10 or more minutes. The denervation is confirmed by the failure in the concentrating response to KCl of the melanophores. When the scale preparations were immersed in KCl solution, the pigment within the xanthophores showed an obious concentration in one to two minutes as well as that of the innervated ones, while the melanophores did not react to KCl (Fig. 2). There was little difference in the reaction time and in the time attained a maximal concentration of the pigment between the denervated and innervated xanthophores. In some of the denervated xanthophores the redispersion of the pigment was recognized after several minutes of the application of KCl. On exchanging the external fluid from KCl solution to physiologicl solution, the pigment within the xanthophores began to disperse and the xanthophores recovered in the expanded state in several minutes. Thus, the concentrating response by K ions was reversible also in the denervated xanthophores. The denervated xanthophores were also able to repeat contraction and expansion when the scale was submerged alternately in KCl and physiological solution. On the other experiments, prior to KCl application, the scales were treated with 10^{-7} *M* adrenaline. When the xanthophores and the melanophores which were maintained in a half expanded state under the influence of the drug were immersed in KCl solution, the former contracted, while the latter expanded (Fig. 3), as was already described elsewhere in the

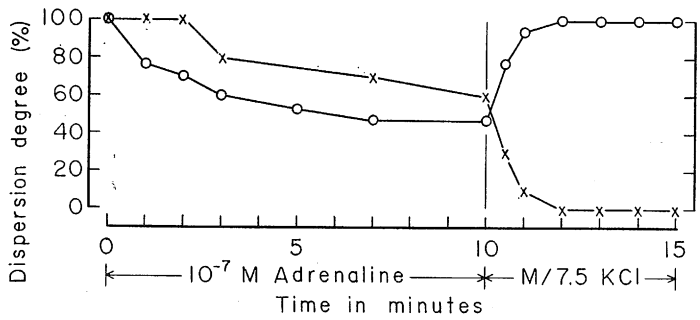


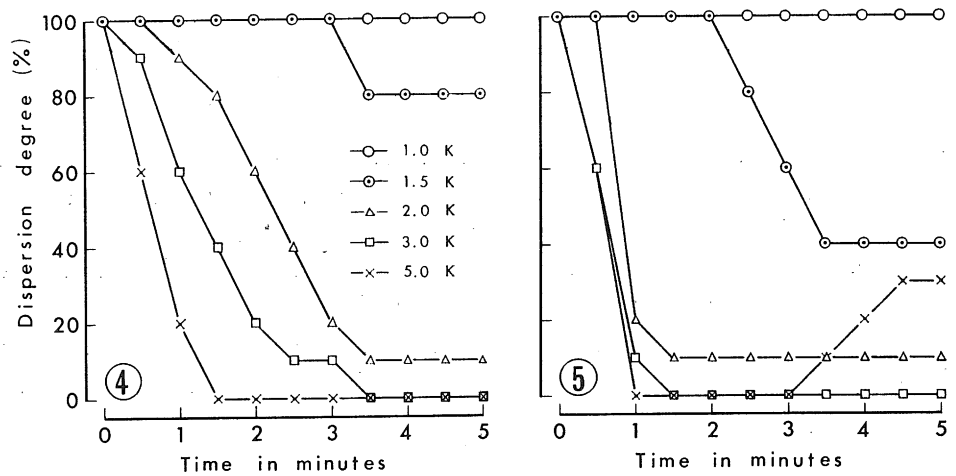
Fig. 3. Responses to *M*/7.5 KCl of a denervated xanthophore (cross symbols) and melanophore (circle symbols) after application of adrenaline. Room temp.: 24.8 °C.

melanophores (Iga, 1962). Thus, it may be asserted that potassium ions did

induced only the concentration response on the xanthophores.

(IV) *Effect of relative concentration of KCl to xanthophore contraction*

The concentration response of the xanthophore pigment evoked by K ions, as well as that of the melanophore pigment, is not of the all or none type, but increases in a graded manner with an increase in the relative amount of potassium ions in the external solution. In order to determine the threshold concentration for the pigment concentration of the xanthophores to K ions, the mixture consisting of *M*/7.5 KCl and *M*/7.5 NaCl mixed in various proportions was applied as the experimental solution. The measurements were carried out on both innervated and denervated xanthophores. The threshold concentration of K which induced a barely visible concentration, is in most of tests less than 1.5 K[1] and more than 1.0 K in the innervated xanthophores. In almost all of the xanthophores examined, when the K concentration reached 2.5 the magnitude of concentration of the pigment approched at that of the full concentration, although the speed of concentration was further increased with an increase in K. Almost the same value, in the threshold and in the concentration required for the maximal contraction, was obtained also in the denervated xanthophores. Each typical example is illustrated in figure 4 in the innervated xanthophore and 5 in the denervated one.



Figs. 4 and 5. Time course of contraction of a single innervated (Fig. 4) and denervated xanthophore (Fig. 5) in various concentrations of K. Room temp.: 24.0 °C.

(V) *Change of the state of the xanthophore during continuous immersion in physiological solution*

1) The 1.5 K refers to the external solution in which *M*/7.5 KCl occupies 1.5 volume parts of the total 10, the remaining 8.5 parts being *M*/7.5 NaCl.

The pigment of the melanophore in an isolated scale keeps a dispersed state in physiological solution for more than an hour. After a few hours, however, the pigment granules began to migrate toward the centrosphere of the cell and the melanophore attains to the punctate state. In due time, the pigment within the melanophore began to disperse again gradually and finally recovered in the fully dispersed state. These pigmentary changes in the melanophore were first reported in a crucian carp, *Carassius auratus* by Iwata et al. (1959a). They designated these periods as the initial dispersion phase, the concentration phase and the final dispersion phase, respectively. In the xanthophores of the wild type and also of the red type of *Oryzias latipes*, similar stational changes of the pigment were recognized. The time course of the stational changes in the xanthophores was perfectly accordant with that in the melanophores. A typical example of these relationships in three kinds of chromatophores, namely the melanophore, the xanthophore and the guanophore, in the same scale was

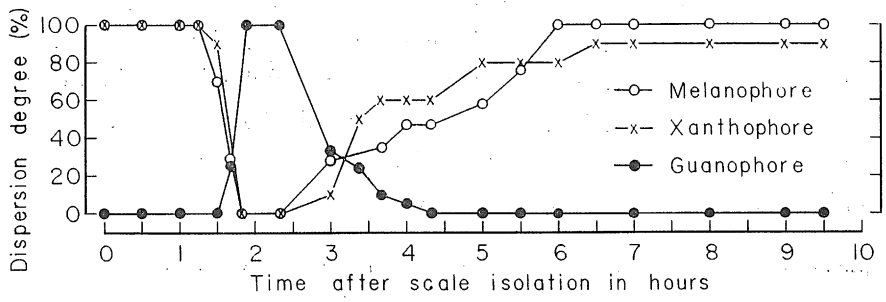


Fig. 6. Changes of the pigmentary state of three kinds of chromatophores during continuous immersion in physiological solution. Room temp.: 25.0–26.5 °C.

graphically illustrated in figure 6. When the xanthophore was treated with KCl solution in a period of redispersing or fully dispersed state of the pigment, the cells responded with an obious contraction. Of course, the cell in the pigment concentration phase kept the punctate state by application of KCl. On the scales performed the denervating operation, the periodic observations were also tried on immersion in physiological solution. In such preparations stational changes of the pigment were not observable in any kinds of three chromatophores.

Discussion

The mechanisms of xanthophore control on the color changes of the teleosts have been studied by many investigators from old time, as well as in the case of the melanophores. By von Frisch (1912), the xanthophores of *Crenilabrus* and *Trigla* were primarily under the influence of the nervous system which caused dispersion and concentration of the pigment. The similar nervous regulation of the xanthophores has been shown also in *Fundulus* by Fries (1927), although these cells of this fish were influenced by a pituitary secretion,

especially in the pigment dispersal. It has been shown in *Fundulus* that the chromatic nerves controlling the activity of the xanthophores are probably consisted of two sets and these permit of independence from these of the melanophores, judging from a disagreement of their behavior of the xanthophores and the melanophores to colored background (Fries, 1931, 1942, Abramowitz, 1936). On the other hand, the xanthophores of the minnow, *Phoxinus*, were controlled dominantly by a pituitary hormone (Giersberg, 1932). Thus, there are two kinds of the mechanisms controlling these color cells of the teleosts, one is mainly nervous and the other humoral. It has been generally accepted that the xanthophores as well as the melanophores of *Oryzias latipes*, are mainly controlled by the nerves, although it is not decided whether a participation of a hypophysial hormone exists in any extent or not. Indeed, it is certain from the experiment I that the pigment concentrating response of the xanthophores is controlling by the nervous system, because the pigment concentration within the yellow cells is induced by electric stimulation of the spinal cord. Relating to the innervation of the xanthophores, it is interesting that these cells underwent their pigmentary change on continuous immersion in physiological solution. The time course of the change of the xanthophore state, moreover, was in full accordance with that of the melanophore state. It may be brought forward that the phenomenon of the stational change of the pigment is one of the evidences on the innervation of the yellow cells in *Oryzias latipes* and that, if it is so, their chromatic nerves lost their function after "the concentration phase", as being pointed out on the melanophores in *Carassius* by Iwata et al. (1959a).

KCl induced an obious concentration of the pigment within the xanthophores, both innervated and denervated, in isolated scales. These results may indicate that potassium ions stimulate directly the xanthophore itself so as to induce concentration of the pigment within the cell. While, it has been pointed out on the fish melanophore system that K ions act selectively on the nerve endings, arousing their pigment concentration (Fujii, 1959, Iwata et al., 1959b). As the xanthophores in a scale isolated from the normal part of the body receive branches from the nerve fibers which remain active, a possible action to the nervous elements of K ions is not decided in distinction from the direct action to the cells. The denervated melanophore expanded to K ions, as also shown in the present experiment III. Therefore, it may be concluded that a direct action of K ions on the color cells is a contraction in the xanthophore, while an expansion in the melanophore. The reversal of the action of potassium ions to two kinds of chromatophores will need to be clear in further investigation.

Summary

1. In the scale xanthophores of the fresh water teleost, *Oryzias latipes* (the wild type and the red type), the responses to KCl and the probable site to be affected by potassium ions were studied.

2. The centripetal migration of the pigment within the xanthophores of this fish was mainly controlled by the nervous system.

3. KCl induced an obious concentration of the pigment within both innervated and denervated xanthophores. No significant difference between the xanthophores and the melanohpores, and also between the innervated and denervated xanthophores was observed in the reaction time and in the threshold concentration of K ions for the pigment concentration of their color cells.

4. The xanthophore pigment in the isolated scale of *Oryzias latipes* underwent three successive stages during continuous immersion in physiological solution, as being observed on the melanophores of the crucian carp and other teleosts. The time course of the pigmentary change of the xanthophores was in accord with that of the melanophores.

5. In three phases, the xanthophores responded only with the pigment concentration to KCl.

6. From these results, the following conclusions may be induced: the action of potassium ions to the xanthophore of *Oryzias latipes* is concentration of the yellow pigment, and that the action site is on the cell itself.

Acknowledgment

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EXPLANATION OF PLATES

Plate 1

The responses to KCl of the innervated (A and B) and denervated chromatophores (C and D) in the isolated scales from the wild type of *Oryzias latipes*.

A : state in physiological solution. The melanophores and the xanthophores expand full and a guanophore is contracted under an expanded melanophore. B : 5 minutes after application of *M*/7.5 KCl. The melanophores and the xanthophores are contracted and the guanophore is expanded. Room temp. : 22.0 °C.

C : initial state in physiological solution. D : 3 minutes after application of *M*/7.5 KCl. The xanthophores show an obious contraction and no response is observed in the melanophores and the guanophore. Room temp. : 23.0 °C.

Plate 2

Changes of the pigmentary state of the chromatophores in an isolated scale during continuous immersion in physiological solution. Room temp. : 26.2—27.0 °C.

A : initial dispersion phase. B : concentration phase. C, D and E : transitional phase to final dispersion. F : final dispersion phase.

Plate 1

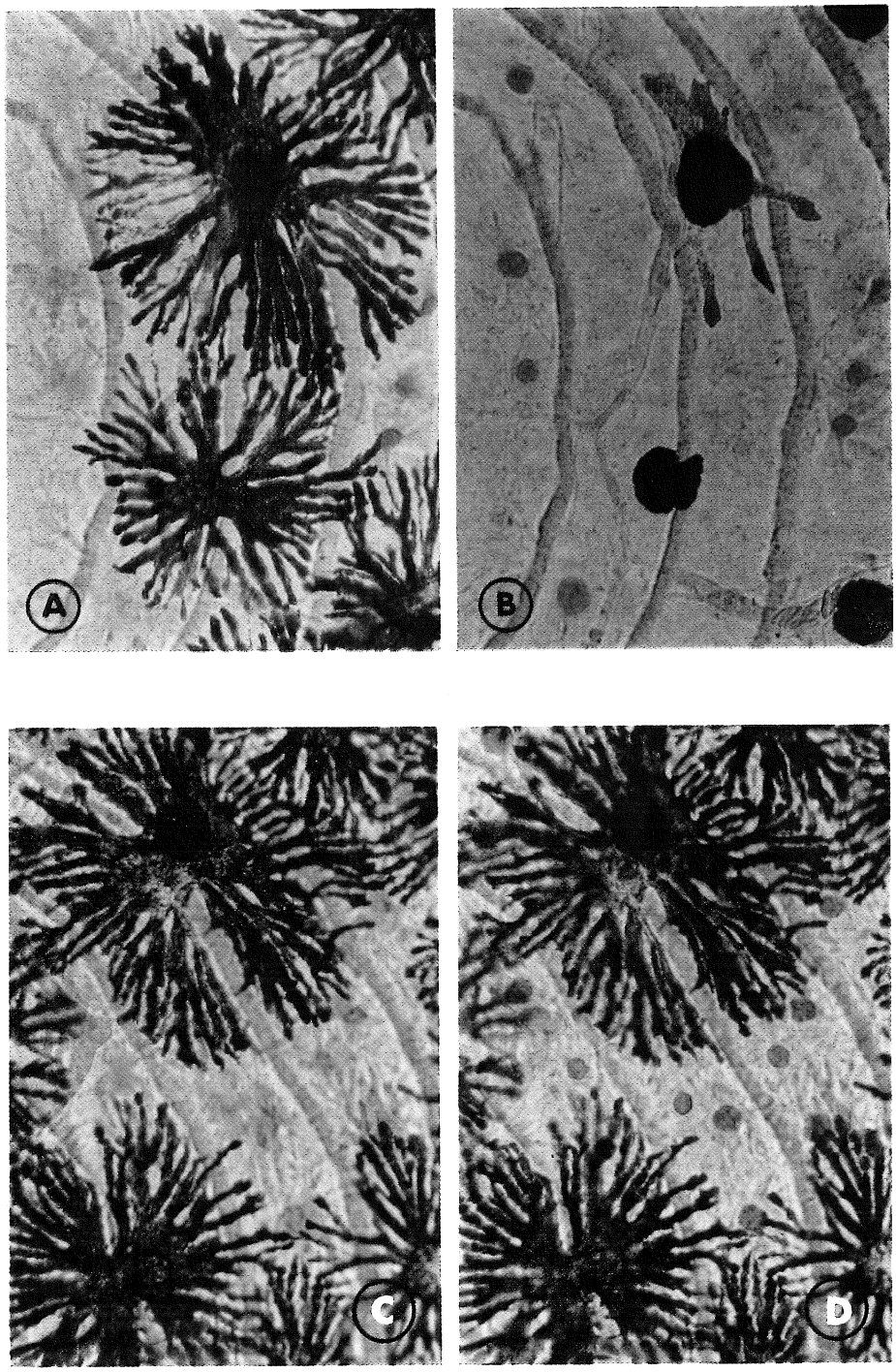

Plate 2