Mem. Fac. Lit. & Sci., Shimane Univ.,
Nat. Sci., **10**, pp. 85–92 Dec. 20, 1976

The Pigment Dispersing Effect of Potassium Ion on Melanophores Induced by X-ray Irradiation in the Goldfish, *Carassius auratus*.

Tetsuro IGA
(*Department of Biology, Shimane University, Matsue 690, Japan*)
(Received September 6, 1976)

ABSTRACT

The responses of the melanophores induced by X-ray irradiation to K ion were examined in the scale of the goldfish, *Carassius auratus*. Melanin-containing cells, melanocytes, within 10 days after irradiation did not show any intracellular displacement of the pigment to isotonic KCl solution and also to 10^{-5} M adrenaline solution. During 2 to 3 weeks after irradiation, newly formed melanophores did not still induced concentration of the pigment granules to KCl solution, but they could respond with rapid concentration of the pigment to 10^{-5} M adrenaline solution. If the melanophores assuming such a responsibility were immersed in KCl solution on the way of recovery from the pigment concentration in physiological solution, which was caused by application of 10^{-5} M adrenaline solution for 5 min, the melanophores showed immediate dispersion of the pigment of a considerable extent, the pigment dispersion persisting during immersion of the solution of KCl. On return to physiological solution, the pigment became again concentrated to much the same degree as that in the physiological solution previous to the treatment. Isotonic NaCl solution did not induced such a rapid dispersion of the melanophore pigment. These results may confirm that K ion has a dispersing effect on the concentrated melanophore pigment by acting directly on the cells, because the melanophores in such a period may not yet receive the supplies of the nerves by which pigment movements within the melanopnores are regulated. In well developed melanophores in irradiated goldfish about a month after irradiation, KCl solution caused concentration of the pigment. Even when these melanophores were in an intermediate state of the pigment, which was induced previously by application of 10^{-5} M adrenaline solution, they responded with only pigment concentration to KCl solution. Thus, it is concluded that K ion possesses two actions on the intracellular movement of the melanophore pigment; one is pigment concentration intermediating the stimulation of the nerve terminals and the other is dispersion of the pigment.

INTRODUCTION

It has been well known that potassium ion induces concentration of the pigment within the melanophores in an isolated scale or an excised strip of fin of teleost fishes. It is generally believed that this cation causes the pigment concentration of the melanophores by exciting the terminals of the pigment concentrating nerve (Fujii, 1959, Iwata, Watanabe and Nagao, 1959). In addition to this pigment concentrating action, another effect of potassium ion on the fish melanophores is now pointed out: when the melanophores of the 3rd type (aneuronic) (Iga, 1975) or in denervated scales of *Oryzias latipes* were immersed in isotonic KCl solution following the treatment with adrenaline, there took place prompt dispersion of the pigment (Iga, 1962, 1976). These observations imply that potassium ion possesses two actions on the intracellular displacement of the melanophore pigment; one is pigment concentration intermediating the stimulation of the nerve endings and the other is dispersion of the pigment. And it is guessed that the latter effect depends on the direct action of potassium ion on the melanophore itself, since the pigment dispersion by isotonic KCl solution occurs conspicuously even after the functional disappearance of the chromatic nervous elements (Iga, 1962, 1976).

On the other hand, it has been pointed out by several investigators that melanophores appears in the skin of xanthic goldfish following irradiation of X-rays (Smith, 1932, Ellinger, 1940, Egami *et al.*, 1962). Etoh and Egami (1963) reported the responsibilities of X-ray induced melanophores of the goldfish to K^+, Na^+, adrenaline and atropine in split preparation of the caudal fins. According to them, in a majority of strips of fins taken from fishes on 18th postirradiation day, the melanophores were responsive to adrenaline, but some of them failed to react to KCl solution. About three weeks or more after irradiation, the response of most of these cells to KCl solution were just the same as those of normal melanophores of the crucian carp, *Carassius carassius*, the melanophores responded with pigment concentration to the solution. From these observations, it is thought that the induced melanophores receive the supplies of the chromatic nerve on about three weeks after irradiation, newly produced melanophores are not innervated. Hence, by using the melanophores at an early stage of appearance, which are not innervated, it may be possible to clear the direct action without intervention of the nervous elements on the melanophores of various ions and drugs.

As the present investigations intended to decide the action point of potassium ion on the fish melanophores, the responsibilities of the X-ray induced melanophores, especially at an early stage of appearance, which are not yet innervated, to isotonic KCl solution were mainly examined.

MATERIALS AND METHODS

Xanthic goldfishes, *Carassius auratus*, 3–4 cm in body length, were used as materials. In these fishes, melanophores were found neither in the skin nor in the fins. The goldfishes, being kept in a small glass tank with 1.5 cm water depth, were totally irradiated with 1000 r of X-rays. The conditions of the irradiation were as follows: 180 kvp, 6 mA, 0.7 mm Cu and 0.5 mm Al filter, target-object distance 30 cm, dose rate in the air 39.5 r per minute. A single irradiation was given to each fish. After irradiation, fishes were reared at the laboratory temperature (20–26°C).

In most fishes surviving for 7 days or more after the irradiation, many melanin-containing cells appeared in both the skin and the fins. The experiments were carried out with the pigment cells in a scale isolated from a darkened area of the skin.

For microscopic observations of the response of the scale melanophore, an isolated scale was fastened epidermal side down to the under surface of a cover glass, which was mounted on a glass vessel filled with physiological salt 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 $NaHCO_3$). Isotonic (M/7.5) KCl solution (pH=7.2 by $NaHCO_3$) and adrenaline solution, prepared by dissolving adrenaline hydrochloride in the physiological saline, were also used as experimental solutions.

To express responses of the melanophores quantitatively, percentage change in apparent length of a given process of the melanophore was read by means of an ocular micrometer, the length at full dispersal of the pigment being taken as 100 and that in a punctate condition of the melanophore as 0 (Iga, 1975).

Experiments were carried out at room temperature.

RESULTS

(I) *Responses of normal melanophores*

The goldfish is colored olive-brown for some time after hatching, but when the fish grows about 2 cm long in body length, depigmentation in the skin begins, the melanophores being first destroyed and then the xanthophores. But this change is not always followed to the end and sometimes stops in the middle of the process, or does not occur at all. Thus, variously colored goldfish are produced, such as olive-brown, black-backed, red, white with red spots, and entirely white (Fukui, 1927).

Responses to potassium ion and adrenaline of the melanophores in a scale isolated from the black area of the skin in the black-backed goldfish were examined for comparison with those of X-ray induced melanophores. These melanophores kept in physiological saline maintained their pigment dispersal. If the solution was replaced with isotonic KCl solution, rapid concentration of their pigment granules took place. On returning to physiological saline, the pigment granules in the melanophores began

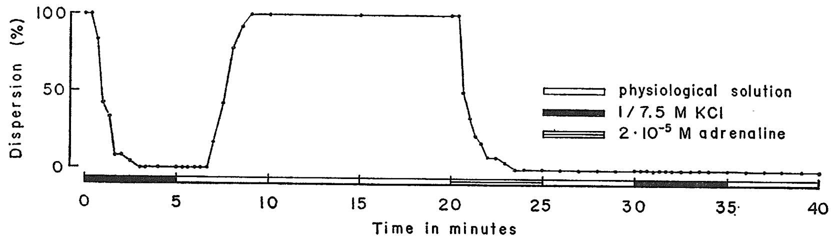


Fig. 1. Typical responses of an isolated scale melanophore of the goldfish, *Carassius auratus*, to M/7.5 KCl solution and 2×10^{-5} M adrenaline solution. Room temp., 25.5°C.

to disperse within a few minutes and attained to their original dispersed state. Then, 2×10^{-5} M adrenaline solution was applied to the scale. The melanophores exhibited concentration of the pigment granules to the adrenaline solution and continued for fairly long time their pigment concentrated state in physiological solution following application of adrenaline solution. On this state of the melanophores, if isotonic KCl solution was applied, the melanophores continued their pigment concentration, or showed again pigment concentration if they were in a half pigment dispersion state in physiological solution. Figure 1 illustrated a typical example of these responses to KCl solution and adrenaline.

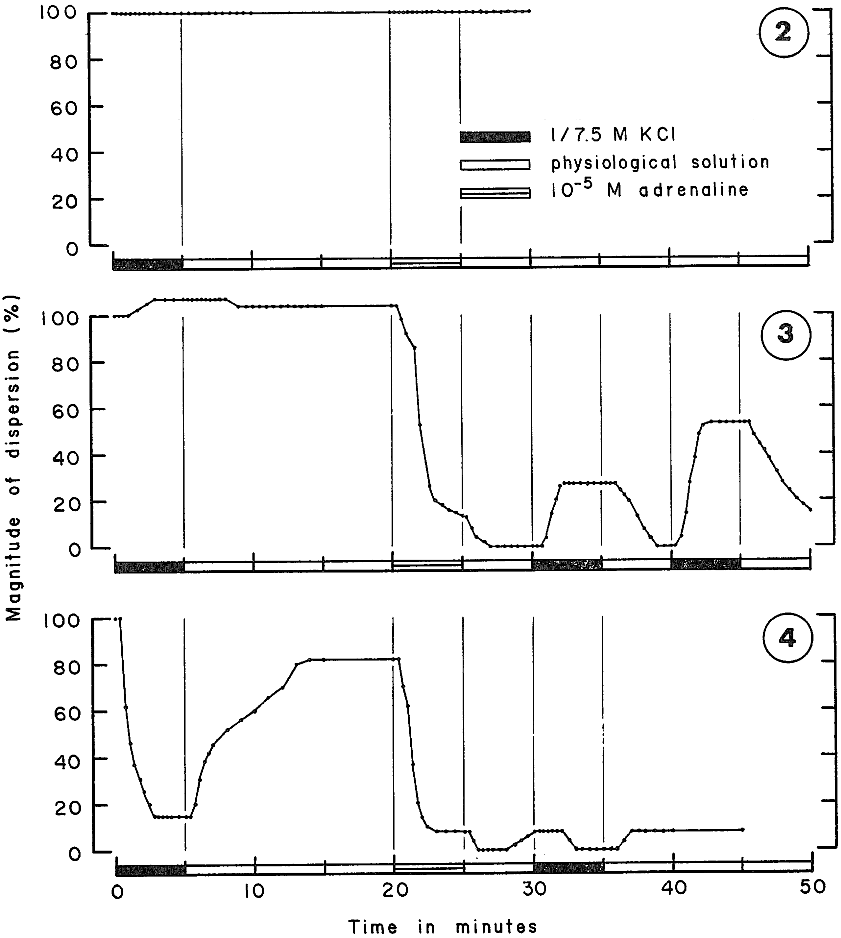
(II) *Responses of X-ray induced melanophores*

Four to five days after exposure of X-rays, melanization of the skin occurred in some of the irradiated fishes. The degree of melanization in the goldfish irradiated differed with each fish and in some of the fishes no recognizable melanization followed. The fishes on 10 to 30 days after irradiation were mainly used in the present experiments.

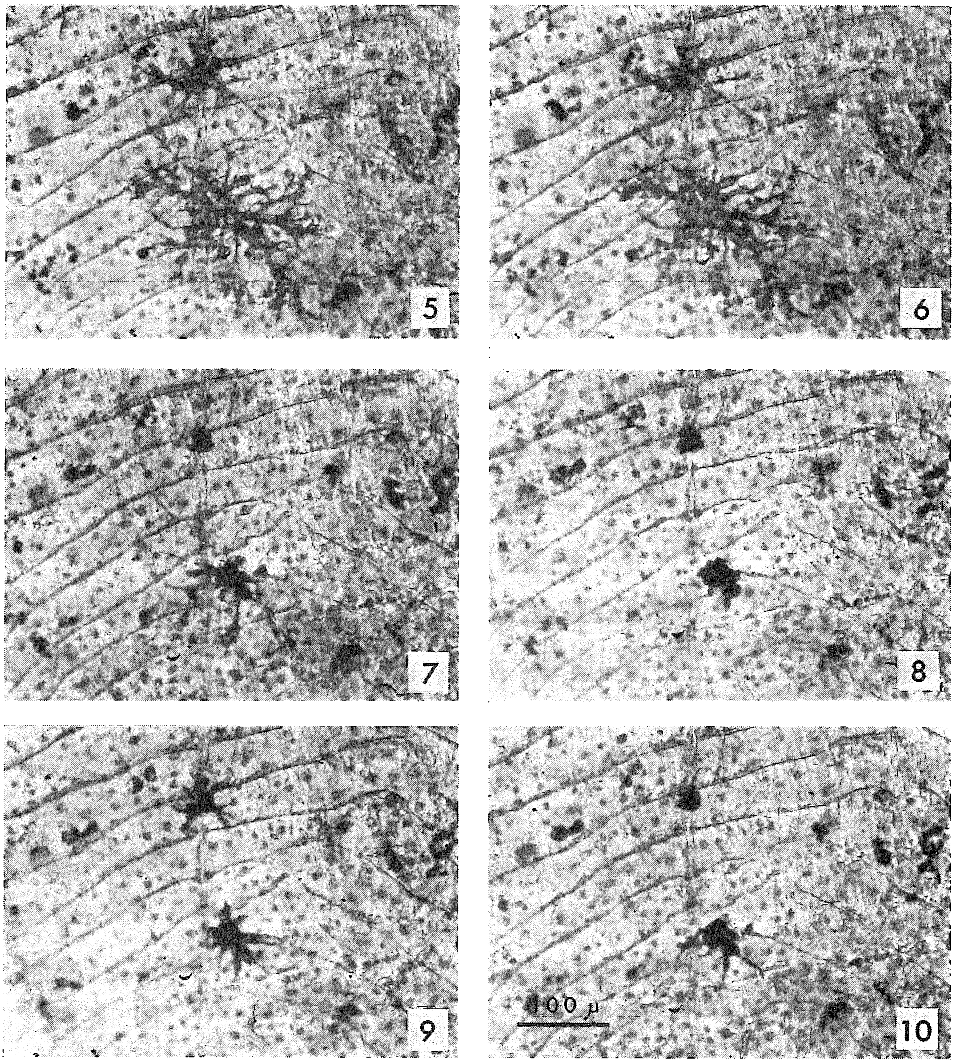
A scale isolated from a melanized area of the skin of the goldfish was kept in physiological solution for 15 minutes, in which melanin containing cells maintained pigment dispersing state. In a majority of the scale isolated from fishes on 10th postirradiation day, a small number of melanin-containing cells were found. These pigment cells still kept the state of their pigment dispersal on application of isotonic KCl solution or of 10^{-5} M adrenaline solution (Fig. 2). These pigment cells should be called melanocytes (Fitzpatrick *et al.* 1966).

About 14 to 20 days after irradiation, the pigment cells were increased in number and the melanophores, within which melanin granules were capable of moving, were found in addition to melanocytes. Although these melanophores yet failed to cause pigment concentration to KCl solution, they responded with pigment concentration to 10^{-5} M adrenaline solution. If these melanophores, being still in a pigment concentrating state by the preceding application of adrenaline for 5 min, were bathed in KCl solution, with which the physiological solution was replaced, they showed immediate pigment dispersion of a considerable extent and the state persisted during immersion in the KCl solution. On succeeding return to physiological saline, the pigment in the melanophores again became concentrated to a certain degree within several minutes (Fig. 3). Such a rapid pigment dispersion of the melanophores was never induced by application of isotonic NaCl solution. The responses to K ion of the melanophores produced by X-ray irradiation in an isolated scale were also shown in Figs. 5–10 with successive microphotographs.

About a month after irradiation, many well developed melanophores were seen in the scale isolated from melanized area of the fish. The reactions of these melanophores to KCl solution or to 10^{-5} M adrenaline solution were the same as those of normal melanophores of the goldfish. On these melanophores showing such a responsibility to KCl solution, only complete pigment concentration was observed on an additional occasion of application of KCl solution to the melanophores on the way of pigment dispersing process in physiological solution after immersion in 10^{-5} M adrenaline solution (Fig. 4).



Figs. 2–4. Typical responses to M/7.5 KCl solution and 10^{-5} M adrenaline solution of melanin-containing cells in three different developmental stages produced by X-ray irradiation in the gold fish, *Carassius auratus*. Fig. 2. 10 days after irradiation. Room temp., 23°C. Fig. 3. 14 days irradiation. When KCl solution was applied to the melanophore in pigment concentrating state left from a preceding application of 10^{-5} M adrenaline solution, the pigment of the melanophore responded with rapid dispersion to the KCl solution. Room temp., 26°C. Fig. 4. 28 days after irradiation. Room temp., 24°C.



Figs. 5–10. Successive treatments of the scale melanophores on 14th day after irradiation with M/7.5 KCl and 10^{-5} M adrenaline solution. Sequence of procedures was as follows: physiological solution (Fig. 5), M/7.5 KCl solution for 5 min (Fig. 6), 10^{-5} M adrenaline solution for 5 min (Fig. 7), physiological solution for 5 min (Fig. 8), M/7.5 KCl solution for 5 min (Fig. 9) and physilolgical solution for 5 min (Fig. 10). Room temp., 26°C.

DISCUSSION

Within 10 days after irradiation, the melanin-containing cells produced by X-ray irradiation were not irresponsible to KCl solution and also to adrenaline solution. These pigment cells should be called melanocytes (Fitzpatrick *et al.*, 1966). These pigment cells gradually developed into melanophores. The newly produced melanophores responded normally to adrenaline with pigment concentration, but they could not cause pigment concentration to KCl solution. About a month after irradiation, most of the melanophores became responsive to both adrenaline and KCl. Present observations, on these points, fairly coincided with those of Etoh and Egami (1963) using the pigment cells in the caudal fin of irradiated goldfish. From these observations it may be reasonable to think that the newly produced melanophores in the present experiments do not receive the supplies of pigment concentrating nerve fibers, the nerve supplies to the cells taking place later, because on the pigment concentration by KCl, the site affected by K ion is the endings of the concentrating nerve fibers (Fujii, 1959a, Iwata, Watanabe and Nagao, 1959).

Of a special interest is another effect of K ion on the melanophores, which are not yet innervated, in a pigment concentrating state following application of adrenaline solution. When these melanophores in pigment concentrating state were immersed in KCl solution, there took place rapid dispersion of the pigment. On return to physiological solution, the pigment concentrated again to much the same degree as the previous pigment concentration. These responses to KCl solution agreed considerably with those on the melanophores of the 3rd type (aneuronic) and the denervated melanophores in the isolated scale of *Oryzias latipes* (Iga, 1962, 1975, 1976).

It has been generally accepted that the melanophores in teleost fishes are supplied with two sets of nerves, one concerned with the concentration of pigment and the other with its dispersion (cf. Parker, 1948). As was already discussed on the pigment concentrating nerve fibers, some problems on the pigment dispersing ones should be argued here. Fujii (1959b), on the tail fin melanophores of goby, *Chasmichthys gulosus*, from the result that there was significant difference in the rate of dispersion response to NaCl solution between innervated and denervated melanophores following application of adrenaline, concluded that Na^+ acts selectively on the endings of dispersing nerves. At the present experiments, however, when isotonic NaCl solution was applied on the newly formed melanophores following application of adrenaline, rapid dispersion of the pigment was never induced, nor was recovery from the pigment concentration induced by preceding application of adrenaline accelerated in the NaCl solution. This may imply that these melanophores do not yet receive the supplies of the dispersing nerve fibers, too.

Thus, it may be concluded that the pigment dispersion by KCl is due to the action of K ion exerted directly on the melanophores.

The author wishes to express his gratitude to professor H. Nagahama of Hiroshima University for his invaluable advice and kindness in reading this manuscript. X-ray irradiation of the goldfishes was done at Matsue city hospital in 1964. Thanks are also due to Mr. Y. Nakamura of the head physician of the department of radio-therapy for providing facilities for the irradiation.

LITERATURE

EGAMI, N., H. ETOH, C. TACHI, K. AOKI and R. ARAI (1962) Role of the pituitary gland in melanization in the skin of the goldfish, *Carassius auratus*, induced by X-ray irradiation. Proc. Japan Acad., **38**: 345–347.

ELLINGER, F. (1940) Roentgen-pigmentation in the gold fish. Proc. Soc. Exp. Biol., N. Y., **45**: 148–150.

ETOH, H. and N. EGAMI (1963) Responses to Na^+, K^+, atropine and adrenaline of melanophores induced by X-irradiation in the fin of the goldfish. Annot. Zool. Japon., **36**: 133–139.

FITZPATRICK, T. B., Jr., W. C. QUEVEDO, A. L. LEVENE, V. J. MCGOVERN, Y. MISHIMA and A. G. OETTLE (1966) Terminology of vertebrate melanin containing cells: 1965. Science, 152: 88–89.

FUKUI, K. (1927) On the color pattern produced by various agents in the goldfish. Folica Anat. Japonica, **5**: 257–302.

FUJII, R. (1959a) Mechanism of ionic action in the melanophore system of fish I. Melanophore-concentrating action of potassium and some other ions. Annot. Zool. Japon., **32**: 47–58.

——— (1959b) Mechanism of ionic action in the melanophore system of fish II. Melanophore-dispersing action of sodium ions. J. Fac. Sci. Univ. Tokyo, **8**: 371–380.

IGA, T. (1962) Actions of potassium ion on scale melanophores of *Oryzias latipes*. Zool. Mag., **71**: 373–374. (In Japanese).

——— (1975) Variation in response of scale melanophores of a teleost fish, *Oryzias latipes*, to potassium ions. J. Sci. Hiroshima Univ., Ser. B. Div. 1, **26**: 23–35.

——— (1976) Action of potassium ion on melanophores in isolated scales of *Oryzias latipes*, with special reference to its pigment-dispersing effect. Ibid. **26**: in press.

IWATA, S. K., M. WATANABE and K. NAGAO (1959) The mode of action of pigment concentrating agents on the melanophores in an isolated fish scale. Biol. J. Okayama Univ., **5**: 195–206.

PARKER, G. H. (1948) Animal colour changes and their neurohumours. Cambridge Univ. Press.

SMITH, G. M. (1932) Eruption of corial melanophores and general cutaneous melanosis in the goldfish (*Carassius auratus*) following exposure to X-ray. Amer. J. Cancer, **16**: 863–870.