Mem. Fac. Lit. & Sci., Shimane Univ.,
Nat. Sci., 11, pp. 63–72 Dec. 20, 1977

Adrenergic Receptors Mediating Pigment Dispersion in Leucophores of a Teleost, *Oryzias latipes*

Tetsuro IGA, Koji YAMADA and Minoru IWAKIRI

Department of Biology, Faculty of Literature and Science, Shimane University, Matsue 690, *Zoological Institute, Faculty of Science, Hiroshima University, Hiroshima* 730 *and Department of Biology, Fukuoka University of Education, Munakata, Fukuoka* 811–41

(Received September 5, 1977)

ABSTRACT

Using leucophores in isolated scales of a fresh-water teleost, *Oryzias latipes*, experiments were designed to characterize the nature of adrenergic receptors mediating the pigment-dispersing response. Adrenaline, Noradrenaline and isoproterenol caused dispersion of the pigment within both innervated and denervated leucophores, indicating that these amines act directly on the cells. The order of potency of these catecholamines for inducing the pigment dispersion within the leucophores was isoproterenol> noradrenaline> adrenaline. Pretreatment with dibenamine or ergotamine, alpha adrenergic blocking agents, did not affect the responses of leucophores to these agonists. On the other hand, propranolol or dichloroisoproterenol, beta adrenergic blocking agents, inhibited the pigment-dispersing effect of these amines. From these results, it was concluded that the adrenergic receptors mediating pigment dispersion within the leucophores are of beta nature.

INTRODUCTION

Melanophores are the most conspicuous and commonly studied type of chromatophores in teleost fishes. As is well known, melanophores of teleost fishes are primarily controlled by sympathetic nervous system. Recent studies have shown that the neurotransmitter taking part in aggregation of the pigment within melanophores may be adrenergic (Fujii, 1961; Scheline, 1963; Scott, 1965; Healey and Ross, 1966; Fujii and Novales, 1972). Furthermore, the latest pharmacological researches have indicated that the adrenergic receptors regulating pigment aggregation in teleost melanophores are of alpha nature (Iga, 1968; Grove, 1969; Reed and Finnin, 1972; Fernando and Grove, 1974a, b; Fujii and Miyashita, 1975).

On the other hand, there have been few physiological studies on fish leucophores except complementary observations (Odiorne, 1933; Fries, 1942, 1958; Miyoshi, 1952; Kinosita, 1963). The leucophores in an isolated scale of *Oryzias latipes* assume the pigment-aggregating state in physiological saline or isotonic NaCl solution and cause pigment dispersion in isotonic KCl solution, while melanophores disperse their

pigment in the former, and induce pigment aggregation in the latter; leucophores and melanophores behave quite oppositely to the ionic stimulation (Miyoshi, 1952). The opposite behavior of these two kinds of chromatophores has been observed also in response to adrenaline in some other fishes (Odiorne, 1933; Fries, 1958).

In recent investigations, the leucophores of *Oryzias latipes* have been shown to be primarily under nervous regulation, and to possess adrenergic receptors which mediate pigment dispersion (Iga, 1975 a). The present experiments were designed to provide more precise information about the nature of adrenergic receptors mediating pigment dispersion within the leucophores of *Oryzias latipes*.

MATERIALS AND METHODS

Scale leucophores of a fresh-water teleost, *Oryzias latipes* were utilized. A scale isolated from the dorso-lateral trunk of the fish was attached epidermis side down to the under surface of a cover glass which was mounted on a glass trough for microscopic observation, in which a physiological salt solution was filled. The physiological salt solution had the following composition: 128 mM NaCl, 2.6 mM KCl, 1.8 mM $CaCl_2$ (pH 7.2 by $NaHCO_3$). Isotonic (133 mM) KCl solution was adjusted to pH 7.2 by $KHCO_3$. In some experiments, 5.0 mM Tris-HCl buffer (pH 7.2) was employed in stead of $NaHCO_3$ or $KHCO_3$ system.

The following drugs were used: *l*-adrenaline hydrochloride (Sankyo, Tokyo), *l*-noradrenaline hydrochloride (Sigma Chemical, St. Louis), *dl*-isoproterenol hydrochloride (Sigma Chemical, St. Louis), dibenamine hydrochloride (Tokyo Kasei, Tokyo), ergotamine tartrate (Tokyo Kasei, Tokyo), propranolol hydrochloride (Sigma Chemical, St. Louis), and *dl*-dichloroisoproterenol hydrochloride (Aldrich Chemical, Milwaukee). Drugs were dissolved in the physiological saline and applied externally to the scale preparation.

In some experiments, denervated leucophores were employed, the procedures for denervation being described in the previous papers (Iga, 1968, 1975 a).

Leucophores generally lie just beneath the melanophores, being brown in appearance in transmitted light, and lose their brilliance. For these reasons, it is rather difficult to distinguish the responses of the leucophores from those of the melanophores in transmitted light. Thus, microscopic observations of leucophores were made mainly with reflected light, under which they clearly appeared silvery or luminously white on a dark background. The use of illumination for microscopic observation was limited to the period when the observation was made, because the continuous light illumination induced pigment dispersion in some of the leucophores.

Response of the leucophore was expressed as a percentage of the length of a given process of a leucophore. The length of the process at the punctate state in the physiological saline was taken as zero and the length at full dispersal of the pigment was taken as 100. Full dispersal was obtained in the KCl solution. Measurements

of the length of the process were made by means of an ocular micrometer.

All experiments were conducted at room temperature (22–24°C).

RESULTS

Effects of catecholamines

Perfusion with the physiological saline for 15 min brought about full pigment aggregation in the leucophores in an isolated scale preparation. Then the scale was placed in the KCl solution for 5 min in order to determine the full pigment dispersion in a test leucophore. On the scale preparation 5 minutes' application of the solution was enough to make the pigment within leucophores disperse completely in the most observations. After being rinsed in the physiological saline for 15 min, the scale was exposed to test solutions containing catecholamines of various concentrations for 10 min. A value of the response obtained at the end of 10 minutes' application of each test solution was estimated as the extent of response to the test solution. The measurements on each concentration of the test solutions were made on 10 cells in different scales.

All the catecholamines investigated were found to cause pigment dispersion

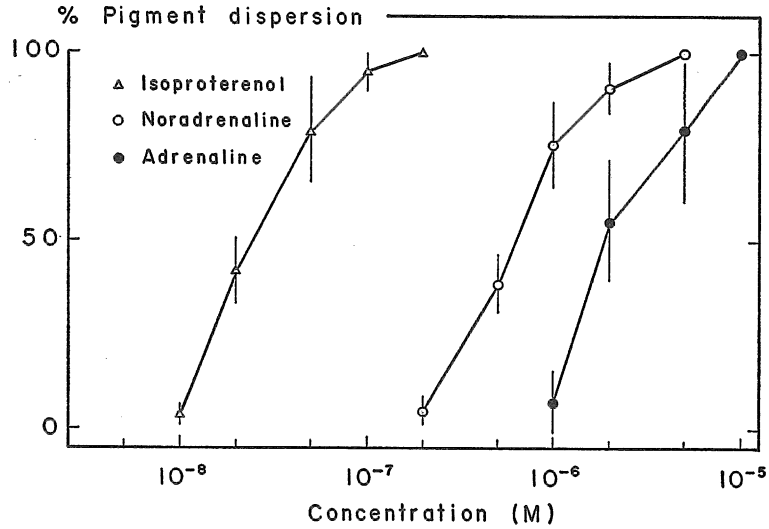


Fig. 1. Concentration-response relations for pigment-dispersing effect of catecholamines on scale leucophores of *Oryzias latipes*. Abscissa, molar concentration of the catecholamines in logarithmic scale. Ordinate, magnitude of response as a percentage of the value to the full response. Each point represents the mean of 10 measurements on different scales. Standard deviation of the mean is indicated by vertical bars.

within leucophores. Figure 1 shows concentration-response curves for the pigment-dispersing effect of catecholamines on the leucophores, where the per cent response of leucophores is plotted to the molar concentration of catecholamines. As indicated in Fig. 1, the minimal concentration required for causing discernible pigment dispersion in the leucophores was found to be about 10^{-6} M for adrenaline, 2×10^{-7} M for noradrenaline and 10^{-8} M for isoproterenol respectively. Full dispersion of the pigment

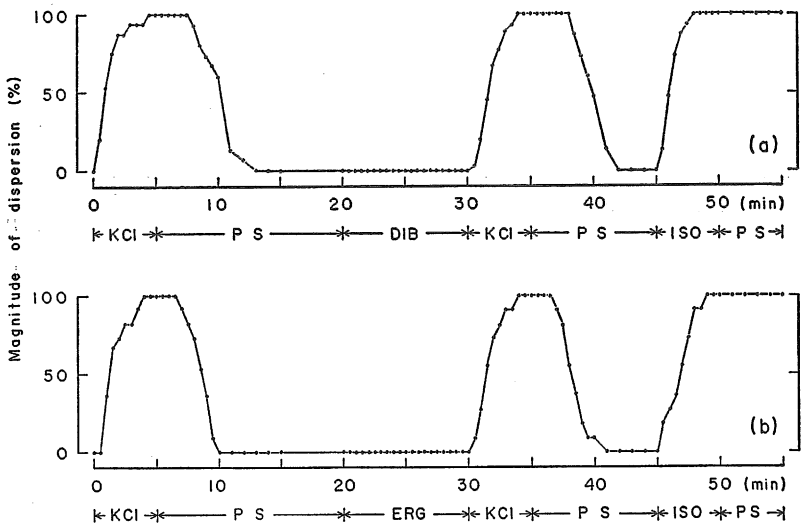
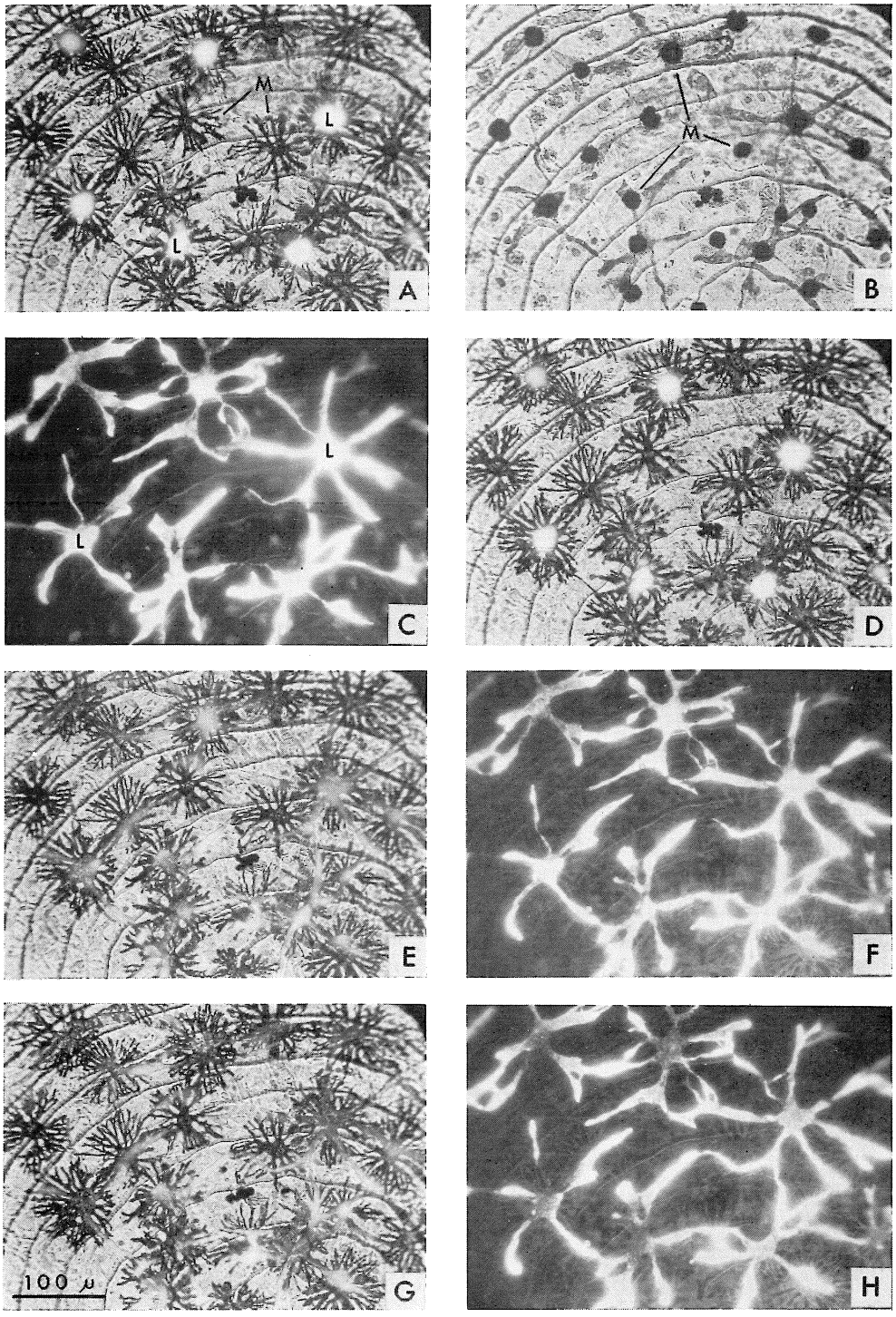


Fig. 2. Typical examples showing the effect of alpha adrenergic blocking agents, 10^{-4} M dibenamine (DIB) (a) and 5×10^{-5} M ergotamine (ERG) (b), on the leucophore response to isotonic KCl and 10^{-5} M isoproterenol (ISO). PS, physiological saline.

Fig. 3. A series of photomicrographs showing the effect of 10^{-4} M dibenamine on the responses of melanophores and leucophores to isotonic KCl and to 10^{-5} M isoproterenol. Photographs of A, D, E and G were taken by using simultaneously both transmitted and reflected lights, B being taken with transmitted light and the others (C, F and H) were done with reflected one. A. States of chromatophores in the physiological saline. Melanophores (M) are in pigment dispersion and leucophores (L) are in pigment aggregation. B and C. 5 min after application of isotonic KCl solution. Melanophores are in pigment aggregation and leucophores are in pigment dispersion. D. 10 min after treatment with 10^{-4} M dibenamine. E and F. 5 min after application of the KCl solution following the treatment with dibenamine. Pigment-aggregating response of melanophores was completely blocked, while pigment-dispersing response of leucophores was unaffected, the leucophores exhibited full pigment dispersion by KCl. Affer the application of the KCl solution, the scale was rinsed with physiological saline for 15 min, in which leucophores became completely punctate. G and H. 5 min after immersion in 10^{-5} M ISO.


M
L
L
A
M
B
L
L
C
D
E
F
100 μ
G
H

within leucophores was obtained at 10^{-5} M for adrenaline, 5×10^{-6} M for noradrenaline and 2×10^{-7} M for isoproterenol respectively. Three concentration-response curves ran almost parallel with each other. The order of relative effectiveness of these agonists for causing pigment dispersion within leucophores was isoproterenol > noradrenaline > adrenaline.

In the experiments with denervated preparations, these three catecholamines also induced pigment dispersion within leucophores, indicating that these catecholamines act directly on the leucophores, through their receptors of the plasma membrane. The receptors, consequently, are of adrenergic ones. On the nature of the receptors, the order of potency of these amines characterized a beta adrenergic receptor-mediated response.

Effects of alpha adrenergic blocking agents

Although the characterization of adrenergic receptors based on their physiological response to agonists was demonstrated, one can distinguish between alpha and beta receptors much more clearly by use of adrenergic blocking agents (Nickerson, 1967). Therefore, effects of adrenergic blocking agents on the pigment-dispersing responses of leucophores to KCl and to these catecholamines were examined in the following experiments.

First, effects of alpha adrenergic blocking agents were studied. As shown in Fig. 2(a), dibenamine (10^{-4} M) had no blocking effect on the pigment-dispersing responses of leucophores to KCl and to 10^{-5} M isoproterenol. On the other hand, pigment aggregation within the melanophores induced by KCl or catecholamines was interfered completely by the pretreatment with dibenamine. The effects of dibenamine on the two kinds of chromatophores are also shown in Fig. 3 as serial photomicrographs. With ergotamine (5×10^{-5} M) we obtained results similar to those obtained with dibenamine; application of ergotamine for 10 min did not affect the responses of leucophores to KCl and to 10^{-5} M isoproterenol (Fig. 2b). Ergotamine had a pigment-aggregating effect on *Oryzias* melanophores, as had been observed in melanophores of some fishes by some other investigators (Fries, 1942, 1958; Fujii, 1961; Pye, 1964; Healey and Ross, 1966), whereas the drug did not affect the state of the leucophores.

Effects of beta adrenergic blocking agents

Adrenergic antagonists known to affect beta receptors were studied for their effects. The pigment-dispersion induced by KCl or catecholamines was antagonized by beta adrenergic blocking agents: When the scales were pretreated with 10^{-4} M propranolol or 10^{-4} M dichloroisoproterenol for 10 min, the pigment-dispersing responses of leucophores to KCl and to isoproterenol of 10^{-5} M, which was the concentration that produced the maximum pigment dispersion within the leucophores, were completely blocked, and the blockade by these agents persisted for fairly long time.

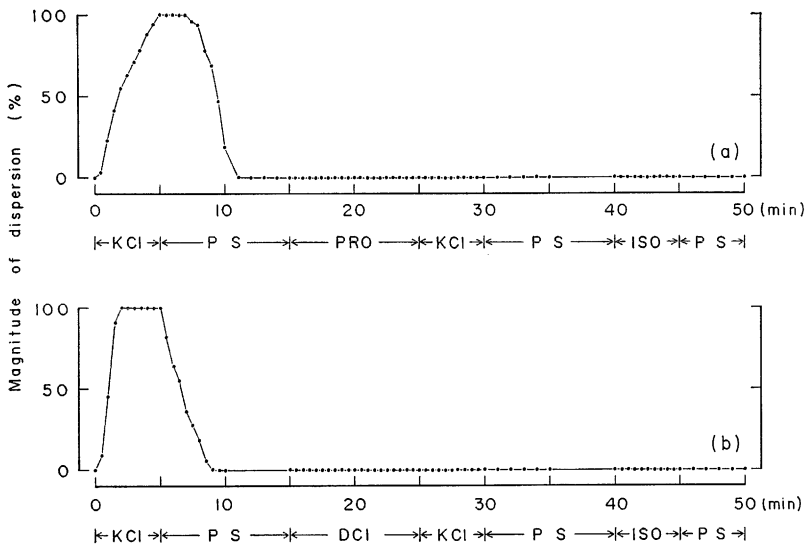


Fig. 4. Typical examples showing the effect of beta adrenergic blocking agents, 10^{-4} M propranolol (PRO) (a) and 10^{-4} M dichloroisoproterenol (DCI) (b), on the leucophore response to isotonic KCl and to 10^{-5} M ISO. PS, physiological saline.

The effects of these agents are illustrated in Fig. 4 and also by a series of photomicrographs in Fig. 5. These blocking agents, though they were sufficient to block the leucophore response in their concentration and also in application time, could not affect the pigment-aggregating response of the melanophores; the pigment aggregation within melanophores was normally induced by KCl or 10^{-5} M adrenaline (Fig. 5). Both propranolol and dichloroisoproterenol, in a high concentration, have a slight effect on pigment aggregation in innervated melanophores.

DISCUSSION

Relatively little is known about properties of fish leucophores on the cellular level. Odiorne (1933) observed that the scale guanophores of *Fundulus* responded with pigment dispersion to adrenaline. Miyoshi (1952) reported that leucophores in isolated scales of *Oryzias latipes* dispersed their pigment granules in KCl solution, while melanophores aggregated their pigment. Leucophores and melanophores in fishes generally behave quite oppositely in response to various stimuli. In connection with the measurements of the melanophore potential, Kinosita (1963) recorded the membrane potential of leucophores in *Oryzias latipes*. The electrical potential was inside negative as in the case of melanophore potential, but the gradient of the electrical potential along the process of the leucophore was always opposite to that along the process of the melanophore under the effect of the same solution.

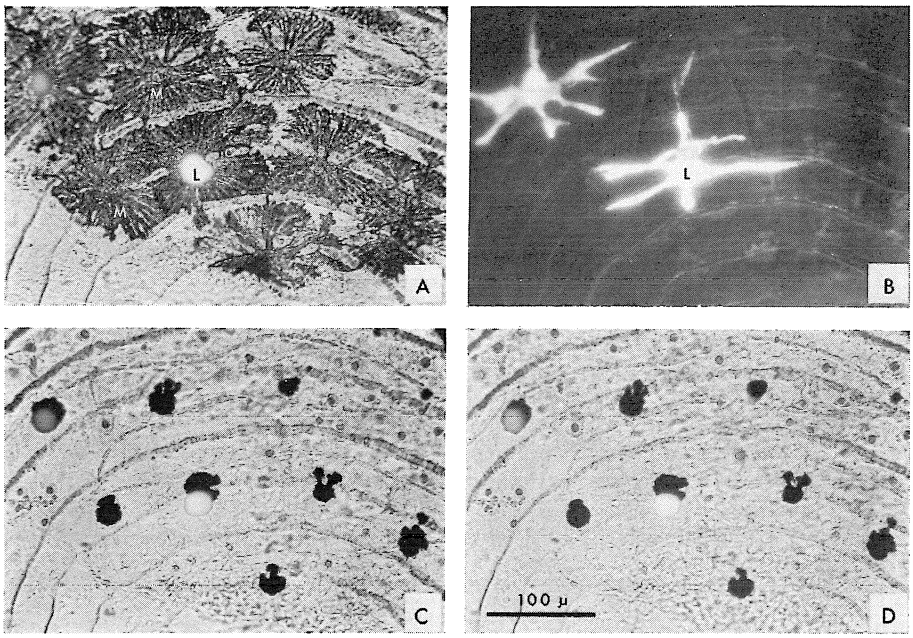


Fig. 5. A series of photomicrographs showing the effect of 10^{-4} M propranolol on the responses of melanophores and leucophores to isotonic KCl and to 10^{-5} M adrenaline. A. States of chromatophores in the physiological saline. L, leucophore. M, melanophores. B. 5 min after application of the KCl solution. C. 5 minutes' application of the KCl solution following treatment with 10^{-4} M propranolol for 10 min. Pigment-aggregating response of the melanophores was normally induced by the KCl solution, while pigment dispersing response of leucophores was completely blocked, the leucophores being still in pigment aggregation. D. 5 min after immersion in 10^{-5} M adrenaline.

The action of catecholamines is mediated through what are referred to as adrenergic receptors. The catecholamines tested here induced pigment dispersion in both innervated and denervated leucophores, indicating that the leucophores of *Oryzias latipes* possess adrenergic receptors. These receptors are generally characterized as being of two types, alpha or beta, each of which usually controls the responses that are opposite in nature to the other. The catecholamines studied in the present experiments dispersed pigment granules within leucophores in the following order of effectiveness: isoproterenol > noradrenaline > adrenaline, a characterization of a beta adrenergic receptor-mediated response (Furchgott, 1967). In addition, this was the reverse order of agonists for aggregating pigment granules in melanophores of the same species (Iga, 1968). Furthermore, the fact that beta adrenergic blocking agents inhibited pigment dispersion within the leucophores to the agonists, whereas alpha adrenergic blocking agents could not affect their responses, indicates that these agonists promote their effect through beta receptors. Thus, it is concluded that the adrenergic receptors mediating the pigment-dispersing response of *Oryzias* leucophores to

adrenergic stimuli are of beta nature.

Meanwhile, recent studies on some amphibians and reptiles have revealed the presence of beta adrenergic receptors in melanophores, which mediate pigment dispersion (Graham, 1961; Goldman and Hadley, 1969a, b; McGuire, 1970; Bagnara and Hadley, 1973). Quite recently it has been discussed by several researchers that melanophores in certain fishes may possess the adrenergic receptors of beta type, which mediate pigment-dispersing response, in addition to ones of alpha type (Reed and Finnin, 1972; Fujii, 1973; Miyashita and Fujii, 1975; Finnin, Dudinski and Reed, 1976; Fujii and Miyashita, 1976).

The pigment dispersion in leucophores of *Oryzias latipes* is induced effectively by adrenergic stimuli. Since the pigment-dispersing response through beta adrenergic receptors has become of major interest lately also in fishes, the present materials may be of much advantage to studies on the nature of beta receptors in chromatophores.

ACKNOWLEDGEMENT

We thank Professor H. Nagahama of Hiroshima University for his interest and encouragement.

REFERENCES

Bagnara, J. T., and M. E. Hadley, 1973. Chromatophores and Color Change. Prentice-Hall, New Jersey.

Fernando, M. M., and D. J. Grove, 1974a. *Comp. Biochem. Physiol.*, **48A**: 711.

———, and ———, 1974b. *Ibid.*, **48A**: 723.

Finnin, B. C., O. Dudinski and B. L. Reed, 1976. *In* Pigment Cell, 3 (Riley, V. ed.), 322. Karger, Basel.

Fries, E. F. B., 1942. *Proc. Nat. Acad. Sci.*, **28**: 396.

———, 1958. *J. Morphol.*, **103**: 203.

Fujii, R., 1961. *J. Fac. Sci. Univ. Tokyo, Sect. IV*, **9**: 171.

———, 1973. *Yale J. Biol. Med.*, **46**: 707.

———, and Y. Miyashita, 1975. *Comp. Biochem. Physiol.*, **51C**: 171.

———, and R. R. Novales, 1972. *In* Pigmentation: Its Genesis and Biologic Control (Riley, V. ed.), 315. Appleton-Century-Crofts, New York.

———, and Y. Miyashita, 1976. *In* Pigment Cell, 3 (Riley, V. ed.), 336. Karger, Basel.

Furchgott, R. R., 1967. *Ann. N. Y. Acad. Sci.*, **139**: 553.

Goldman, J. M., and M. E. Hadley, 1969a. *J. Pharmacol. Exp. Therap.*, **166**: 1.

———, and ———, 1969b. *Gen. Comp. Endocrinol.*, **13**: 151.

Graham, J. D. P., 1961. *J. Physiol.*, **158**: 5 P.

Grove, D. J., 1969. *Comp. Biochem. Physiol.*, **28**: 37.

Healey, E. G., and D. M. Ross, 1966. *Comp. Biochem. Physiol.*, **19**: 545.

Iga, T., 1968. *Zool. Mag., Tokyo*, **77**: 19.

———, 1975a. *J. Sci. Hiroshima Univ., Ser. B. Div.* 1, **26**: 23.

———, 1975b. *Zool. Mag., Tokyo*, **84**: 362.

Kinosita, H., 1963. *Ann. N. Y. Acad. Sci.*, **100**: 992.
McGuire, J., 1970. *Arch. Dermatol.*, **101**: 173.
Miyashita, Y., and R. Fujii, 1975. *Comp. Biochem. Physiol.*, **51C**: 179.
Miyoshi, S., 1952. *Annot. Zool. Japon.*, **25**: 21.
Nickerson, M., 1967. *Ann. N. Y. Acad. Sci.*, **139**: 571.
Odiorne, J. M., 1933. *Proc. Nat. Acad. Sci.*, **19**: 750.
Pye, J. D., 1964. *J. Exp. Biol.*, **41**: 535.
Reed, B. L., and B. C. Finnin, 1972. *In* Pigmentation: Its Genesis and Biologic Control (Riley, V. ed.), 285. Appleton-Century-Crofts, New York.
Scheline, R. R., 1973. *Comp. Biochem. Physiol.*, **9**: 215.
Scott, G. T., 1965. *Limnol. Oceanogr.*, **10**: R230.