J. exp. Biol. (1979), **78**, 47–57
With 5 figures
Printed in Great Britain

FLUORESCENCE AND LUMINESCENCE OF ISOLATED PHOTOPHORES OF *PORICHTHYS*

BY F. BAGUET AND A. M. ZIETZ-NICOLAS

Laboratoire de Physiologie Animale,
Université catholique de Louvain, Belgium

(*Received* 6 *March* 1978)

SUMMARY

1. Isolated photophores of *Porichthys* exposed to u.v. light (365 nm) exhibit a green fluorescence localized in the photocytes and a bluish fluorescence originating from the lens-like body.

2. The luminescence of photophores evoked by epinephrine, nor-epinephrine or potassium cyanide decreases with the intensity of the green fluorescence of the photogenic tissue; the bluish fluorescence of the lens-like body does not change significantly.

3. The total amount of light emitted is a linear function of the decrease of the greenish fluorescence intensity. The slope of the regression line is maximal in response to epinephrine or nor-epinephrine $5 \cdot 10^{-4}$ M and potassium cyanide 10^{-3} M. It decreases significantly for higher concentrations.

4. The decrease of fluorescence observed during the light emission is tentatively explained by oxidation of a luciferin present in a fluorescent form in a fresh photophore.

INTRODUCTION

The photophores of the southern Californian fish, *Porichthys*, emit a greenish fluorescence under u.v. light (Barnes, Case & Tsuji, 1973), and it is assumed that this is correlated with the fish's ability to luminesce, for the following two reasons: (i) photophores of larvae are able to luminesce only after the 28th day, i.e. when they become detectably fluorescent (Anctil, 1977), (ii) photophores of adult *Porichthys* from the Puget Sound, which do not fluoresce, are unable to luminesce (Barnes *et al.* 1973).

In the present work we intended (1) to identify the fluorescent structures inside the isolated photophore and (2) to correlate the fluorescence changes with the luminescence induced by putative neuromediators (epinephrine or nor-epinephrine) or a potent stimulator, potassium cyanide (KCN).

Our results give direct evidences for the presence of two fluorescent structures in the photophore: photocytes, which exhibit a greenish fluorescence, and the lens-like body, which shows a bluish fluorescence. The light production induced by epinephrine, nor-epinephrine and potassium cyanide involves a significant decrease of the green fluorescence which is directly correlated with the total amount of light produced. It is suggested that the green fluorescence present in the photocytes is associated with a luciferin which is transformed into a non-fluorescent metabolite during the light emission.

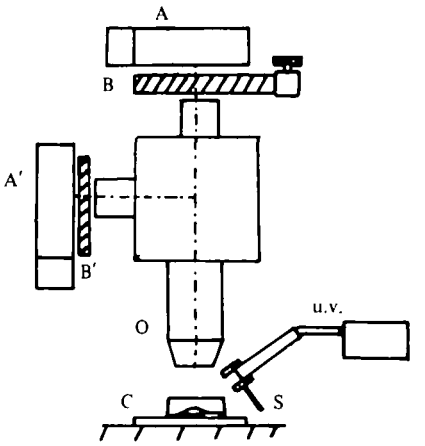

Fig. 1. Basic set-up for recording luminescence and fluorescence. The photophore, held on a glass slide by a perspex ring (C) under the objective of the microscope was exposed to the pen-ray u.v. light source. Luminescence signal is recorded through the 150 AVP photomultiplier (A) equipped with a sliding continuously variable interference filter (B); fluorescence signal is collected on the PM 270 C photomultiplier (A') equipped with a 470 nm interference filter (B'). The u.v. light beam can be stopped by the shutter (S).

MATERIALS AND METHODS

I. *Dissection of the photophores*

Specimens of *Porichthys myriaster* air-shipped by the Bio-Marine supply house Cy (Venice, California) were kept in large tanks (600 l) provided with aerated running sea water from the North Sea (15–17 °C). The animals were fed twice a week on freshly cooked shrimps (*Crangon*).

Fish were anaesthetized by partial immersion in a tray containing sea water and quinaldine (1·5 ml/l sea water) at 18 °C. Following anaesthesia, a strip of skin with 5–7 gastric or pleural photophores, using Greene's terminology (1899) was excised and maintained in a small vessel filled with cold (10 °C) air-saturated saline (Baguet & Case, 1971). After 5–10 min, one photophore was isolated from the strip, and the layer of epidermal cells covering the photophore was dissected out under a microscope using fine scissors and forceps (Dumont no. 5). The 'skinned' photophore was placed with its light emitting area facing upward on a glass slide previously covered with a non-reflecting piece of black tape. In order to prevent any movement of the light organ, the skin surrounding the photophore was gripped by a piece of 1 mm mesh black tulle clamped on the glass slide by a perspex ring (3 mm high and 16 mm in diameter); the ring and the tulle were pasted with silicon grease (Apiezon, L type).

II. *Solutions*

The preparation was immersed in a saline of the following composition: NaCl 231 mM; KCl 8 mM; $CaCl_2$ 2 mM; $MgCl_2$ 2 mM, adjusted to pH 7·3 with Tris buffer (20 mM) at 20 °C. Epinephrine, nor-epinephrine hydrochlorides or potassium cyanide were dissolved in appropriate volumes of saline immediately before use.

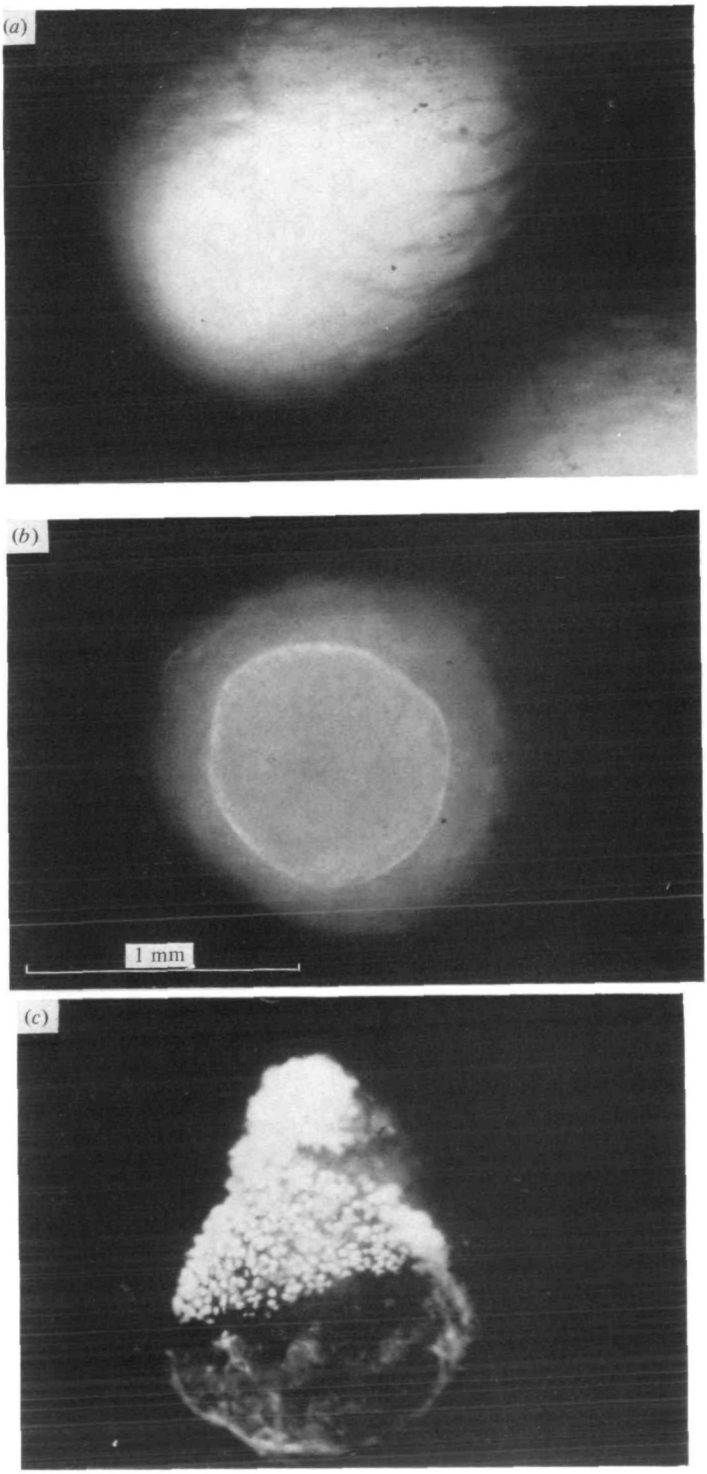

Fig. 2. (*a*) Diffuse greenish fluorescence of a photophore of *Porichthys* exposed to 365 nm u.v. light. (*b*) Same photophore after the removal of the epidermal layer. (*c*) Photocytes and lens-like body drawn out of the light organ. Note the particular arrangement of the photocytes. It is obvious that the green fluorescence is localized in the small ovoid cells corresponding to the photocytes.

III. *Recording fluorescence and luminescence*

The isolated photophore mounted in the small chamber was placed under a Reichert Zetopan microscope equipped with a microphotometer. A pen-ray ultraviolet light source (type II scIL) covered by a phosphor and a G-278 filter selecting the 365 nm wavelength in the emission spectrum was put close to the objective (10×). This overhead illumination of a skinned photophore reduced considerably the absorption of u.v. light by the tissues surrounding the internal structures of the light organ. The highly fluorescent signal obtained from a freshly skinned photophore was collected through the objective and sent simultaneously to the photomultiplier of the microphotometer (150 AVP) equipped with a continuously variable interference filter with selectable diaphragms, and to a second photomultiplier (PM 270 C, International light) through a 470 nm interference filter (Fig. 1). In this case, the apparatus was used as a fluorimeter. When the luminescence signal alone was recorded, the u.v. light source was covered by a black shield. Luminescence was collected through the objective on the 150 AVP photomultiplier without a filter. Owing to the low transmission (20%) of the interference filter, no luminescence signal passed through the 470 nm interference filter. By using this set-up alternatively as a fluorimeter or a photometer, it was possible to follow fluorescence and luminescence signals without interference.

IV. *Calibration of luminescence*

A quantitative estimation of light production was obtained with a tritium irradiated phosphor (Betalight, by Saunders Roe, Nuclear Enterprises Ltd.) of light-emitting area 1·1 mm^2. This circular luminescent area corresponds to that area of photophores isolated from *Porichthys* of medium size, i.e. 25 cm length, used for the present experiments. The spectrum of light emitted by the source shows a peak at 470 nm, corresponding to the peak of the light spectrum observed *in vitro* on the isolated luciferin-luciferase system of *Porichthys* (Cormier, Crane & Nakano, 1967). The source has been calibrated in quanta s^{-1} in the Department of Physics at Princeton University (Dr G. T. Reynolds). For calibration, the light source was in the same location as the photophore.

V. *Calculation of results*

Our results were analysed using conventional statistics; the number of photophores used for the determination of the mean value of a parameter is indicated by n and each mean value is expressed with its standard error (mean ± S.E.M.).

RESULTS

I. *Localization of fluorescent structures*

An isolated photophore exposed to 365 nm light shows, in our experimental conditions, a diffuse greenish fluorescence (Fig. 2*a*). After skinning, i.e. removing the epidermal layer covering the light organ, one can easily distinguish a greenish disc limited by a light bluish fringe standing out against a greenish background (Fig. 2*b*). By pulling the interior of the light organ with fine tungsten needles a translucent mass

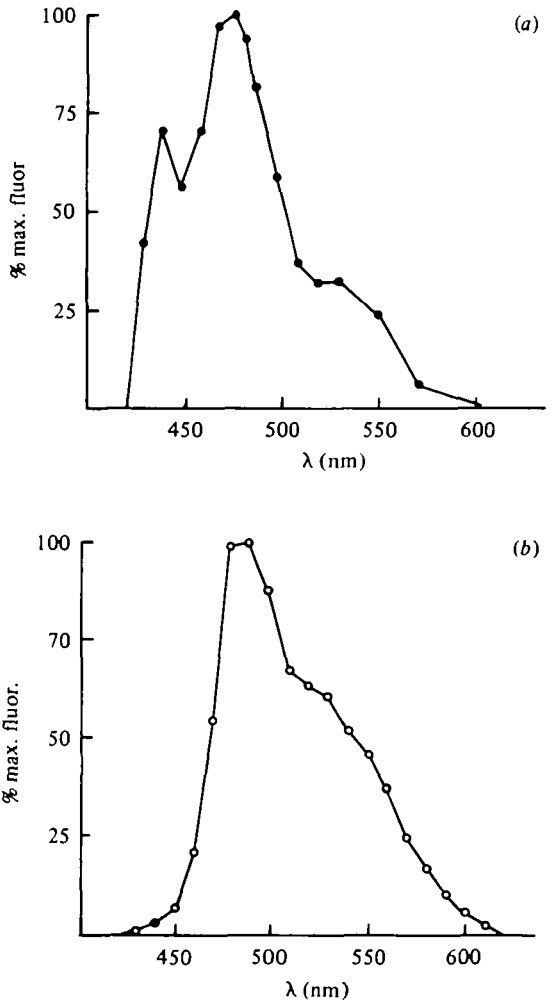

Fig. 3. Comparison of corrected fluorescence spectra of photocytes (*b*) and of the lens-like body (*a*) isolated from the photophore and immersed in saline (20 °C). The fluorescence excitation wavelength is 365 nm.

can be extracted. Excited by 365 nm light, this mass exhibits the typical fluorescent pattern shown in Fig. 2(*c*): a bluish spherical structure half-covered with a cluster of small green ovoid cells that gather together at the top to form a tuft. The corrected fluorescence spectrum of one of these cells shows a peak at 490 nm (Fig. 3*b*); the spectrum measured in any region of the blue sphere shows a first peak of low amplitude at 440 nm and a second of high amplitude at 470 nm (Fig. 3*a*). As the green cells originate from the bottom of the photophore and are overlaid with a spherical structure, we suggest that they correspond respectively to the photocytes and the lens-like body described in histological studies (Strum, 1969).

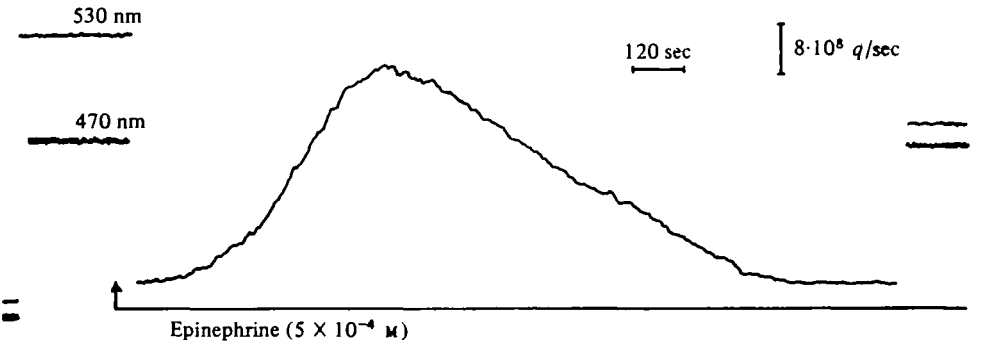

Fig. 4. Original record of the fluorescence of an isolated photophore exposed to 365 nm u.v. light, originating from the photocytes (530 nm, fine trace) and the lens-like body (470, thick trace), before and after the light response to addition of epinephrine $5 \cdot 10^{-4}$ M in saline (arrow).

II. *Fluorescence and luminescence*

(A) *Resting photophores*

Half an hour after the dissection, the skinned photophores in saline (20 °C) are non-luminous and remain so even during exposure to u.v. light (365 nm). The fluorescence level of the light organ measured at 530 nm shows a slow continuous exponential decrease. The time constant for the rate of the exponential decrease calculated on 18 photophores is $189 \cdot 3 \pm 17 \cdot 4$ min. This slight decrease occurs spontaneously without any detectable light emission and does not depend on the fluorescence intensity of the organ measured at the beginning of the u.v. exposure. The intensity of the 470 nm fluorescence signal does not usually change during this period of time.

(B) *Stimulated photophores*

(1) *Epinephrine stimulation.* After following the time course of the fluorescence of a resting photophore for 10 min, epinephrine was introduced into the chamber, the u.v. light source was covered by the shutter, and the luminescence of the photophore recorded.

Fig. 4 shows a typical light emission and the corresponding fluorescence changes measured on an isolated photophore in response to epinephrine $5 \cdot 10^{-4}$ M. In this particular case, the light emission starts 50 s after application of the chemical and reaches a maximal value (L_{max}) 10 min afterwards. Then the luminescence decreases slowly and extinction is complete 25 min after the beginning of the stimulation. The total amount of light produced by the photophore is calculated from the surface area under the light emission curve. The 530 nm fluorescence level measured immediately after the extinction is lower than that measured before the stimulation; on the other hand the 470 nm fluorescent signal does not change. The difference between the intensity of the fluorescence level after the light response and the extrapolated fluorescence level recorded before the stimulation gives a measure of the decrease in fluorescence which occurred during the light emission (ΔF).

Twenty-two photophores were stimulated separately with either one of the three epinephrine concentrations, 10^{-4}, $5 \cdot 10^{-4}$ and $5 \cdot 10^{-3}$ M. The light production is maximal with $5 \cdot 10^{-4}$ M epinephrine: the mean values ($n = 8$) of the three parameters charac-

Table 1. *Mean values* ($\pm$ 1 $\times$ S.E.) *of different parameters of luminescence and fluorescence of isolated photophores in response to epinephrine, nor-epinephrine and potassium cyanide* ($5 \cdot 10^{-4}$ M *for each substance*)

	Epinephrine	Nor-epinephrine	KCN
Total light (10^{10} quanta)*	104·2 ±24·0	102·5 ±21·0	106·0 ±27·0
Duration (min)†	18·8 ± 1·8	15·9 ± 1·5	16·3 ± 1·0
L_{max} (10^7 quanta/s)‡	132·0 ±30·0	156·0 ±30·0	160·0 ±32·0
ΔF (a.u.)§	73·7 ±10·3	51·1 ± 8·6	47·7 ±14·0
F_1 (a.u.)‖	180·0 ±20·0	169·3 ±27·5	168·0 ±36·0
$\Delta F/F_1$	0·40± 0·03	0·30± 0·04	0·33± 0·08
n¶	8	11	8
$b \pm S_b$ (10^{10} quanta/ΔF)**	1·80± 0·50	2·00± 0·50	1·40± 0·40

* Total amount of light produced.
† Time duration of the light response.
‡ Peak intensity of light.
§ Decrease of the green fluorescence level (arbitrary units).
‖ Level of green fluorescence before stimulation (arbitrary units).
¶ Number of photophores.
** Regression coefficient ± standard error.

terizing the light response (peak of light, duration of the light emission and the total amount of light produced) and the corresponding fluorescence changes are summarized in Table 1. The light response lasts 18·8 ± 1·8 min and the total light emitted is 104·2 ± 24·0 10^{10} quanta. At $5 \cdot 10^{-3}$ M, the magnitude and the duration of luminescence are dramatically lowered: the photophore glows for only 7·0 ± 1·1 min and the total amount of light emitted corresponds only to about 10% of that produced at $5 \cdot 10^{-4}$ M epinephrine.

All the photophores studied showed a decrease of their fluorescence level after the light response to any of the different epinephrine concentrations. Fig. 5 shows the total amount of light emitted plotted as a function of the fluorescence decrease (ΔF) for $5 \cdot 10^{-4}$ M epinephrine concentration. Regression analysis shows that there is a significant correlation between the two phenomena. The meaning of b, the slope of the calculated regression line, is straightforward: it gives a quantitative measure of the relation between luminescence and fluorescence decrease. In this case it indicates that for a unitary fluorescence decrease, 1·80 ± 0·50 10^{10} quanta are emitted. This is twice as large as that emitted at 10^{-4} M and nine times as high as that emitted at $5 \cdot 10^{-3}$ M.

(2) *Nor-epinephrine stimulation.* In our experimental conditions, nor-epinephrine prompts the isolated photophore to light up at a lower concentration than epinephrine. At $5 \cdot 10^{-5}$ M, the duration of the light emission is short (3·6 ± 1·3 min) and the magnitude of the peak of light (44·0 ± 12·0 10^7 quanta/s) is low. At $5 \cdot 10^{-4}$ M, nor-epinephrine induces a much more prolonged and much more intense light emission (Table 1): the peak of light, the duration of the response and the total amount of light emitted are similar to that observed in response to epinephrine at the same concentration. The response to $5 \cdot 10^{-3}$ M nor-epinephrine is not significantly different from the response to $5 \cdot 10^{-4}$ M. The light emission recorded in response to three different concentrations of nor-epinephrine is associated with a singificant decrease of the fluorescence intensity of the photophores. The total amount of light emitted during the response is proportional to the decrease of the fluorescence level; the amount of light produced per unit of fluorescence decrease, given by the slope of the regression line for $5 \cdot 10^{-4}$ M nor-

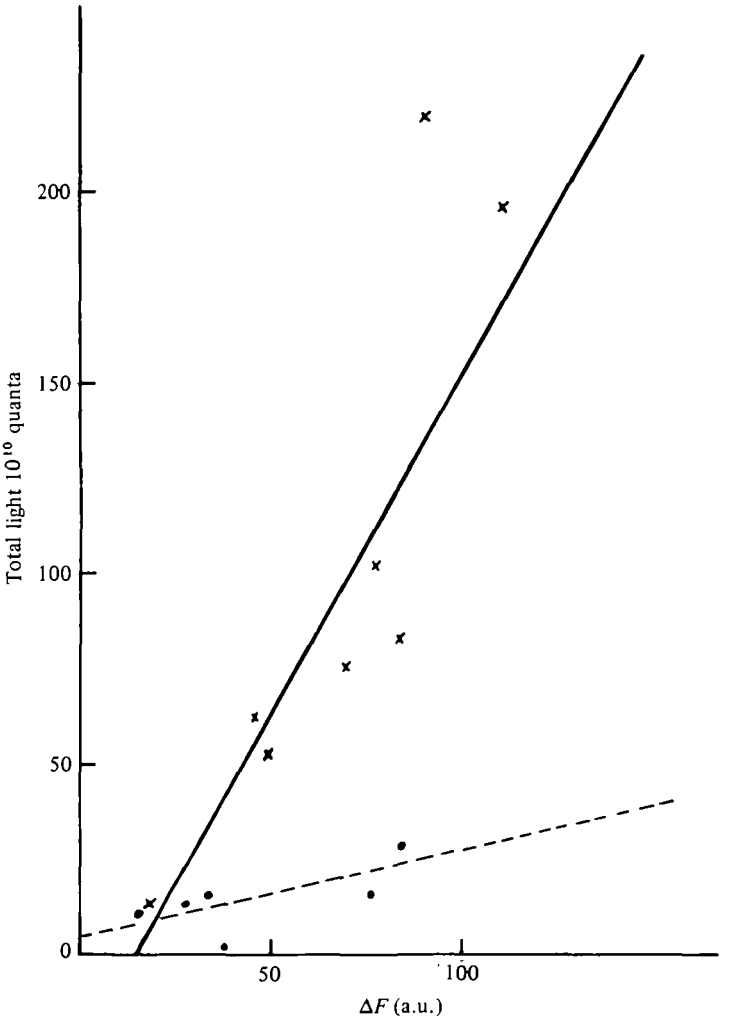

Fig. 5. Relationship between the total amount of light emitted in response to epinephrine $5 \cdot 10^{-3}$ M (●) and $5 \cdot 10^{-4}$ M (×) and the decrease of the fluorescence intensity of the photocytes. In ordinates, total light expressed in 10^{10} quanta; in abscissa, difference of the fluorescence level measured before and after the light response, expressed in arbitrary units (a.u.).

epinephrine, is very similar to that calculated for the response to $5 \cdot 10^{-4}$ M epinephrine (Table 1). At $5 \cdot 10^{-3}$ M, the slope of the regression line is lowered to $1 \cdot 30 \pm 0 \cdot 40$ 10^{10} quanta/ΔF.

It is concluded that the maximum light emission per unit of fluorescence decrease occurs for nor-epinephrine as for epinephrine, at a concentration of $5 \cdot 10^{-4}$ M. On the other hand, the photophores do not exhibit desensitization to nor-epinephrine at high concentration as was observed with epinephrine.

(3) *Potassium cyanide stimulation.* Potassium cyanide (10^{-3} M) has been reported to be a potent luminescent stimulator of isolated *Porichthys* photophores (Baguet, 1975). Anctil & Case (1978) reported that KCN produces ultrastructural damage in nerve terminals synapsing on photocytes and suggested that luminescence is due to a release

of a catecholaminergic transmitter, probably nor-epinephrine. If this is the case, we may expect a response to KCN similar to that obtained with nor-epinephrine.

Thirty-six photophores were stimulated with KCN at three different concentrations, $5 \cdot 10^{-4}$ M, 10^{-3} M and $5 \cdot 10^{-3}$ M. Table 1 shows that at $5 \cdot 10^{-4}$ M-KCN, the magnitude and the duration of the light emission are similar to those recorded in response to epinephrine or nor-epinephrine at concentrations of $5 \cdot 10^{-4}$ M. At 10^{-3} M-KCN, the magnitude of the response is exceptionally high ($580 \pm 120 \times 10^7$ quanta/s) but the duration of luminescence is reduced to $9 \cdot 9 \pm 1 \cdot 2$ min. At $5 \cdot 10^{-3}$ M, the response is as short as at 10^{-3} M but the peak of light and the total amount of light produced are half as high. These results show that the optimal concentration of KCN which induces luminescence is 10^{-3} M. Regarding the fluorescence variations involved in the light production, Table 1 shows that the mean values of the decrease of the green fluorescence level (ΔF) is not significantly different from that measured in response to $5 \cdot 10^{-4}$ M epinephrine or nor-epinephrine. Though the coefficient b of the regression line between luminescence and fluorescence decrease is somewhat lower ($1 \cdot 40 \pm 0 \cdot 40$ 10^{10} quanta/ΔF), it is not significantly different from the slopes calculated for epinephrine or nor-epinephrine. However at high concentration ($5 \cdot 10^{-3}$ M), the slope b is significantly decreased to $0 \cdot 46 \pm 0 \cdot 20$ 10^{10} quanta/ΔF.

DISCUSSION

I. *Fluorescence of resting photophores*

The present results show that the photophores of *Porichthys* contain two types of tissue with different fluorescent properties when irradiated at 365 nm u.v. light: the green fluorescence is limited to the photocytes, whereas the blueish fluorescence originates from the lens-like tissue. The photophores of most of the living deep-sea fish also exhibit two types of fluorescences in the same conditions of u.v. irradiation (Baguet & Nicolas, 1976): generally, one fluorescent mass of cells is located deep in the light organ and covered by the other fluorescent tissue. So far there have only been a few reports concerning the functional significance of these tissues. Ultrastructural studies of the lens-like body reveals that it is composed of a large amount of very thin canalicules (Anctil & Case, 1978) and its function may be to collimate the light emitted in a given direction. On the other hand, it is well established that any light source tested on the isolated lens-like body passes through it without spectral modification (Tsuji *et al.* 1975).

Our results strongly suggest that, in the case of *Porichthys*, the lens-like body and the blue fluorescent substance associated with it are not necessary for light production in an isolated photophore: the blue fluorescence does not change during light emission and moreover its removal does not affect it. We feel that this tissue is an accessory structure associated with the photogenic tissue and does not interfere with the short-dated light reaction. The photocytes, which look like small ovoid cells 30 μm diameter, are filled with a greenish fluorescent substance that is essential for the light production in response to a chemical stimulus, since only fluorescent photophores produce light.

II. *Fluorescence change and light production*

Many bioluminescent systems in invertebrates exhibit *in vitro* specific fluorescent properties when irradiated by u.v. light. When examined *in vivo*, the photogenic cells of luminescent Echinodermata and Polychaeta contain particles that become fluorescent only after the light emission. Brehm & Morin (1977) reported that KCl isotonic to sea water is found to produce intense luminescence in *Ophiopsila californica* and *Amphipholis squamata* (Echinodermata; class Ophiuroidea). The luminescent sites become fluorescent after stimulation, the fluorescence intensity of a site being directly related to the capability of the site to produce light. Living elytra isolated from the polychaete *Acholoe astericola* respond to a train of electrical stimuli by a series of flashes originating from small granules, the photosomes (Bassot & Bilbaut, 1977). The fluorescence intensity of each granule increases after each flash so that the elytra which emitted the largest amount of light show also the most intense fluorescence.

A luciferin-luciferase reaction with oxygen requirement was first demonstrated on the polychaete *Odontosyllis* by Harvey (1952). Shimomura, Johnson & Saiga (1963) have reported that *in vitro*, luciferin prior to reaction with luciferase shows a very weak fluorescence; after its reaction with luciferase, the product is highly fluorescent. These studies suggest that in these invertebrate light-emitting organs an oxidation product of the light reaction may be responsible for the fluorescence properties.

Our experiments demonstrate opposite results in the epipelagic teleostean luminescent fish *Porichthys*, the photocytes exhibiting a strong fluorescence when they are non luminous and any light emission involving a decrease of fluorescence. A similar decrease of fluorescence has recently been observed in photophores of the bathypelagic fish *Argyropelecus hemigymnus* after epinephrine and nor-epinephrine stimulation (Baguet & Marechal, 1978). The biochemical mechanism for luminescence in *Porichthys* and probably in bathypelagic fish consists of a luciferase catalysed oxidation of luciferin by oxygen (Tsuji *et al.* 1971; Tsuji *et al.* 1977). *Porichthys* luciferin appears to be similar, if not identical, to the luciferin of *Cypridina hilgendorfii*, a small marine ostracod crustacean (Cormier *et al.* 1967; Tsuji *et al.* 1971). Since *Cypridina* luciferin fluoresces in the green *in vitro*, whereas oxyluciferin, its oxidized product, fluoresces weakly (Tsuji *et al.* 1975), the decrease of fluorescence observed during the light emission of a living photophore should correspond to the oxidation process of luciferin.

It is clear then that the products of oxidation of the luciferin system in a living photophore of *Porichthys* have fluorescent properties different from those characterizing the luminescent system of *Acholoe*, *Ophiopsila* or *Amphipholis*.

(A) *Response to epinephrine and nor-epinephrine stimulation*

In our experimental conditions, the intensity and the duration of the light response increase with epinephrine and nor-epinephrine concentration up to $5 \cdot 10^{-4}$ M. At higher concentration ($5 \cdot 10^{-3}$ M) the total amount of light emitted in response to epinephrine is reduced about 7 times and the duration of luminescence is halved. On the other hand at $5 \cdot 10^{-3}$ M nor-epinephrine, the light response is not significantly affected. The photophore of *Porichthys* seems to show a selective desensitization to epinephrine at high concentration. In the case of the photophores isolated from the deep-sea luminescent fish *Argyropelecus hemigymnus*, Baguet & Marechal (1978) reported that

for concentrations higher than 10^{-5} M (epinephrine) and 10^{-4} M (nor-epinephrine), both neuromediators inhibit luminescence. At lower concentrations both are potent stimulators. Thus, the adrenergic receptors of the light organs from *Porichthys* and *Argyropelecus* have probably different specific properties. The green fluorescence originating from the photocytes of *Porichthys* photophores decreases in intensity, in proportion to the amount of light produced with the different concentrations of epinephrine and nor-epinephrine tested. Assuming that the decrease of fluorescence corresponds to the oxidation of luciferin present in the photocyte, the value of the slope b of the regression line between fluorescence and light emitted should characterize quantitatively the net amount of luciferin involved in the light emission. The value of b can thus be taken as an index of the efficiency of the light emission. In this case, in response to $5 \cdot 10^{-4}$ M epinephrine or nor-epinephrine, the values of b are respectively $1 \cdot 80 \pm 0 \cdot 50$ and $2 \cdot 00 \pm 0 \cdot 50$ 10^{10} quanta/ΔF and it is concluded that the efficiency of the light emission is similar in both cases. At higher concentrations of both neuromediators ($5 \cdot 10^{-3}$ M) the efficiency of the light emission is lowered, especially in response to nor-epinephrine, since the value of b is only $0 \cdot 20 \pm 0 \cdot 07$ 10^{10} quanta/ΔF. One way to explain this effect is to suppose that nor-epinephrine at high concentration induces oxidation of luciferin without luminescence. We have recently observed that oxidizing substances applied to the isolated photophore induce a spontaneous decrease of fluorescence without any light emission. However the lack of knowledge concerning the pathways of luciferin metabolism cautions against any rigorous interpretation.

(B) *Response to potassium cyanide stimulation*

The optimal concentration of KCN necessary to evoke a bright glowing of an isolated photophore of *Porichthys* is 10^{-3} M: the amplitude of the light emission which lasts about 10 min is exceptionally high, three times as high as that measured at the two other concentrations.

Luminescent glow of a light organ upon application of potassium cyanide is an unusual but not exceptional feature among bioluminescent systems: 10^{-3} M-KCN applied to the marine fireworm *Odontosyllis* causes spectacular increases in the intensity of emitted light (Shimomura, Beers & Johnson, 1964). The authors reported that cyanide activates the luminescent reaction in extracts containing luciferin and luciferase, crude or partially purified. No information is available concerning a possible effect of KCN on the luciferin-luciferase system of *Porichthys* photophores *in vitro*. In a study devoted to ultrastructural correlates of luminescence in *Porichthys* photophores Anctil & Case (1978) provide evidence that KCN alters nerve endings in contact with the photocytes. These authors suggest that KCN induces luminescence by altering the synapses and liberating a neuromediator, probably nor-epinephrine (Anctil & Case, 1978), that lights up the photophore. Our results provide evidence that the relationship between luminescence and fluorescence decrease in response to $5 \cdot 10^{-4}$ M or 10^{-3} M-KCN is similar to that measured in response to a direct application of $5 \cdot 10^{-4}$ M epinephrine or nor-epinephrine. However at $5 \cdot 10^{-3}$ M-KCN, the magnitude of the light emission decreases just as is observed in response to a direct application of epinephrine. Although these results are not incompatible with the assumption that there is an indirect effect of KCN via the nerve terminals, they do not rule out the possibility of a direct non-specific stimulatory effect on the photocytes.

REFERENCES

ANCTIL, M. (1977). Development of bioluminescence and photophores in the midshipman fish, *Porichthys notatus*. *J. Morph.* **151**, 363–396.

ANCTIL, M. & CASE, J. F. (1978). Ultrastructural correlates of luminescence in *Porichthys* photophores. II. Effects of metabolic inhibitors. *Cell. Tiss. Res.* (in the Press).

BAGUET, F. & CASE, J. (1971). Luminescence control in *Porichthys* (Teleostei): excitation of isolated photophores. *Biol. Bull.* **140**, 15–27.

BAGUET, F. (1975). Excitation and control of isolated photophores of luminous fishes. *Prog. Neurobiol.* **5**, 97–125.

BAGUET, F. & NICOLAS, A. M. (1976). Fluorescence properties of isolated photophores during the light emission induced by chemical stimulation. *Abst. Int. Cong. Photobiol.* p. 323.

BAGUET, F. & MARECHAL, G. (1978). Luminescence of photophores of *Argyropelecus hemigymnus* stimulated by epinephrine and nor-epinephrine. *Comp. Biochem. Physiol.* (in the Press).

BARNES, A. T., CASE, J. F. & TSUJI, F. I. (1973). Induction of bioluminescence in a luciferin deficient form of the marine teleost, *Porichthys*, in response to exogenous luciferin. *Comp. Biochem. Physiol.* **46A**, 709–723.

BASSOT, J. M. & BILBAUT, A. (1977). Bioluminescence des élytres d'Acholoe. IV. Luminescence et fluorescence des photosomes. *Rev. Biol. Cell.* **28**, 163–168.

BREHM, P. & MORIN, J. (1977). Localization and characterization of luminescent cells in *Ophiopsila californica* and *Amphipholis squamata* (Echinodermata: Ophiuroidea). *Bio. Bull.* **152**, 12–25.

CORMIER, M. J., CRANE, J. M. & NAKANO, Y. (1967). Evidence for the identity of the luminescent system of *Porichthys porosissimus* (fish) and *Cypridina hilgendorfii* (Crustacean). *Biochem. Biophys. Res. Commun.* **29**, 747–752.

GREENE, C. W. (1899). The phosphorescent organs in the toad fish *Porichthys notatus* Girard. *J. Morph.* **15**, 667–696.

HARVEY, E. N. (1952). *Bioluminescence.* New York: Academic Press.

SHIMOMURA, O., JOHNSON, F. H. & SAIGA, Y. (1963). Partial purification and properties of the *Odontosyllis* luminescence system. *J. cell. comp. Physiol.* **61**, 275–292.

SHIMOMURA, O., BEERS, T. R. & JOHNSON, F. H. (1964). The cyanide activation of *Odontosyllis* luminescence. *J. cell. comp. Physiol.* **64**, 15–21.

STRUM, J. M. (1969). Fine structure of the dermal luminescent organs, photophores, in the fish, *Porichthys notatus*. *Anat. Rec.* **164**, 433–462.

TSUJI, F. I., HANEDA, Y., LYNCH, R. V. & SUGIYAMA, N. (1971). Luminescence cross-reactions of *Porichthys* luciferin and theories on the origin of luciferin in some shallow-water fishes. *Comp. Biochem. Physiol.* **40A**, 163–179.

TSUJI, F. I., NAFPAKTITIS, B. G., GOTO, T., CORMIER, J. M., WAMPLER, J. E. & ANDERSON, J. M. (1975). Spectral characteristics of the bioluminescence induced in the marine fish, *Porichthys notatus*, by *Cypridina* (Ostracod) luciferin. *Mol. Cell. Biochem.* **9**, 3–8.

TSUJI, F. I., DELUCA, M., BOYER, P. D., ENDO, S. & AKUTAGAWA, M. (1977). Mechanism of the enzyme-catalyzed oxidation of cypridina and firefly luciferins studied by means of $^{17}O_2$ and $H_2{}^{18}O$. *Biochem. Biophys. Res. Commun.* **74**, 606–613.