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LUMINESCENCE OF *ARGYROPELECUS* PHOTOPHORES ELECTRICALLY STIMULATED

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Abstract—1. Isolated photophores of the living bathypelagic fish *Argyropelecus hemigymnus* respond to a strong (25–80 V) and long (4–15 msec) electrical stimulus by a brief flash emission (F response).

2. Weaker and shorter stimuli are subthreshold, but when repeated at a frequency equal to or higher than one per second, the photophores respond by a slow light emission (S response); it is characterized by a long emission latency time, at least 2 sec duration and by a progressive increase in the rate of light production during the stimulation period. The maximal amplitude of the response is more than 300 times lower than the response of the photophore of the epipelagic luminescent fish Porichthys.

3. A train of strong (35 V) and long stimuli (4 msec) applied at high frequency (100/sec) quench temporarily the luminescence of spontaneous luminescent photophores. The inhibitory response is reversible: after the end of stimulation, the light production returns to the level recorded before the stimulation.

4. The inhibitory response to electrical stimulation is tentatively explained either by the presence of an inhibitory nervous system or by the liberation of an excess of adrenalin, the possible natural neuromediator that inhibits luminescence of isolated photophores at high concentration.

INTRODUCTION

In the first study of the light responses of isolated photophores to electrical stimuli in bathypelagic fish, Baguet & Maréchal (1976) summarized their findings as follows. Photophores of *Argyropelecus* and of *Chauliodus* (called F photophores) produce brief flashes which fatigue rapidly but never fuse. Some differences were seen between flashes of *Argyropelecus* (type Fa) and *Chauliodus* (type Fb), but the dominant fact was that the flashes lasted a few milliseconds only, not much longer than an action potential. For this reason the term "lumispike" was coined to designate the F response.

One of us showed that other photophores, the type of which is found in *Porichthys*, produce a slow and low light production. These photophores were designated S photophores. Their light responses are very similar to those of the twitch and the tetanus of striated muscles, for this reason the terms "lumitwitch" (abbreviated in "litch") and "luminus" were coined to designate respectively the fused and the unfused light responses of S photophores.

Further studies have shown that these descriptions are substantially correct, but that the photophores of *Chauliodus* are much more different from those of *Argyropelecus* than was thought by these authors. A detailed study of the electrical excitability of *Chauliodus* photophores has already been published (Christophe *et al.*, 1979). In the present paper we report a detailed study of the electrical excitability of *Argyropelecus* photophores. The findings have led to slight redefinition of the above-mentioned terms: *Argyropelecus* photophores produce lumispikes when stimulated by strong electrical currents, but produce luminuses when stimulated by weak electrical currents. Contrary to *Chauliodus*, they never produce litches (i.e. brief flashes which can fuse into a luminus) even if they are stimulated with weak stimuli.

METHODS

1. *Collection of fish*

Specimen of *Argyropelecus hemigymnus* Cocco were either captured in a handnet from the shallow waters of the Strait of Messina or collected along the shores of the Sicilian coast near Torre di Faro (Baguet & Marechal, 1976; Baguet & Marechal, 1978). They were brought to the laboratory (Stazione di Idrobiologia, Ganzirri) in cooled seawater, stored in a refrigerator at 7°C and used the same day. It was impractical to restrict the study to fish which still moved in the aquarium, their number being too small. We have therefore included in this study fish which did not swim anymore at the time they were taken for dissection. As it was important to use animals which were as fresh as possible, we have discarded the fish which lacked any of the three characters which have shown to disappear rapidly after death (Baguet & Marechal, 1978). The experiments were made in January 1977, February 1977 and January 1978.

2. *Dissection of the photophores and electrical stimulation*

The ventral photophores of 40 fish have been dissected as described in Baguet & Marechal (1978). The strips thus obtained contained 24 photophores, 12 on either side. Two L-shaped platinum electrodes (diameter: 50 μm) were laid at each end of the strip and connected to a square pulse constant voltage stimulator (Janssen JSI0198).

The electrical stimulation presented two problems. The first stemmed from the low excitability of the organs, especially for a single stimulus. The strong current which had to be used polarized the electrodes and led to rapid inexci-

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tability. The second problem was that a current flowing from the rear of the organ to its front (i.e. when the front electrode was negative) was much more effective than a current flowing in the reversed direction (i.e. when the front electrode was positive): the former was called "cathodic" and the later was called "anodic" stimulation. The problems were partly solved by the use of a composite stimulus: the square pulse of cathodic current was followed at an interval of 1 msec by a square pulse of anodic current, both pulses having the same intensity and duration. In this paper a "stimulus" means a "double shock" of that type, the parameters (voltage–duration) being given for the first pulse only. No influence was noted if the first pulse of the double shock was anodic and the second one was cathodic. Except for a more rapid exhaustion, cathodic pulses had the same effects than "double shocks".

3. *Measurement of the light emission*

The preparation was transferred to a small Perspex chamber immediately after dissection and laid perpendicularly to a photomultiplier PM 270 (international light) in such a way that the emitting area of the photophores faced the photocathode at a distance of 2.0 cm. The light sensitivity was calibrated with a standard light source (betalight, peaking at 470 nm) held at the place of the tissue. The response was recorded on a 20 cm flat-bed recorder (Servogor) which had its full scale response within 0.30 sec. In some experiments the signals were displayed on a Tektronix 5100 storage oscilloscope and recorded with a Polaroid camera.

The light source has been calibrated in quanta/sec 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.

RESULTS

1. *The F and S responses*

Photophores of *Argyropelecus hemigymnus* respond to electrical stimulation either by a brief flash (fast response, F) or by a steady glow (slow response, S). The two types of response have been obtained on the same organ and differ by many properties, among which the parameters of the electrical stimulation which trigger them are most important in practice, since they are easily controlled by the experimenter who can at will produce either one of these responses.

The F response is obtained when the stimulus is strong (25–80 V) and long (4–15 msec); these two parameters are equivalent in the sense that the same increase of the flash intensity can be obtained either by an increase of the voltage or by an increase in the duration. If the stimulus is stronger than 80 V or longer than 16 msec, the organ is irreversibly damaged and has lost it ability to give any response (F and S). If the stimulus is weaker than 25 V or shorter than 4 msec, there is no response.

The flash begins 3–5 msec after the stimulus, peaks 0.5 to 3 msec later and extinguishes in 4–7 msec. It has been described for the first time by Baguet & Marechal, 1976 (cf. their Fig. 2).

When the stimulus is repeated at 1/sec (Fig. 1a), the flashes vary in size, being at times stronger than 50 Mquanta/sec and at times so weak as to be barely detectable (less than 5 Mquanta/sec). The flashes never fuse as the extinction of each of them is complete in a few milliseconds. Less than 20 msec after the last stimulus the only glow remaining is that which was observed in the unstimulated fresh photophores; this basal glow is lower than 10 Mquanta/sec (Baguet & Maréchal, 1978). In most preparations, the basal glow increases steadily during the experiment (cf. below). However it has been impossible up to now to ascertain whether the increase of the basal glow is a late result of F (or S) stimulations.

The S response is obtained only if the stimulus is weak (5–20 V) and short (1–8 msec). Such a stimulus is subthreshold and does not induce neither an F nor an S response if it is isolated; however if it is repeated at a frequency equal to or higher than one per second, the S response appears slowly, while the F response is never observed. The S response is thus slow and obtained by summation of subthreshold stimuli. Figure 1b shows a typical example. The organ is stimulated by weak stimuli (10 V, 1 msec) at 1/sec. The first 30 stimuli are ineffective. After this long latency, the light production increases slowly during the next 240 stimuli. A few seconds after the last stimulus, it peaks at 10.8 Mquanta/sec, a value not much larger than a fifth of many F responses. Then it decreases slowly, requiring more than 2 min to be extinguished. This slow rate of extinction is a further characteristic which differentiates markedly the S response from the F response.

A second train of stimuli immediately after extinction of an S response induce a normal S response without potentiation. If it is delivered before the light response is completed, the light responses summate.

2. *The effect of the frequency of stimulation on the F and S responses*

The effect of an increase of the stimulus frequency is markedly different on F and S responses.

F responses. The flashes remain discrete when the frequency of stimulation is increased from 1 to 2, 4 or 10/sec. The responses are very similar to those shown in Fig. 1a. When the stimuli are prolonged for several seconds, the flashes decrease progressively; as might be expected, the rate of fatigue is faster the higher the stimulus frequency (Baguet & Maréchal, 1976). For some "threshold" frequency between 10 and 20/sec, the response stops altogether. The existence of a recovery process is easy to demonstrate. For example, if a preparation is stimulated 10 sec at 10/sec (65 V, 8 msec) it responds with a series of flashes similar to those shown in Fig. 1a. If this train is repeated at 5 min intervals, no obvious changes are observed. If this train is repeated at 60 sec intervals, the intensity of the flashes decreases markedly during the train of stimuli, this effect being the more noticeable the more the train of stimuli is repeated.

S responses. An increase of the stimulation frequency has three effects: (a) shortening of the latency; (b) acceleration of the light production; (c) increase of the light maximum.

In Fig. 2, the light intensity of S responses and their corresponding emission latency times are plotted as a function of the stimulation frequency. Each point corresponds to a mean value calculated on 4 different preparations, each being stimulated at 2 min intervals during 10 sec at five different frequencies (stimuli, 10 V and 1 msec duration). The magnitude of the light response increases with the frequency. At 4/sec, the response is characterized by a long emission latency time (7.7 ± 1.9 sec, SEM) and a low rate of light

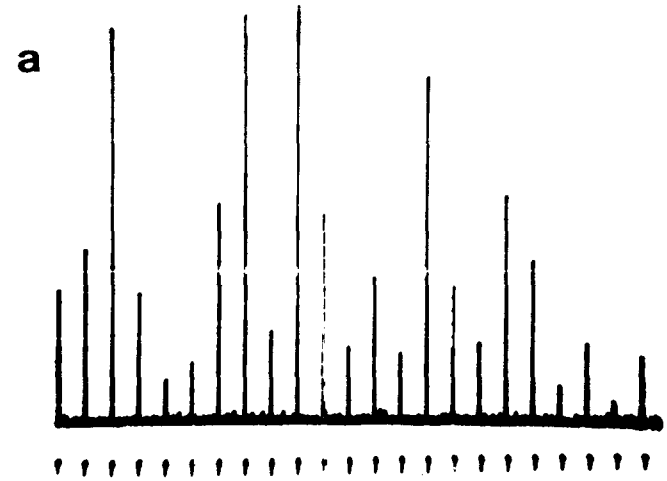

Fig. 1a. F response. The organ is stimulated with 25 V, 8 msec at 1/sec for 23 sec. The flashes are displayed on an oscilloscope as vertical lines, the height of which being equal to the maximal intensity of the flash. This is because the flash is very short relative to the sweep rate (0.5 cm/sec). The stimuli are signaled on the lower trace. Vertical bar 20 Mquanta/sec.

emission (4.3 ± 0.8 Mquanta/sec at peak). At 8/sec, the emission latency time decreases significantly to 3.3 ± 0.2 sec and the amplitude of the light response is about 3 times higher (10.7 ± 3.8 Mquanta/sec). At 20/sec, the latency time is shorter than that observed at 8/sec (2.3 ± 0.3 sec) and the amplitude of the light emission is twice as high. At 50/sec, though the amplitude of the response is twice as high as that measured at 20/sec, the emission latency time is similar (2.1 ± 0.2 sec). At the high frequency of 100 stimuli per second, the amplitude reaches the highest value (52.7 ± 6.1 Mquanta/sec), however the emission latency time is not significantly different from that measured at 20 or 50/sec.

3. *Kinetic of the extinction*

After a train of stimuli (10 V, 1 msec) applied at a frequency ranging from 1 to 100 per second, a characteristically long extinction latency time (3–5 sec) always occurs between the last stimulus and the first detectable decrease of the light level of the S response. After this substantial extinction latency time, the luminescence time course looks like one of the curves shown in Figs 3a, b, c.

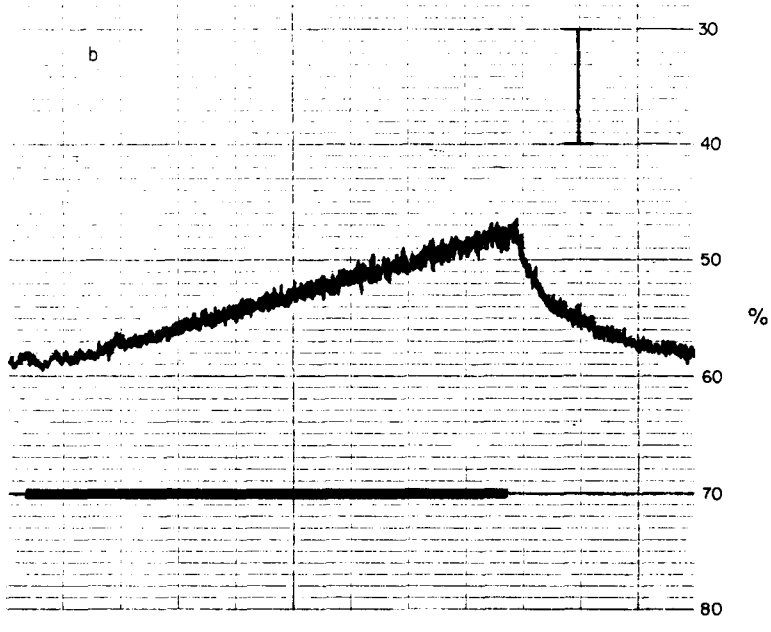

Fig. 1b. S response. The organ is stimulated with 10 V, 1 msec at 4/sec for 450 sec. The stimuli are shown on the lower trace. Note that the scales are different in a and b. Control exeperiments have shown that S and F responses do not depend on the type of the stimulus (cathodic, anodic or "double shock"; see Method). Vertical bar: 9 Mquanta/sec.

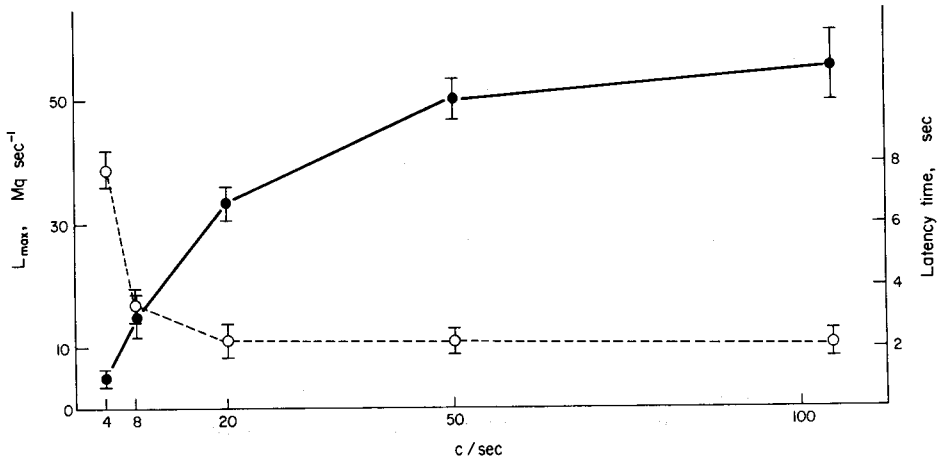

Fig. 2. Influence of the frequency of weak and short stimuli (10 V, 1 msec, 10 sec) on the light maximum (●) and the emission latency time (○) of the S response. Mean values with standard error of mean, calculated on four preparations.

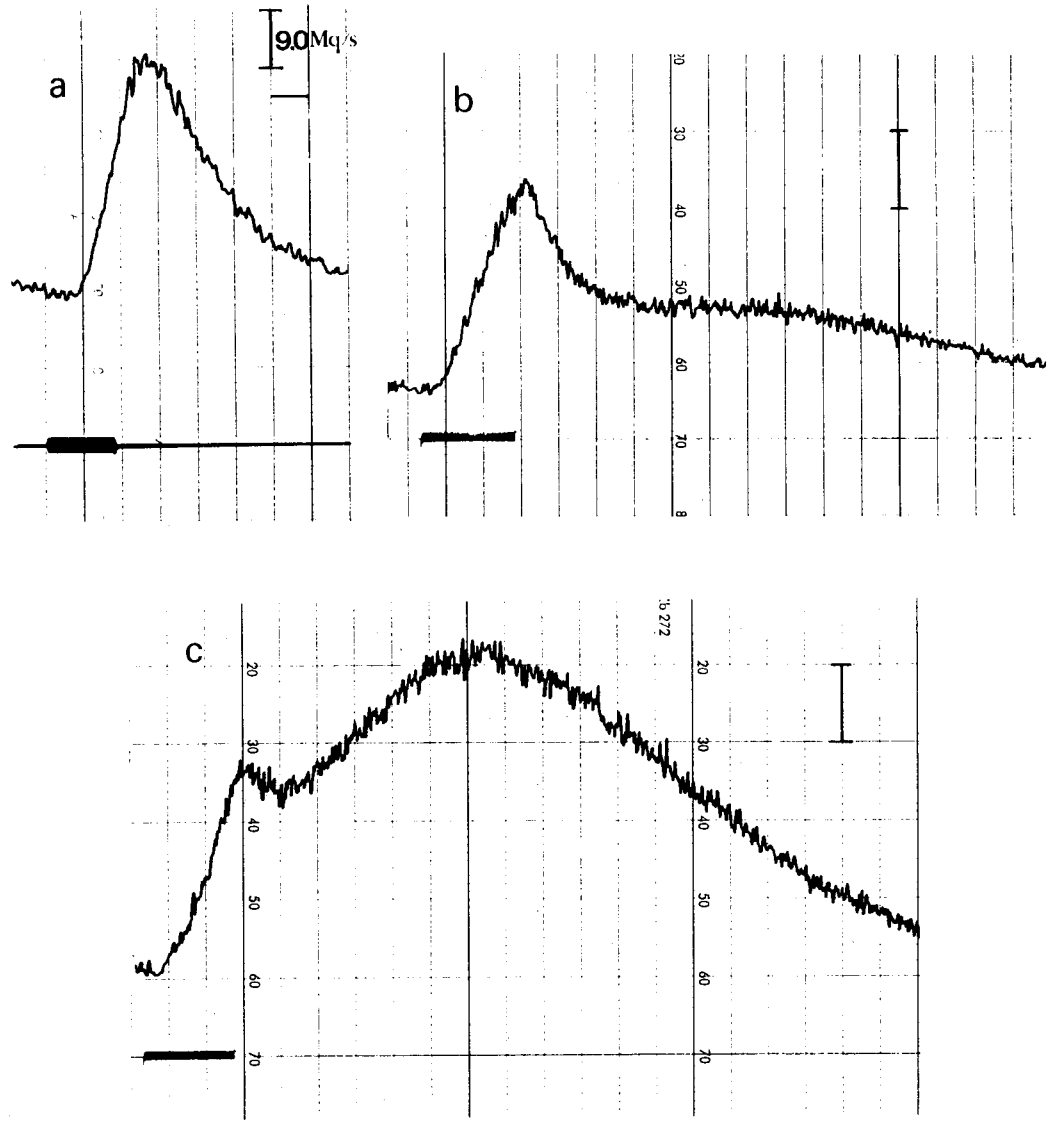

Fig. 3. Kinetic of the S response from three different preparations stimulated by a 5 sec train of stimuli (10 V, 1 msec, 10/sec). Vertical bar: 9 Mquanta/sec.

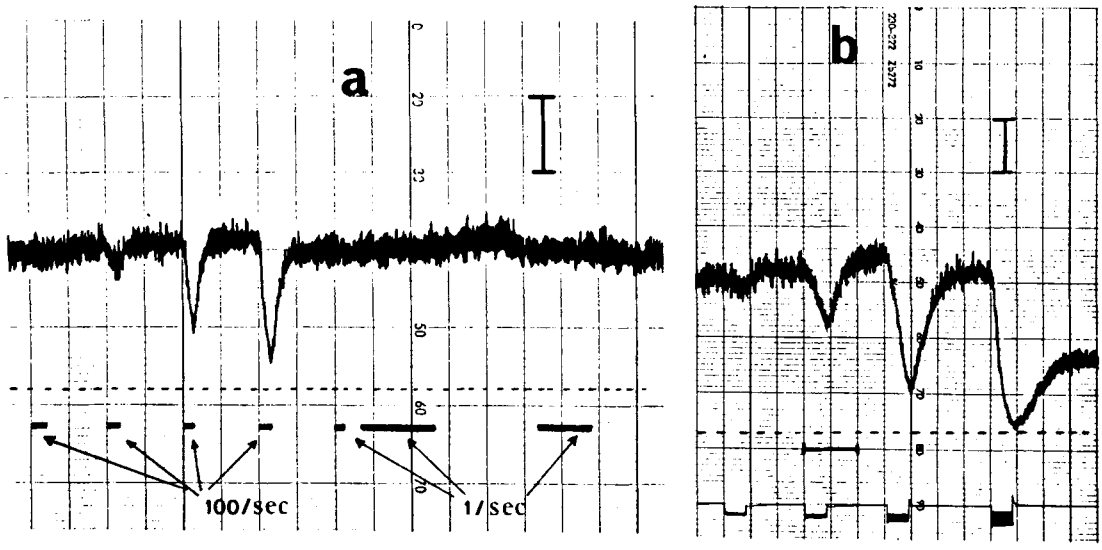

Fig. 4. A glowing preparation stimulated by trains of stimuli of different strength (a) and different duration (b); the horizontal bars correspond to the stimulation period. (a) Stimuli of 1 msec duration, 100 sec, have no effect when the strength is 25 V (first bar). The decrease of the light level is more marked when the strength is raised successively to 35, 40 and 45 V. The same stimuli (45 V) applied at 1 sec have no effects. (b) Glowing inhibition phenomenon induced by four trains of stimuli (35 V, 100/sec); the duration of the stimuli increases from 1 to 4 msec, from the first to the fourth stimulation. Vertical bar: 9 Mquanta/sec.

In Fig. 3a, the light production decreases progressively and extinction is complete in about 20 sec. In some photophores, extinction presents two distinct phases as shown in Fig. 3b: after a rapid extinction period, lasting 5–10 sec, the slope of the curve changes suddenly and the extinction rate decreases appreciably. In those photophores complete extinction lasts 60–80 sec. In other cases, after the first phase of extinction, the light production increases again and a second peak of light, much longer than the first, appears about 30 sec after the end of the stimulation. In this case, complete extinction can require 4–5 min (Fig. 3c).

The kinetics of the extinction phase suggest a classification of the photophores into two categories: *photophores I* that exhibit a simple extinction curve as illustrated in Fig. 3a and *photophores II* corresponding to those presented in Fig. 3b, c. Stimulation seems to trigger two light phenomena: one rapid light emission that is maximal at the end of the stimulation and one delayed that reaches a maximal value during the extinction phase of the first light phenomenon. Thus, photophores I, lack the delayed light emission. On the other hand, in photophores II, the maximal amplitude of the delayed luminescence can be so large that the light production continues to increase for several seconds after the end of the stimulation period. The magnitude of the second peak of light varies widely among the preparations and is sometimes higher than the first peak of light.

4. *Inhibition of light emission by a train of stimuli*

In four series of photophores isolated from four different Argyropelecus, stimuli of low strength (1–15 V) and short duration (1 msec) of different frequencies did not induce an S response but they began to glow slowly and progressively after the end of stimulation. After four trains of stimuli repeated at 60 sec interval, the light production can be as high as that measured at the peak of an S response. However, a train of strong and long stimuli at high frequency, quench temporarily the luminescence (Fig. 4a): at 100 sec, 25 V, 1 msec a train of 10 sec does not modify the glow, but it depresses it if the strength of the stimuli is increased (compare the effects of the four trains of stimuli in the left par of Fig. 4a). After the end of stimulation, the light production returns to the level recorded before the stimulation. On the other hand, a low frequency stimulation (1/sec) is without effect whatever its duration or its strength (compare the three trains of stimuli in the right hand part of Fig. 4a). Thus, a glowing preparation can be quenched by this stimulation at high frequency but not by a stimulation at low frequency.

Figure 4b shows the effects on the same preparation of increasing the duration (1, 2, 3 and 4 msec) of inhibitory stimuli (4 trains of 35 V, 100/sec at 10 sec interval).

The amplitude of the extinction increases with the duration of stimuli. The inhibition is completely reversible as long as the duration of the stimuli does not exceed 2 msec. As a matter of fact, after the third stimulation the light production returns to about 85% of that measured before the first stimulation; after the fourth stimulation the recovery is less than 50%. However in both cases the rate of recovery is similar, the light production being stabilized 20 sec after the end of stimulation.

DISCUSSION

Photophores from *Argyropelecus* collected in January from the strait of Messina do not luminesce when isolated from the fish and immersed in saline at room temperature (15°C). Their electrical stimulation

evokes two types of light emission, called F and S responses, depending on the parameters of the electrical stimuli.

1. *The F response*

The F response is a brief flash produced by a photophore stimulated with a cathodic stimulus of high strength (25 V at least) and long duration (more than 4 msec). This type of response has also been reported recently for the photophores isolated from the bathypelagic fish *Chauliodus sloanei* (Christophe & Coll, 1979). However the flash of *Argyropelecus photophores* differs from that of Chauliodus in two respects:

(i) In *Chauliodus*, the flash is evoked by an anodic pulse which must last at least 8 msec, whilst in *Argyropelecus* the flash is emitted in response to a cathodic pulse that lasts only 4 msec.

(ii) In *Chauliodus* the flashes last at least 60 sec, as long as the interval between stimuli does not exceed 500 msec. On the other hand, *Argyropelecus* photophores stimulated in the same conditions never flash longer than 5–10 sec.

In these two bathypelagic fishes the magnitude of the flashes emitted *in vitro* ranges from 3.3 to 3.7 Mquanta/sec. Though both preparations share the property to flash, the mechanism controlling the production of light appears to have different properties of excitability. The rapid fatigue of *Argyropelecus* photophores cannot be explained by a rapid exhaustion of some substrate involved in the luminescent reactions because isolated photophores stimulated by epinephrine can glow during 3–5 hr at an intensity higher than 3 Mquanta/sec (Baguet & Marechal, 1978). On the other hand, it is possible that the activation of the mechanism that triggers the luminescent reactions by electrical current, is progressively inhibited when the application of the electrical stimulus is prolonged. In this hypothesis the fatigue phenomenon is to be explained by some deterioration of the coupling mechanism between excitation and intracellular luminescent reactions.

In the epipelagic luminescent fish, *Porichthys*, flashing of photophores requires stimuli of high strength (30 V) and long duration (7 msec): the usual response to a low strength electrical stimulation is a slow luminescence similar to the S response of *Argyropelecus* photophores.

2. *The S response*

Light production. When stimuli which are subthreshold, singly, that is stimuli of short duration (1 msec) and low strength (10 V) are repeated at a frequency of at least 1/sec, a slow light emission is elicited. This luminescence, called "S response" is characterized by a long emission latency time, at least two seconds duration, and by a progressive increase in the rate of light production during stimulation periods as long as 20 sec. A similar "S response" is also observed on *Porichthys* photophores stimulated by trains of short duration and low strength; however the "S response" of *Porichthys* photophores differs somewhat from the response of *Argyropelecus* photophores:

(i) In *Porichthys* the stimuli must be applied at a frequency of 5/sec, at least, to evoke the light response, instead of 1/sec in *Argyropelecus.*

(ii) The maximal amplitude of the light response measured after a train of 20 sec is as high as 10^3 Mquanta per sec and per photophore in *Porichthys*, whilst it is only about 3 Mquanta per sec in *Argyropelecus.*

(iii) In *Argyropelecus*, the "S response" is not potentiated by successive stimulations. On the contrary, in *Porichthys*, the light response can be 4–5 times increased by four successive stimulations applied at 60 sec intervals (Baguet, 1975). Baguet & Maréchal (1976) proposed to call such a fused luminescent response a "luminus" by analogy with a "tetanus", the fused contractile response of a striated muscle. However, in this sense, a luminus supposes the fusion by summation of the individual responses evoked by each electrical stimulus, the fusion increasing with the frequency of stimuli. Thus is not the case in *Argyropelecus* or *Porichthys* photophores where no individual responses are detectable at any frequency tested.

The knowledge of the responsiveness of *Argyropelecus*, *Chauliodus* and *Porichthys* photophores to trains of stimuli has now advanced so far that we can propose a definition and a classification of luminuses.

A luminus is a sustained light production obtained by summation of electrical stimuli. Two varieties of luminuses are known:

1. Q type: a luminus obtained by fusion of individual lumitwitches (Baguet & Maréchal, 1976). Induced by low voltage and short duration stimuli.

2. S type: a luminus obtained by summation of subthreshold electrical stimuli.

Chauliodus photophores produce luminuses of Q type, while *Argyropelecus* and *Porichthys* photophores produce luminuses of S type. Recently, we observed that *Myctophum* photophores also produce S type luminuses (in preparation).

Extinction phase. There is a long delay, on the order of 2 sec, between the last stimulus and the beginning of the light extinction. Two types of photophores can be recognized by their extinction rate: those of type I that are characterized by a rapid extinction taking about 20–30 sec, and those of type II showing after a short extinction period of about 5 sec a spontaneous secondary production of light.

In *Porichthys* photophores, with a train of stimuli longer than 10 msec, spontaneous glowing also occurred after the light response: however after such a behaviour these photophores usually do not longer respond to electrical or chemical stimulation (Baguet & Case, 1971). In *Argyropelecus* photophores, when the spontaneous glowing is present, it does not affect the responsiveness to a next train of stimuli. It is possible to modify the intensity of this second luminous peak; for example, after treatment with propranolol, a β-adrenergic blocking factor, it is possible either to increase the second luminous peak or in the case of photophores of type I to induce the development of this secondary production of light.

3. *The control mechanism of the light response*

The excitatory mechanism. In a pharmacological study on isolated photophores of *Argyropelecus*,

Baguet & Maréchal (1978) have reported that the light organs are very sensitive to epinephrine and respond to the neuromediator by a long-lasting luminescence. This suggests that the ventral photophores are innervated by an excitatory nervous system. Our results suggest that this efferent nervous system has a low threshold to electrical stimulation: repetitive stimuli of low strength and short duration stimulate nervous terminals and the liberation of the excitatory neuromediator, presumably epinephrine, inducing luminescence of the photophores. After the stimulation, the extinction corresponds to a spontaneous, passive deactivation of the photophore at a rate limited by the rate of hydrolysis or uptake of the neuromediator liberated. When, for some unknown reason, the activity of the excitatory system is disturbed, the photophore lights up spontaneously and can maintain a high luminescence during at least 4 hr. This glow can be temporarily inhibited by appropriate electrical stimuli.

The inhibitory mechanism. There are three possible mechanisms to explain the light inhibition:

(i) Inhibitory nervous mechanism with a higher threshold to electrical stimulation than the excitatory nervous mechanism: present evidences show that inhibition of glowing photophores occurs only on application of train of stimuli of at least 5 msec duration, 25 V strength, at the frequency of 100/sec. Further experiments are necessary to investigate the nature of a possible inhibitory neurotransmitter; we know that acetylcholine does not stimulate isolated photophores (unpublished results) but we do not know how far acetylcholine might inhibit luminescence.

(ii) Electrical stimulation of the photocytes membrane. Assuming that the cellular membrane of the photocyte is the support of events coupling the extracellular excitation to the intracellular luminescent reactions, it is conceivable that luminescence is controlled by membrane depolarisation. It is possible that appropriate stimuli can temporarily hyperpolarize the photocytes membranes and consequently inhibit the membrane events controlling the light reactions.

(iii) Excess of excitatory neuromediator. Baguet & Maréchal (1978), reported that epinephrine at concentrations higher than 10^{-5} M inhibits luminescence of *Argyropelecus* photophores. Strong electrical stimulation might rapidly release epinephrine in inhibitory concentrations. In this case it is unnecessary to invoke the liberation of a second neuromediator to explain the inhibition phenomenon: the same neuromediator, namely epinephrine, might trigger the luminescence and initiate the extinction of the photophore depending on its concentration. This concentration, in turn, would depend on the character of the electrical stimulation.

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