

FATIGUE OF THE LUMINESCENT RESPONSE OF *CHAETOPTERUS*

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(Text-figs. 1-6)

When stimulated in some suitable manner *Chaetopterus* secretes a luminescent material into the surrounding sea water. The luminescent secretion is discharged from glands which are widely dispersed over the surface of the animal; the most conspicuous are two glandular patches on the dorsal surface of segment XII. Secretory material is forced out of the cells by some contractile process; discharge is not merely the result of secretion pressure. Under repetitive stimulation the intensity of the luminescent response decreases owing to the intervention of fatigue. Fatigue has been interpreted as a gradual exhaustion of luminescent material in the glandular cells (Nicol, 1952*b, c*). The present investigation seeks to analyse the onset and progress of fatigue in greater detail.

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MATERIAL AND METHODS

Fresh specimens of *C. variopedatus* were employed. These were stimulated by brief condenser shocks from an electronic or mechanically operated circuit. The luminescent response was recorded by means of photomultiplier tube (RCA 931), direct coupled amplifier, and oscilloscope. Deflexions of the spot on the oscilloscope screen were photographed with moving paper.

RESULTS

Diminution of Response under Repetitive Stimulation

Specimens were stimulated electrically for different periods and at different frequencies. When subjected to a series of shocks, with long intervals between stimuli, the response takes the form of separate flashes. Fig. 1 shows a series of records obtained by administering an electrical shock every 10 min. Each shock evokes a luminescent response, which quickly rises to a peak, and gradually decays over the course of 5 min. The intensity of consecutive flashes is plotted in Fig. 2. The first few responses are of about equal magnitude, but thereafter intensity rapidly decreases. The decay curve approaches a quarter

ellipse in form. At this very slow rate of stimulation, 1 per 10 min., it is reasonable to assume that no fatigue of the contractile mechanism is occurring, and that diminution of the response is due to intervention of other factors.

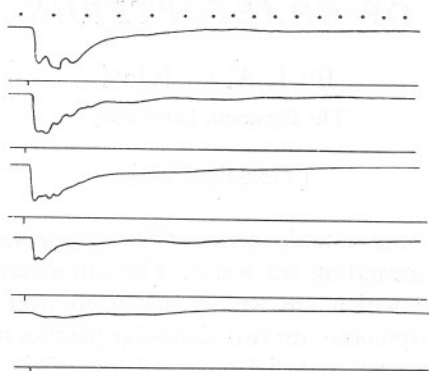


Fig. 1. Fatigue of the luminescent glands of *Chaetopterus* (glands on segment XII). Five consecutive records from the same animal, responses separated by an interval of 10 min. Stimulus on each occasion was a single condenser shock, shown as a pip on the lower line. Luminescent response appears in these records as a deflexion downwards of the middle trace. Time scale above, 1 per 30 sec. Paper speed halved after 2½ min.

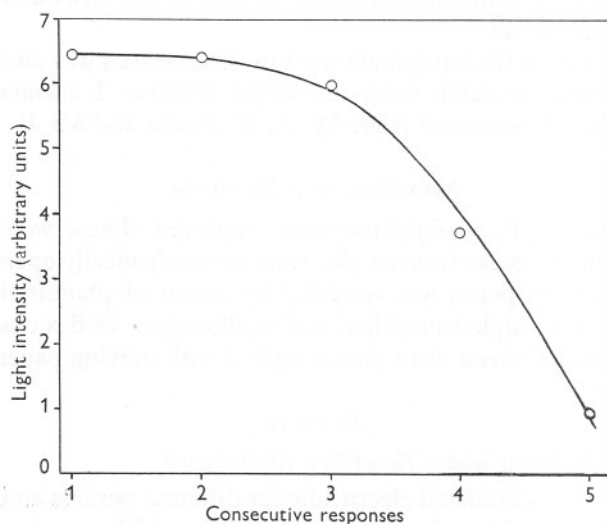


Fig. 2. Curve showing decrease of intensity of consecutive luminescent responses. Each response evoked by a single stimulus.

A burst of stimuli at slow rates (2–6 per min.) evokes recognizably discrete responses which summate, especially at higher frequencies (Fig. 3). When stimulation is maintained at these frequencies for some time, the response (light intensity) gradually falls off. With rest periods of 5 min. between

successive bursts of stimuli, each successive period of stimulation evokes a luminescent response which is progressively less than that of its predecessor (Figs. 3B, 4A). A point of some importance for the argument which will be

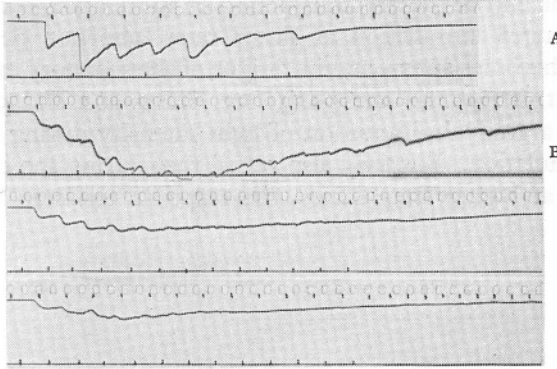


Fig. 3. A, luminescent responses evoked by a burst of stimuli (8 stimuli at 2 per min.) for 4 min. Time scale, 1 per 30 sec. B, responses to repetitive stimulation. Three consecutive periods of stimulation at 6 per min. for 2 min. Intervals of 5 min. between periods of stimulation. Time scale, 1 per 10 sec.

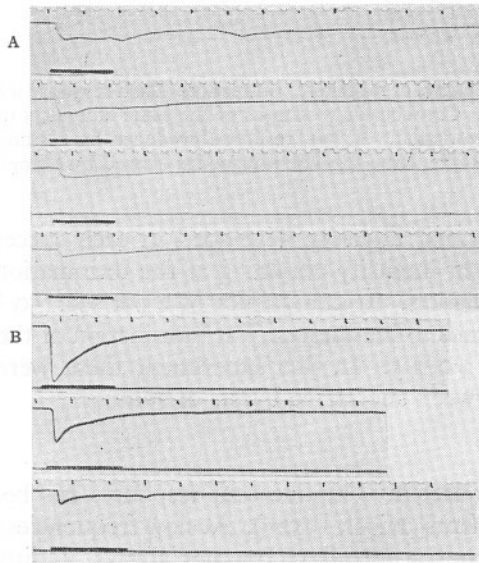


Fig. 4. A, consecutive responses to repetitive stimulation. Each response was evoked by a 50 sec. burst at 1 per sec. Interval of 5 min. between successive periods of stimulation. Duration of stimulation is shown in heavy black on lower line of each record. Time scale, 2 per min. B, responses to repetitive stimulation. Experimental conditions and details of recording similar to above, except that a frequency of 2 per sec. was used.

developed is that the maximal intensity developed in each response (after the first) is about equal to that developed at the termination of the previous period of stimulation and, in any event, is no greater. At a stimulation frequency of 1 per sec., the ratio of maximal light intensity at the beginning of a response to intensity at the end of the previous period of stimulation is unity.

With prolonged stimulation at faster rates, above 1 per sec., another phenomenon becomes apparent, viz. fatigue of the secretory mechanism. This is brought out in Figs. 4B and 5A (50 sec. bursts at 2 and 5 per sec.). The records show a gradual diminution of light intensity during each successive period of stimulation. But they also reveal that during the course of a prolonged burst at higher frequencies, the response tends to fall off rapidly.

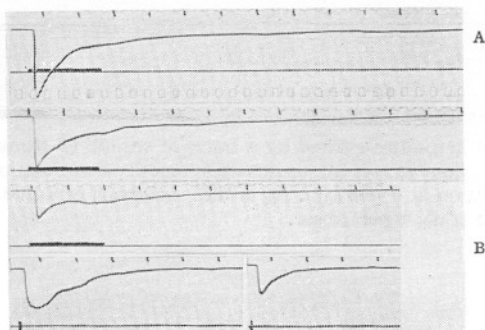


Fig. 5. A, responses to bursts of repetitive stimulation. Each response was evoked by a 50 sec. burst at 5 per sec. Consecutive responses of the same animal; 5 min. interval between each period of stimulation. B, left, response produced by 2 stimuli (interval between shocks 0.5 sec.). Right, same specimen, response evoked by a single shock. Time scale, 2 per min.

Moreover, the maximal intensity developed in each successive response is greater than the light intensity obtaining at the termination of the previous period of fast stimulation. At a frequency of 2 per sec. (50 sec. burst), ratios of initial luminescence to luminescence at termination of the previous period of stimulation are 2.0–2.5. In this experiment there were rest periods of 5 min. or more between successive bursts of stimuli.

Summation

Summation of consecutive luminescent responses has been demonstrated in previous experiments (Nicol, 1952*c*). At slow frequencies, from 2 to 12 per min., separate responses are often, but not always, additive, and light intensity may rise during progress of stimulation (Fig. 3). Fusion of discrete responses occurs above 30 per min. An attempt made previously to demonstrate facilitation in the luminescent response was unsuccessful, and fresh experiments were devised in which the nerve cord was stimulated by strong

shocks, instead of direct stimulation of the luminescent glands. Comparison was made of 1 versus 2 condenser shocks; in the latter event the two stimuli were separated by an interval of 0.5 sec. As previously noted, the response following two stimuli is sometimes equal to, or less than, that resulting from a single stimulus (Nicol, 1952c). But with the present technique some records were obtained (two out of five) in which the response produced by a pair of stimuli was significantly greater than that evoked by a single stimulus (Fig. 5B). Magnitude of response (maximal intensity, total light) produced by two stimuli was not more than twice that produced by a single shock. This appears to preclude facilitation and to demonstrate that increment of response, under repetitive stimulation, is due to summation.

Recovery from Fatigue

Earlier attempts to demonstrate recovery of the luminescent response after fatigue were unsuccessful. A few observations suggest that a rest of several hours following stimulation permits recovery of the luminescent response (Nicol, 1952b). Recovery of luminescence has been investigated in the following manner. Whole specimens were stimulated with a prolonged burst of shocks at slow frequencies, and records were obtained of the response. Electrodes were placed either directly on the glandular cells, or over the ventral nerve cord of segment XII. With electrodes in the latter position stimulation invariably caused autotomy of the anterior region as well, and these preparations were used only to investigate recovery over short periods not exceeding 24 hr. After stimulation the specimens were returned to sea water. Those animals to be examined in 24 hr. or later were placed in empty *Chaetopterus* tubes. Whole animals soon demonstrated normal activity by fabricating new sections on cut ends of the tubes. Thirty-three animals were investigated.

It was found that specimens varied considerably in time taken to achieve complete recovery. After periods of 4½, 24 and 48 hr., animals displayed 10–100% of the luminescent response (original response rated at 100%) (Fig. 6). After 72 hr. all specimens which were examined gave a luminescent response equal to or greater than that originally recorded. Complete recovery of the luminescent response, following exhaustive stimulation, therefore, can take place within 5 hr.

HISTOLOGICAL OBSERVATIONS

The histology of the luminescent glands of *Chaetopterus* is reviewed in an earlier paper (Nicol, 1952a). On the dorsal surface of segment XII the glands consist of dense aggregations of elongated cells packed with secretory granules. The secretory contents form a bag-shaped mass invested by a cytoplasmic sheath, the cell wall. The small nucleus lies at the base of the cell, from which

fibres run to the basement membrane. The cell walls are strongly argento-philic, suggesting a dense groundwork, but no structural differentiation is apparent. Interspersed between the glandular cells are numerous ciliated cells. These cells have been carefully examined in preparations stained with iron haematoxylin or treated with silver protargol. It is found that the ciliated cells are very slender elongated elements lying between the luminescent cells. Distally the ciliated cells flare out into trumpet-shaped expansions which partially cover the external region of the luminescent cells. They bear long cilia on their external surfaces; the cilia arise in the usual way from basal granules, from which a cone of fibres can be followed a short distance into the cell towards the nucleus.

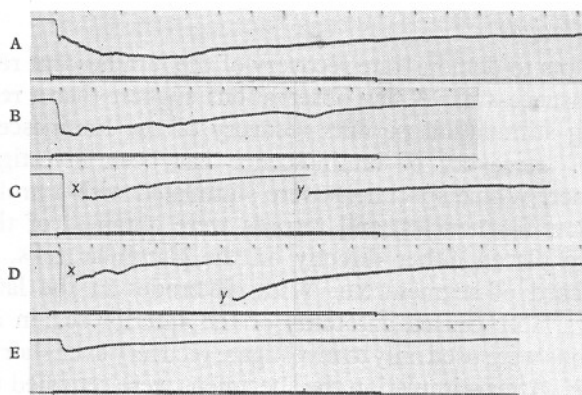


Fig. 6. Fatigue of luminescent response and recovery from fatigue. Stimulation in each record, 5 min. burst at 30 per min. Time scale, 1 per 30 sec. A, first response; B, second response from the same specimen after an interval of $4\frac{1}{2}$ hr.; C, first response from another specimen; D, second response from the same after an interval of 24 hr.; E, another response, 3 min. after D. At x, amplification was reduced to one-fifth and increased again at y.

Ciliated cells in the intestine of *Lumbricus* are said to possess contractile intracellular fibrils which assist in the extrusion of the secretory material from contiguous glandular cells by exerting compression on the latter (Millott, 1948). The appearance of these cells is not dissimilar to that seen in the luminescent glands of *Chaetopterus*, except that I have not been able to satisfy myself that any system of strongly developed intracellular fibrils extends through the entire length of the ciliated cell. The appearance of the luminescent cells when secreting strongly suggests that the glandular contents are being forcibly expelled, presumably by contraction of the cytoplasmic sheath of the glandular cell, which may contain contractile protein. Secreting cells differ in appearance from quiescent cells in that the glandular contents of the former are pushed towards the free surface of the cell while the base of the cell is compressed and

tapering. The ciliated cells serve to drive the discharged luminescent material towards a ciliated tract on the median dorsal surface, in which it is carried anteriorly, and dispersed away from the animal.

DISCUSSION AND CONCLUSIONS

The data which have been secured in this investigation can be summarized as follows. A single stimulus evokes a bright response, and repetitive stimuli result in summation of luminescent responses. There is no evidence for facilitation in the neuro-effector system. Repetitive stimulation at slow rates (below 1 per sec.) brings about gradual exhaustion of the luminescent response, as evidenced by the effect of further stimulation after a short period of rest. Complete recovery of luminescent ability is achieved in 4-5 hr. in some specimens, longer in others. Under repetitive stimulation at fast rates, above 1 per sec., the response quickly decays during the course of stimulation, but recovers after a brief interval of 5 min. so that the maximal intensity developed during the second period of stimulation is greater than that measured towards the end of the previous period of stimulation. Histological examination of the luminescent glands reveals that much secretion is still present in the glandular cells, even after exhaustive stimulation. The following hypothetical interpretation is offered to account for these facts.

Prolonged stimulation at high frequencies (above 1 per sec.) probably brings about gradual fatigue of the contractile mechanism responsible for extruding the luminescent material. Consequently, the cell is no longer able to maintain its contraction, and extrusion of luminescent material declines. A rest period of 5 min. is sufficient to allow recovery of contractile efficiency, and to permit another maximal response. The correspondence to muscle fatigue is obvious. Curves of tetanic contraction recorded from the longitudinal muscle of the sabellid *Branchiomma*, for example, show a similar sequence of events, i.e. rapid decline of tension and contraction-height during the course of stimulation, and recovery of contractile ability after a period of rest, so that the initial tension developed by the rested muscle is greater than the tension developed during the terminal period of stimulation in the previous contraction (Nicol, 1951). This similarity, of course, sheds no light on the physico-chemical events responsible for fatigue, but it emphasizes that the same sort of contractile mechanism is operating in the discharge of luminescent material as in muscular contraction.

In the absence of any other apparent mechanism it is suggested that contraction is accomplished by the cytoplasmic sheath of the glandular cells. These may be endowed with contractile proteins capable of exerting pressure and producing movement.

The progressive diminution of light intensity which occurs in successive discrete responses is due to factors other than contraction-fatigue. Part of this decrease must result from progressive exhaustion of luminescent material

available for discharge. At first sight this would seem to provide an adequate explanation for the gradual decline in brightness of successive responses. There are several reasons for considering this explanation inadequate. First, if glands are electrically stimulated to exhaustion until the luminescent response becomes very weak, and are then mechanically disrupted, a great deal more light is produced. Secondly, if glands electrically stimulated to exhaustion are examined histologically, it becomes evident that a large amount of secretory material is still present inside the cells. It is evident, therefore, that some other factor is operating which is not merely fatigue of the contractile mechanism, causing decreased efficiency of contraction during prolonged activity, and resulting in conservation of luminescent material intracellularly.

It is noteworthy that the curve showing progressive diminution of light intensity in successive isolated responses has the form roughly of a quarter ellipse. That is, the first few responses (2 or 3) maintain a high intensity, after which maximal light intensities fall off at an increasing rate. Similar curves showing decrease of intensity in consecutive luminescent responses of polynoids take the form of rectangular hyperbolas. That is, the intensities of luminescent responses after the first one fall off at a decreasing rate along an asymptotic curve. In these latter animals the controlling factor is probably progressive exhaustion of intracellular luminescent material, or some other substance controlling oxidation of the latter.

Any suggestions concerning the factor responsible for this apparent fatigue of luminescent ability which develops progressively in isolated consecutive responses of *Chaetopterus* must be speculative, with the indirect information at hand. Attention should be directed first of all to the saccular organization of the glandular cells. Once the cell starts to secrete a distal pore opens on the cell surface, and secretory granules are forced out. A fresh unstimulated glandular cell will be distended and its wall stretched. When stimulated it will compress the cell contents and the pressure will find relief on extrusion of some of the cellular contents. A small proportion of the intracellular secretory material is lost with each contraction. A second stimulus and contraction will find the cell less distended than on the first occasion. From this viewpoint the glandular cell can be regarded as a semi-fluid hydraulic system in which pressure exerted on an enclosed column leads to movement of the latter and compensatory pressure-release once the cell opens to the outside. An analogy is at hand in the fluid-filled cavities of soft-bodied animals such as annelids. But in these forms the fluid-volume remains constant, and provides a hydrostatic system against which the muscles can operate. The contents of the glandular cell, however, are gradually dissipated during successive responses. On the assumption that the contractile mechanism of the cell behaves like muscle under stretch, it may be expected to produce maximal compression when stretched or expanded, and the force which it develops will fall off as it

progressively diminishes in pace with reduction of cellular contents and cell-size. The curve in Fig. 2 showing progressive diminution in light intensity of successive responses is very similar to a curve for striated muscle when tension developed is plotted against decreasing length along the x -axis.

It is impossible from these data, and probably impossible from experiments designed in this way, to obtain a true picture of the course of recovery and restitution of secretory material in the luminescent cells. Even after an exhaustive stimulation of the luminescent glands the cells show no visible decrease of secretory material, and the problem cannot be resolved by histological means. The luminescent response is regenerated to full intensity in some 4–5 hr., even in isolated heads severed from the rest of the body. This possibly could be due to restitution of glandular material. It could also result from temporary intake of sea water, restoring cell volume.

This hypothesis and these analogies are presented for argument. As a tentative hypothesis it has this to commend it. It provides an explanation of the progressive decrease in intensity of successive responses. It accounts for progressive exhaustion of the secreting (contractile) mechanism in the face of high residual levels of intracellular luminescent material. And it evokes no new principles not already described for contractile tissues.

SUMMARY

Further experiments have been carried out on the luminescent glands of *Chaetopterus* in an attempt to provide a functional explanation of the mechanism of glandular secretion. Attention has been confined to the luminescent glands in segment XII, which were excited by electrical stimulation. Responses have been followed and recorded by photomultiplier tube and oscilloscope.

A bright response is evoked by a single stimulus, and repetitive stimulation at rates slow enough to allow separate responses to be distinguishable produces summation. Paired stimuli provide no evidence for facilitation. The intensity of the response produced by two stimuli is not more than twice that produced by one.

Evidence is adduced for the participation of three factors in fatigue of the luminescent response. At rapid rates of stimulation the contractile mechanism responsible for extruding the secretory material becomes fatigued. At very slow rates of stimulation, below that producing fatigue of contractility, light-intensity falls off in consecutive responses. This is only in small part due to gradual exhaustion of available luminescent material, since the glandular cells still contain large amounts of secretion even after stimulation to apparent functional exhaustion. The hypothesis is advanced that the contractile mechanism exerts maximal contraction when fully stretched in a cell heavily loaded with secretory material, and its efficiency decreases as it shrinks with reduction of cell volume.

Complete recovery of luminescent ability, to intensities initially recorded, was attained by some specimens in 5 hr.; all specimens examined showed complete recovery in 72 hr.

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