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STUDIES ON *CHAETOPTERUS VARIOPEDATUS* (RENIER). III. FACTORS AFFECTING THE LIGHT RESPONSE

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(Text-figs. 1–26)

Luminescence in *Chaetopterus* is an extracellular phenomenon and is due to the secretion of photogenic material by certain glandular cells in definitely circumscribed regions of the body. These gland cells are eosinophilic elements scattered singly or massed together in dense aggregations in certain regions. They are particularly abundant in the epithelium covering the distal surface of posterior notopodia, and in two glandular areas on the dorsal surface of the aliform notopodia. These two regions also display the brightest luminescence (Nicol, 1952*a*).

The gland cells producing the photogenic material are under nervous control in *Chaetopterus*, and secrete only as the result of stimulation. A preliminary investigation of light production in this animal has established certain facts about the nature of nervous regulation (Nicol, 1952*b*). With electrical stimulation it has been found that a single shock, above threshold strength, will induce secretion and luminescence, and that the amount of secretion, and hence luminescence, is augmented by increasing the number of stimuli. The luminescent powers of the animal are soon fatigued or exhausted, however, under repetitive stimulation, and after a few shocks the light tends to diminish. Moreover, it was also noticed that at low frequencies of stimulation the light response was confined to the region directly stimulated, but as the frequency was raised the response showed a tendency to spread to other parts of the body. This effect was most pronounced in the posterior region of *Chaetopterus*.

The present study is concerned with analysing in greater detail the factors concerned with regulation of the magnitude of the luminescent response. In particular, attention has been concentrated on the effect of altering the frequency and number of impulses. The regions found most favourable for study were the aliform notopodial light glands and the notopodia of the posterior region, and quantitative investigations were confined to these structures.

I am indebted to Dr W. R. G. Atkins, F.R.S., for the loan of a galvanometer and camera used in this investigation, and to Mr F. J. Warren for technical assistance. Part of the expenses incurred in this research was defrayed by a grant-in-aid of scientific investigations from the Royal Society.

Material and Methods

The methods of recording luminescence have already been described (Nicol, 1952*b*), and involve the employment of a multiplier photocell, and a sensitive galvanometer or oscilloscope. More use of the latter instrument was made in the present investigation. Electrical stimuli consisted of condenser discharges from an electronic stimulator, unless otherwise stated, and were delivered through platinum electrodes laid on the surface of the animal.

Observations

Luminescence in the Aliform Notopodia

The experiments described in this section consisted of stimulating the light glands on the aliform notopodia, and of recording the light which resulted. The effects of altering the frequency and number of stimuli were explored.

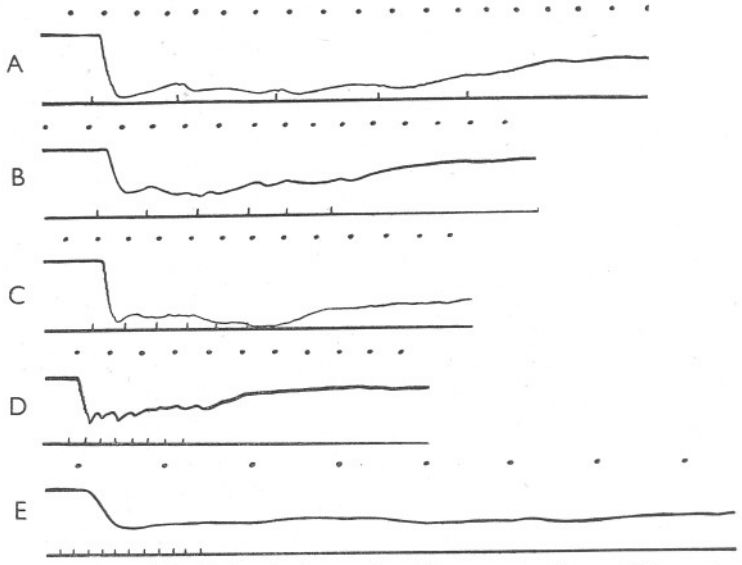


Fig. 1. Stimulation of the light glands on the aliform notopodia at different frequencies. Each record was obtained from a different specimen. A, stimulation 1 per 30 sec.; B, 1 per 15 sec.; C, 1 per 10 sec.; D, 1 per 5 sec.; E, 3 per 5 sec. Time scale above each record, 1 per 10 sec. Stimuli shown on bottom lines. In A–D separate inflexions can be seen, in relation to the separate stimuli. In E, however, the response curve becomes smoothed out. Oscilloscope records.

Following a suggestion made by Professor A. V. Hill, F.R.S., I have prepared a series of records to permit a comparison of the general character of the luminescent response with records of muscular contraction (Fig. 1). Stimuli were delivered at low frequencies of 2–36 per min., and the responses were photographed at low camera speeds. At the lower frequencies (Fig. 1 A–D)

a definite inflexion can be seen corresponding to each stimulus. These inflexions result from the discharge (secretion) of photogenic material. Above a rate of 30 per min. the separate inflexions tend to become smoothed out (Fig. 1 E) and a response curve somewhat resembling a curve of tetanic contraction is produced. At the low frequencies employed in this series of experiments the response to the first stimulus is maximal, and subsequent responses do not exceed the initial response. The rate of decay of luminescence, however, is very slow, and successive stimuli tend to maintain the initial level of light intensity. The reduced response to the second and succeeding stimuli is due to some kind of fatigue, and it is not possible to demonstrate summation at these low frequencies (below 1 per sec.).

In a previous communication (Nicol, 1952*b*) some observations were presented to show that by increasing the number of stimuli the height of the resultant light response was increased in consequence. This effect has been explored further to determine the nature of the summation process involved and the relative roles of frequency and number of impulses in determining the magnitude of the response. A very extensive series of experiments was carried out on the light glands of the aliform notopodia, and over 150 animals were used.

In order to have comparable records showing the differential effects of varying the experimental conditions, say the frequency of stimulation, it is necessary to be able to make repeated observations on the same animal. Fatiguing of the light response is very marked in *Chaetopterus*, however, and recovery has not been observed in any period of time that would be advantageous for experimentation. The experiments to be described consisted of stimulating an animal in a given way, allowing a rest period of 5 min. in which decay of luminescence could occur, and then stimulating once more at a higher rate or for a longer period. Periods of stimulation were kept as brief as the experiment would allow. These experiments have been carried out on the assumption that the response to a second stimulation, which duplicates the first stimulation, may be equal to the first response or less than the first response, but will not be greater. Furthermore, if fatigue should occur, it is possible that any augmented response, resulting from increasing some characteristics of the stimulation, may be sufficient to offset the fatigue effect, and reveal itself as an increment over the first response.

In general, it has been found that when a specimen is subjected to successive periods of identical stimulation, and the conditions of the experiment are maintained constant, the succeeding responses fall below the level of the initial response.

Effect of Increasing the Frequency of Stimulation

To determine any possible effect dependent upon frequency of stimulation, specimens were excited electrically for two periods, once at a low frequency,

and again after a period of rest, at a higher frequency. To eliminate the influence of varying the number of impulses, the duration of the stimulation period was regulated so that the same number of impulses (within 10%) was administered on each occasion. Impulse duration and voltage were kept constant. Frequencies which were used ranged from 1 to 33 per sec., and the number of stimuli varied from three to twenty-one in the different experiments.

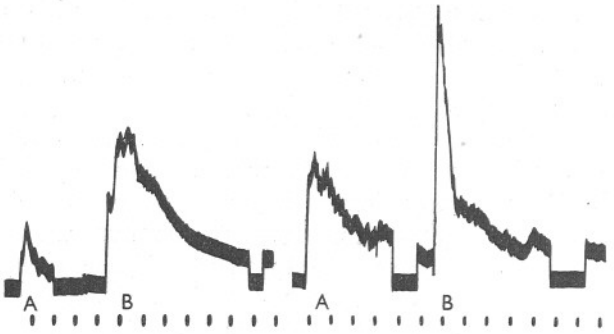


Fig. 2 (*left*). Stimulation of the light glands on the aliform notopodia. A, response to a single electrical stimulus; B, twenty-nine stimuli at 1 per sec.

Fig. 3 (*right*). A record showing the effect of stimulating the light glands at different frequencies. A, $4\frac{1}{2}$ sec. burst at 2 per sec.; B, 1 sec. burst at 9 per sec. Time scale below both figures, 1 per min.

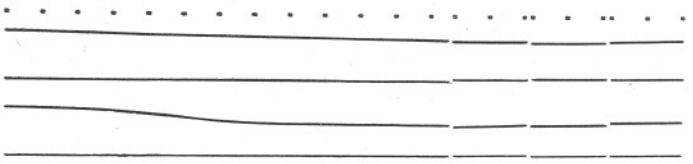


Fig. 4. Oscilloscope records of light produced by stimulation of the light glands on the aliform notopodia at different frequencies. Top record, 2 sec. burst at 5·5 per sec. Bottom record, 0·5 sec. burst at 23·4 per sec. Time scale, 1 per 2 sec. Interruptions in the records are intervals of 20 sec.

A number of definite and clear-cut positive results was obtained in which the second burst of stimuli, at a higher frequency, resulted in a much larger response than that evoked by the previous stimulation at a lower rate. The increment lay both in the initial response peak, and in the total amount of light produced. A typical record, obtained by photographing a galvanometer deflexion, is shown in Fig. 3. In this experiment the first stimulation consisted of a 4·5 sec. burst at 2 per sec., the second stimulation of a 1 sec. burst at 9 per sec. The peak intensity of light resulting from the higher frequency is about twice as great as that evoked by the lower frequency, but the total light is about the same in both. In other records initial and total light showed a pronounced increase (Figs. 4, 5).

Effect of Increasing the Number of Stimuli

By increasing the number of stimuli it is possible to increase the amount of light produced, but the amplitude of the effect varies with the specimen. An augmentation of the light response on increasing the number of stimuli first becomes apparent at a frequency of about one per sec. (Fig. 2).

Increasing the number of stimuli has the same kind of augmentative effect as raising the frequency, but the effect is more pronounced and is more easily

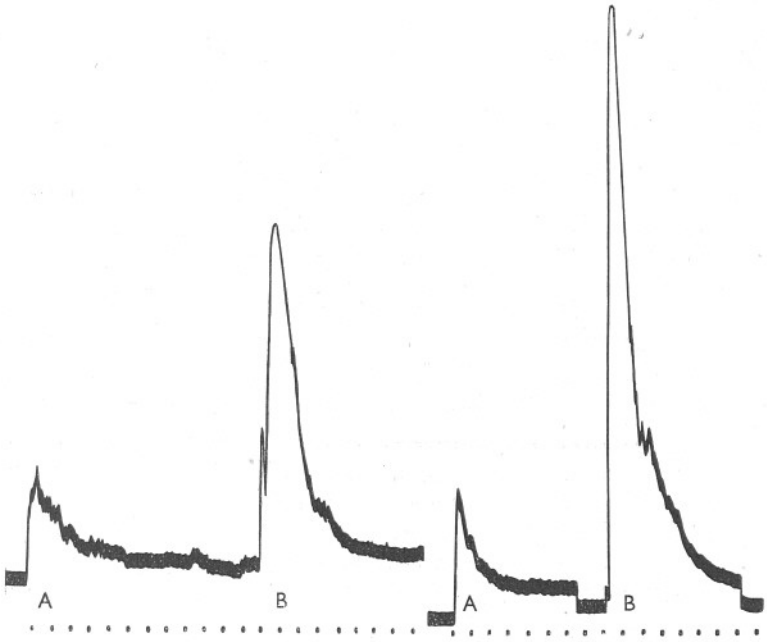


Fig. 5 (*left*). Galvanometer records of light produced by glands of the aliform notopodia. A, twenty shocks at 5·5 per sec. for 3·6 sec.; B, nineteen shocks at 32·2 per sec. for 0·6 sec.

Fig. 6 (*right*). A record of light produced by glands of the aliform notopodia after dissection and removal of the nerve cord. A, a burst of eighteen stimuli at 1·3 per sec. for 14 sec.; B, eighteen stimuli at 9 per sec. for 2 sec.

The upper half of the second response curve in both records (B) has been extrapolated on the basis of visual readings of galvanometer deflexions. Time scale, 1 per min.

elicited. The height of the response, that is the initial intensity, and the total light emitted, that is the amount of secretion, are both increased by prolonging the duration of stimulation (Fig. 7).

It is possible to consider two mechanisms that could be operating in these responses, one a simple effect of summation in the effector, and the other a facilitatory effect induced by the build-up of an excitatory state under prolonged rapid stimulation. Facilitation, if present, could be peripheral or central, since the preparation consisted of the whole animal. The data presented above are not adequate to allow a choice among these alternatives, and

towards this end the experiments described in the following four sections were carried out. These experiments consisted of testing the effects of various anaesthetics on the luminescent response; stimulating the nerve cord in contradistinction to the peripheral field; stimulating pieces of glandular tissue which were severed from the nerve cord; and comparing the effects of one versus two electrical stimuli. The results may be anticipated by stating that they favour the concept of a peripheral contractile mechanism controlling secretion, and capable of summation under repeated stimulation.

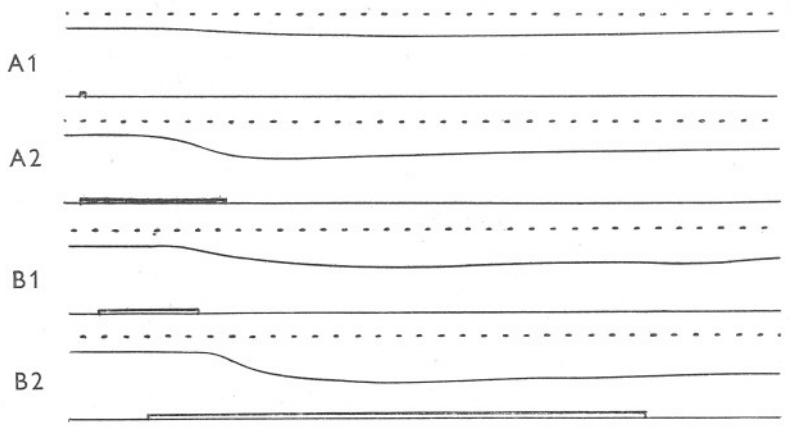


Fig. 7. Effect of number of impulses on light production in *Chaetopterus*. Oscilloscope records of luminescence in the aliform notopodia. A1 and A2 from the same animal, stimulated at a frequency of 25 per sec. A1, burst of 0·2 sec. duration; A2, burst of 5 sec. duration. B1 and B2 from another animal, stimulated at 2·2 per sec. B1, duration 4 sec.; B2, duration 22 sec. Stimulation represented by horizontal lines at bottom of records; time scale above each record 1 per sec.

Effects of Anaesthetics on the Luminescent Response

Several anaesthetics, that are known blocking agents of nervous tissue, were employed in an attempt to prevent nervous transmission without halting the activity of the peripheral effector concerned with the luminescent response. The anaesthetics employed were chloretone (acetone chloroform), MS. 222 (tricaine methansulphonate : Rothlin, 1932), cocaine, stovaine, procaine, eucaine, ether, and isotonic magnesium chloride ($MgCl_2.6H_2O$, 7·3%).

Stimulation of the Anaesthetized Gland Cells

In the following experiments the animals were anaesthetized for varying periods of time, and the light glands were then stimulated directly by placing a pair of platinum electrodes on them. The animals were then placed in running sea water for several hours to wash out the anaesthetic, following which they were stimulated as before. Stimulation consisted of 10 or 20 sec. bursts at 5 per sec.

Chloretone (0·1%). Specimens were immersed in this anaesthetic for 5–30 min. After 10 min. the anaesthetic caused a marked diminution in the amount of light produced, which was only about a third of that given off by the same animal free of anaesthetic (Fig. 8).

MS. 222. Animals immersed in this anaesthetic for 10–30 min. showed greatly reduced luminescence under electrical stimulation when compared with the same individuals free of anaesthetic (Fig. 9). The results were similar to those obtained with chloretone.

Stovaine (0·5%) and *eucaine* (0·5%). These two drugs were tried at several concentrations and for various periods to determine effective dosages and action times. Concentrations of 0·5% acting for 15 or 30 min. proved effective in greatly reducing luminescence while still permitting subsequent recovery (Figs. 10, 11). A peculiarity of many of the records obtained is the occurrence of a small primary peak before the luminescence reaches its maximum in specimens from which the anaesthetic has been washed out.[1]

Cocaine. Solutions of cocaine hydrochloride were used at concentrations of 0·5% (pH 7·6) and 1% (pH 7·3). After acting for 30–60 min., these solutions greatly reduced the luminescence produced by electrical stimulation (Fig. 12).

Procaine at concentrations 0·5–2%, acting up to 30 min., failed to significantly reduce the amount of luminescence. Solutions of 4% were then made up, and the pH was returned to 8·0 with NaOH. Procaine, at this concentration, greatly reduced luminescence in 30 min. (Fig. 13).

Ether (0·1%) failed to significantly reduce luminescence in 1 hr.

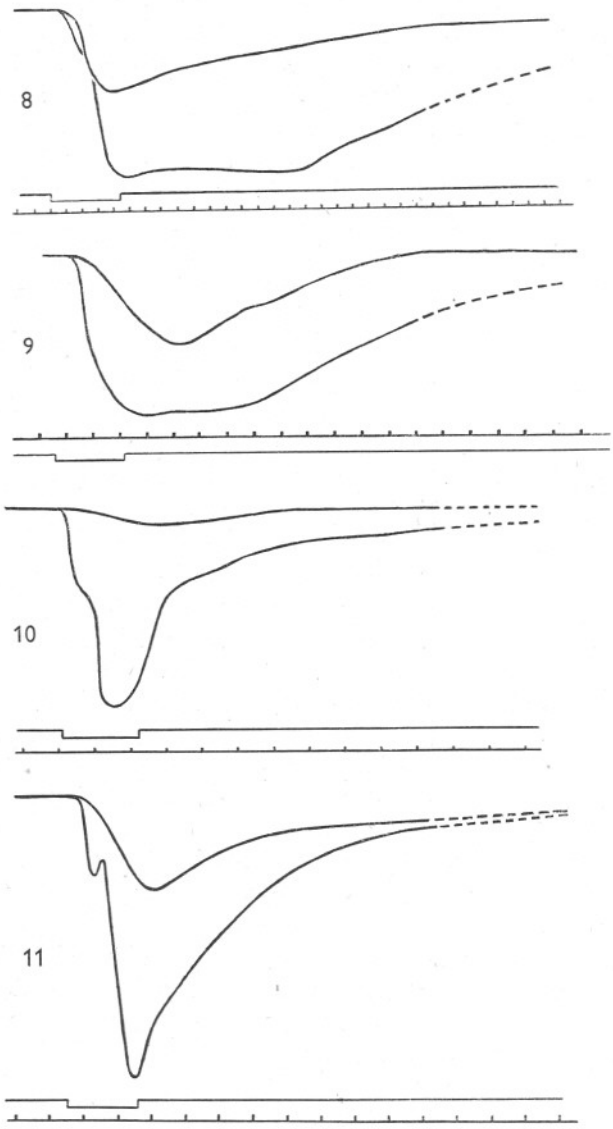
Isotonic *magnesium chloride* (pH 8·2). After immersion for 15–30 min. in this solution the animals showed greatly reduced luminescence to electrical stimulation (Fig. 14).

The noteworthy feature of all these results (except with ether) is that the anaesthetic greatly diminishes but fails to completely abolish the luminescent response. The drugs greatly reduce the intensity of light, and appear to restrict its appearance to a narrowly confined region immediately underneath the electrodes. The responses under anaesthesia are interpreted as suggesting that nervous transmission has been blocked, but that the luminescent effector is still responding directly to electrical stimulation.

Stimulation of the Nerve Cord in Anaesthetized Animals

Evidence for this viewpoint has been sought by stimulating the nerve cord directly. Specimens were anaesthetized as before, using MS. 222 for 30 min. They were then pinned out on a platform with ventral side uppermost and with the dorsal light glands in the aliform notopodia exposed through an aperture situated above a mirror. Fine silver electrodes arranged to give

[1] This effect, also seen in other records, is due to quantitative differences in the secretion produced by the two luminescent glands of the aliform notopodia.



Figs. 8–11. Luminescent responses of specimens when anaesthetized, and after recovery from anaesthesia. The upper curve in each record is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Stimulation, indicated as a horizontal interval, consisted of a 10 sec. burst at 5 per sec. Fig. 8, chloretone 0·1 % for 10 min. Fig. 9, MS. 222 1/2000 for 30 min. Fig. 10, stovaine 0·5 % for 30 min. Fig. 11, eucaine 0·5 % for 30 min. Time scale in Fig. 8, 1 per 2 sec.; time scales in Figs. 9–11, 1 per 5 sec.

localized stimulation were then inserted into the nerve cord in the mid-ventral surface of segment XII, and the preparation was subjected to prolonged repeated stimulation. It was found that normal animals responded to

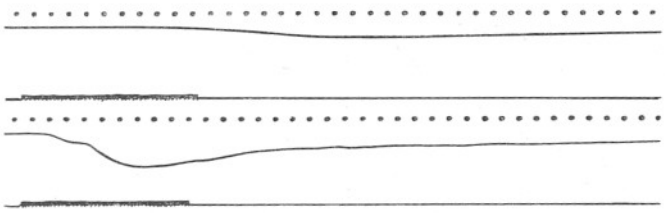
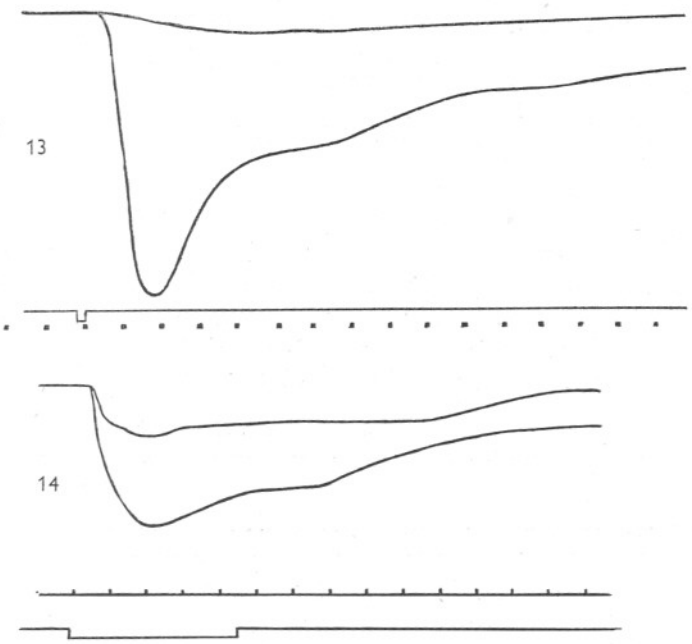


Fig. 12. Luminescent response of a specimen narcotized with cocaine hydrochloride (0·5 %) for 52 min. The upper curve is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Stimulation, 10 sec. burst at 5 per sec. Time scale above, 1 per sec.



Figs. 13, 14. Luminescent responses of specimens when anaesthetized, and after recovery from anaesthesia. The upper curve in each record is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Fig. 13 (*above*), procaine hydrochloride 4 % for 28 min. Stimulation, 1 sec. burst at 5 per sec. Fig. 14 (*below*), isotonic $MgCl_2$ for 15 min. Stimulation, 22 sec. burst at 5 per sec. Time scale in both records, 1 per 5 sec.

stimulation of the nerve cord by a bright flash along the aliform notopodia and by the release of luminous secretion in the light glands at the bases of those structures. After anaesthetization, however, repeated stimulation failed to produce any light.

This is strong evidence that the anaesthetics employed do actually stop nervous transmission, while allowing the peripheral effectors to respond directly to electrical stimulation. If the assumption be made that the anaesthetics have little or no effect on the activity of the glandular effectors, certain conclusions can be drawn about the effect of electrical stimulation of the light glands on the aliform notopodia as carried out in this investigation. Direct stimulation of the effector cells apparently produces much less light than stimulation of the nerves supplying them. This may be due to the effectors having a higher threshold than the nerve fibres so that, with a given voltage, fewer glandular cells would be affected than nerve fibres. Or it may result from the spatial organization of the nerve fibres themselves such that very localized stimulation of peripheral nerves initiates impulses that spread through a widely distributed network (not necessarily non-synaptic), and thereby reach the entire glandular area. In either event it follows that electrical stimulation of the peripheral light gland causes excitation of the nerve fibres supplying the glandular cells as well as direct excitation of the effector cells. But the major part of the light is due to indirect stimulation of the light cells via the nerve fibres, and only a small fraction is ascribed to direct stimulation of the effector cells themselves.

The Effect of Raising the Voltage in Anaesthetized Specimens

If the slight luminescence of anaesthetized specimens is due to a higher threshold in the responding structure or to the small field directly affected it is to be expected that a greater response could be secured by raising the voltage. With this in mind experiments were carried out as follows. Animals were anaesthetized in isotonic $MgCl_2$ and the light glands in the aliform

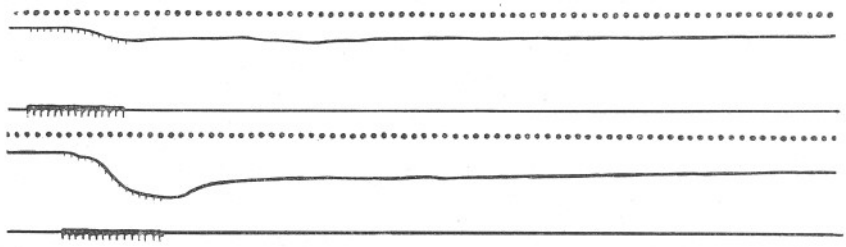


Fig. 15. Records of light produced by the photogenic glands of segment XII, demonstrating the effect of raising the stimulation-voltage in anaesthetized animals. The preparation was narcotized with isotonic $MgCl_2$ for 28 min., and it was then stimulated at 9V. (*upper record*), and 49V. (*lower record*). Stimulation consisted of a burst of 15 pulses at 84 per min. Time scale above each record, 1 per sec.

notopodia were then stimulated at different voltages (charging voltages of the condenser, 9V.–210V.). A relay-operated stimulator activated by an electronic device was employed for these experiments. Luminescent responses at low voltages were small, but were greatly increased by raising the voltage (Fig. 15).

Moreover, by raising the voltage it was possible to secure responses equal in magnitude to those obtained from normal animals. These results favour the interpretation that in anaesthetized animals the gland cells are being directly stimulated and that raising the voltage overcomes the higher threshold of these elements, or brings more into activity.

The Effect of Prolonging the Period of Stimulation in Anaesthetized Specimens

The action of narcotics in blocking nervous transmission while still allowing the glandular cells to react presented the possibility of testing the responses of the effectors to direct stimulation for varying periods. Animals were narcotized with isotonic $MgCl_2$ (pH 8·2) and MS. 222 (1/2000), and the light glands of the aliform notopodia were then stimulated through electrodes placed directly upon them. Stimulation consisted of short bursts at 5 per sec.,

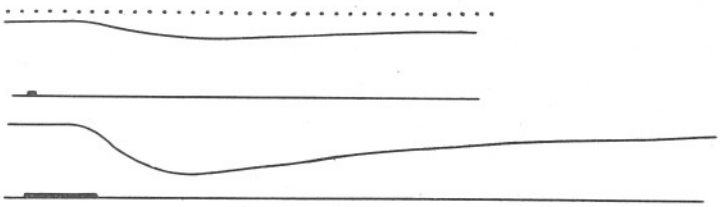


Fig. 16. Response of the light glands of the aliform notopodia in a specimen narcotized with MS. 222 (1/2000) for 11 min. The light glands were stimulated directly. Upper curve, response to a 1 sec. burst at 5 per sec.; lower curve, response to a 10 sec. burst at 5 per sec. Time scale above, 1 per 2 sec.

first for 1 sec., then again for 10 sec. Considerably increased amplification was used in recording the responses. In these preparations it was found that the second period of more prolonged stimulation produced a much greater luminescent response (Fig. 16).

It is emphasized that the luminescent responses recorded from these narcotized animals were very weak, and were only a small fraction of those obtained from normal animals. The explanation is advanced that the small response is due to the excitation of only a small number of gland cells under direct stimulation. The fact that the same kind of augmented response can be obtained both in narcotized and in untreated animals by increasing the number of stimuli suggests that the processes involved are taking place peripherally, in the effector cells, and not in efferent nervous pathways.

Light Produced by Stimulation of the Nerve Cord in Normal Animals

For comparison with the effects of peripheral stimulation some experiments have been carried out in which the nerve cord has been stimulated and the resultant luminescent response recorded. The arrangement was similar to that described above, p. 119. The animal was spread out with the dorsal side downwards: the basal light glands of the aliform notopodia

overlay an aperture which was focused on a photocell lying below. Fine silver wire electrodes, giving localized stimulation, were inserted into the nerve cord on the median ventral surface, and stimuli were applied. Periods of stimulation were regulated so that the effects of short and prolonged bursts could be compared.

These experiments gave results similar to those obtained by stimulating peripherally, with the electrodes placed on the surface of the light glands. Two or three stimuli were delivered at a rate of 3 per sec., the response was recorded, and the light was allowed to fade; then a burst of thirty to forty stimuli at the same rate was applied, and a record was made of the response. In the majority of specimens (four out of five) the response to the second prolonged burst was appreciably greater than the previous response to fewer stimuli; in one specimen it was about the same (Fig. 17).

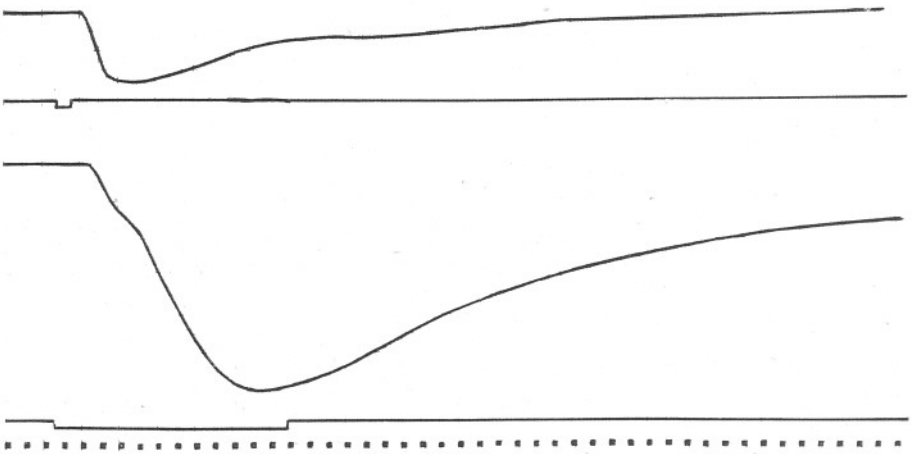


Fig. 17. Light produced by the aliform notopodia as the result of localized electrical stimulation of the nerve cord in segment XII. Upper tracing, three stimuli; lower tracing, thirty-eight stimuli. Frequency of stimulation, 3·3 per sec. Periods of stimulation are shown as horizontal intervals below each record. Time scale below, 1 per sec.

This is considered to be significant in view of the onset of fatigue or exhaustion previously discussed. The salient point that emerges from these results is that an augmentation of the luminescent response can be obtained by prolonging the period of nervous stimulation, that is, an increase in the number of nerve impulses brings about a greater response and produces more light. This result, read in conjunction with the previous conclusions relating to peripheral stimulation, lends weight to the viewpoint that when stimuli are delivered to the periphery they excite nerve fibres which in turn affect the glandular cells; reasons are presented above for considering that direct stimulation of the effector cells is a minor consequence. This point is of some importance in the present investigation since the animal

does not lend itself readily to stimulation through the nerve cord, with simultaneous recording of the luminescent response, and the majority of quantitative results were obtained by applying stimuli through electrodes resting on the basal light glands of the aliform notopodia.

The Responses of Specimens from which the Nerve Cord had been Removed

The reverse experiment to the preceding one has been carried out, namely stimulation of the peripheral light glands without involving the central nervous system (nerve cord). In the usual experimental arrangement, with electrodes resting on the dorsal surface of the animal, it is likely that solely efferent paths are involved rather than the nerve cord; but excitation of the latter, and consequent central regulation of the response, are not excluded. The following experiment was undertaken to resolve this difficulty, and the results indicate that the events responsible for augmentation of the light response on increasing the duration or frequency of stimulation occur at the periphery.

The worms used in these experiments were first anaesthetized by immersion in isotonic $MgCl_2$ for 15 min. The anterior regions were cut off at the junction between segments XII and XIII, and the ventral surface was removed from these anterior fragments. This operation removed the nerve cord (central nervous system) and left pieces which consisted of dorsal body wall and gut, and which contained the light glands of segment XII. The experimental material was afterwards washed in running sea water for several hours. These pieces were then stimulated either at two different frequencies or with short and long bursts of stimuli.

This experimental material showed the same responses as intact worms containing central nervous system. The response to a second period of stimulation at a higher frequency was significantly greater than after the first burst of stimuli at a lower frequency (Fig. 6). The response was also augmented by increasing the number of stimuli (Fig. 18). Both the maximal height of response and the total light produced were greatly increased by stimulating at a higher frequency or for a longer period. In addition, the rate of rise of light intensity showed a pronounced increment. It follows that the augmentatory effect, whatever its nature, that is produced by more stimuli or faster rates of stimulation, can be a peripheral affair, and experimentally is not dependent on the central nervous system and centrally located nerve cells.

Histological sections of the aliform notopodia, impregnated with silver (Holmes's method and Bodian's activated protargol), have revealed some details of innervation. The nerve cord, situated mid-ventrally, gives off small nerves which proceed peripherally underneath the epidermis. The nerve fibres in these nerves are very small and it has not been possible to distinguish terminal details of peripheral innervation, but the multiplicity of fibres and

their close proximity to the glandular photogenic cells form a structural basis for the physiological mechanisms reported above.

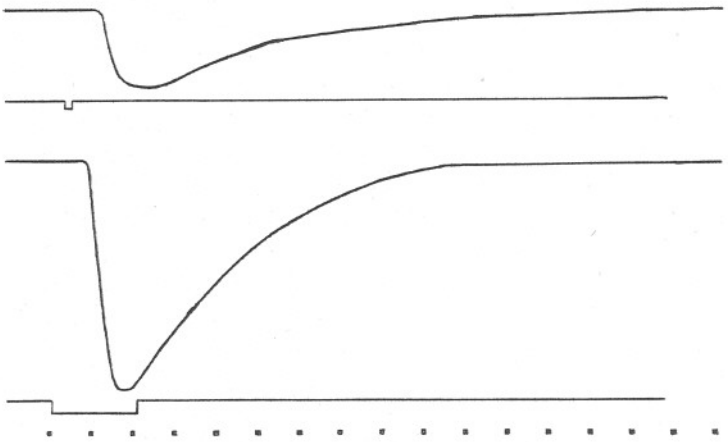


Fig. 18. Record of light produced by the light glands in the aliform notopodia of a preparation from which the nerve cord has been removed. Upper record, three stimuli; lower record, 10 sec. burst (fifty stimuli). Frequency of stimulation, 5 per sec. The tracing below each record indicates the period of stimulation. Time scale below, 1 per 5 sec.

Comparison of the Effects of One versus Two Stimuli

In order to secure critical data on whether the augmentation of the light response is due to a process of summation in the effectors (luminescent gland cells), or to facilitation at the neuro-effector junction, specimens have been stimulated with a single stimulus and with a pair of stimuli, and the responses compared. Two sets of experiments were carried out as follows.

In the first set of experiments each animal was stimulated twice. It was stimulated by a single impulse; then, after allowing a period for decay, two electrical stimuli were applied. The two impulses were separated by an interval of 0·25 sec. In these experiments all the results obtained were negative. Of seventeen specimens examined a few gave about the same amount of light with two stimuli as with one; the majority, however, gave off even less light following the second period of stimulation (Fig. 19). It was concluded that a fatigue effect was operating here, in that the first stimulus had partially exhausted the light glands; any augmentative effect due to two stimuli would be masked by the previous partial evacuation of the light glands.

A second approach to the problem was essayed by stimulating separate specimens either with a single stimulus, or with two stimuli (interval 0·25 sec.). Forty-six animals were used, half of which were stimulated with a single impulse, and half with two stimuli. Since the experiments lasted several hours and there was the possibility of an alteration in the experimental conditions during that time, the animals were divided into small groups. In

each group several animals were stimulated with one stimulus, and a corresponding number with two stimuli. The mean response of each group was determined, and from these values weighted means were calculated for the whole assemblage. (Measurements of response refer to displacement of the response curves.)

Group	Mean responses of each group (in arbitrary units)	
	one stimulus	two stimuli
1	10·0	5·2
2	2·9	4·3
3	11·4	13·7
4	10·3	12·0
5	9·6	5·4
6	10·2	7·7
Weighted means	8·3	7·8

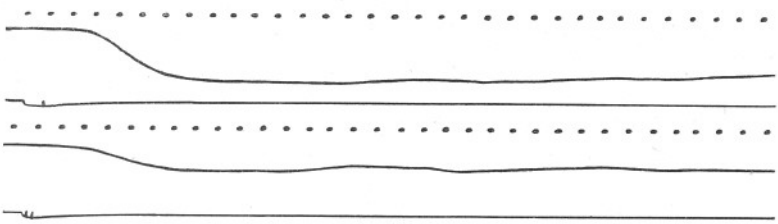


Fig. 19. Records of light produced by the photogenic glands on the aliform notopodia. *Upper record*, the response to a single stimulus; *lower record*, the response of the same specimen to two stimuli (interval of 0·25 sec.). Stimulation indicated below each record. Time scale above, 1 per sec.

To reduce the possibility that some partly exhausted specimens were vitiating the results, a comparison was made of the twenty maximal responses. Means for these were 14·2 units for one stimulus (ten animals) and 12·5 units for two stimuli (ten animals).

In these experiments two stimuli produced rather less light than a single impulse. The actual fact that the mean response was slightly less for two stimuli than for one, however, is certainly a statistical accident. If two stimuli were to give a disproportionate response, equal to many times that produced by a single impulse, then it would be expected that it would be revealed by the technique employed, and might be sufficient even to offset partial exhaustion resulting from a previous stimulus. Other records demonstrate, moreover, that a series of repeated stimuli do give a significantly greater response than one or a few stimuli (Figs. 2, 7, 15, 16).

Facilitation due to build-up of an excitatory state seems to be ruled out by these results, but they can be explained by summation in a contractile tissue in the light glands. They suggest a mechanism in which the first stimulus brings about a large response (or contraction) and subsequent stimuli are responsible for only small additional increments. This explanation of peripheral summation is in line with the results of experiments reported above (p. 123),

in which augmentation of the light response was obtained by stimulation of the photogenic glands in narcotized animals, presumably due to direct excitation of the effector-gland cells. The subject is discussed more fully in a later section.

Rate of Rise of Light Intensity and Temporal Characteristics of the Luminescent Response

The augmented responses observed in some of the experiments described above obviously invite comparison with results obtained in investigations of neuromuscular functioning. From experiments on crab muscle Pantin (1936) was able to show that as the frequency of electrical stimulation was raised, there was a corresponding increase both in the maximal tension developed and in the rate of rise of tension. These studies in neuromuscular functioning have prompted further analyses of the light response of *Chaetopterus*.

Measurements made on those records that show an increased response to a higher frequency of stimulation, or to more prolonged stimulation, also reveal a corresponding increase in the rate at which the luminescent response develops (Figs. 4, 7). Data from five experiments in which the frequency of stimulation was raised are presented below.

Specimen	Frequency of stimulation (per sec.)	Rate of rise per sec. (mm.)
1	1·3	4·0
	9·0	8·9
2	1·3	1·7
	9·0	2·8
3	1·3	1·3
	9·0	3·8
4	2·0	3·0
	9·0	4·9
5	5·5	2·4
	32·2	4·9

Mean increment at lower rate, 2·5 mm./sec.
Mean increment at higher rate, 5·1 mm./sec.

In these experiments the frequency of stimulation was raised 1/2–7 times and the rate of rise of light intensity increased about twofold at the higher frequencies. The rate of rise of light intensity is also significantly increased by increasing the number of stimuli (Fig. 7). An increased response, therefore, reveals itself in three ways: by a rise in peak intensity, by an increase in the rate of rise of light intensity, and by an increase in the total amount of light.

Detailed analyses of a large number of response curves have yielded information about certain temporal features of the light response and the rate of increment of light intensity. The following data were extracted from each record: the rate of rise of the response curve, the time taken to reach maximal height, the maximal height of the response, and the rate of decay for a period

extending up to 30 sec. after the beginning of the response. Due to the great variation between individual specimens, it has been necessary to reduce the records to a comparable basis, and this was accomplished by expressing all intensities as a percentage of the maximal response, set at 100. Fig. 20 shows a representative plot for a series of nineteen specimens. In this figure two curves represent records which showed maximal and minimal rates of increment, and a third curve represents mean values for all specimens.

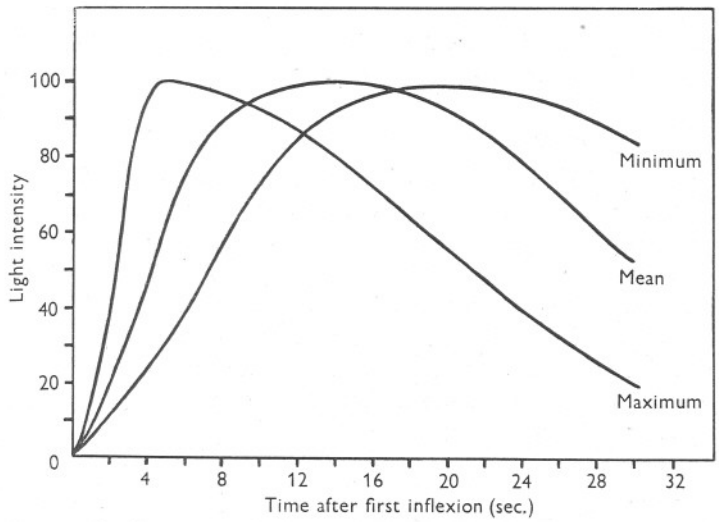


Fig. 20. Curves showing temporal characteristics of the luminescent response evoked by electrical stimulation (2·3 sec. burst at a frequency of 6·2 per sec.). Maximal and minimal curves are selected records showing fastest and slowest rates of rise of light intensity. The mean curve is based on the average for all records (19). All data have been replotted as a percentage of maximal response (= 100).

Mean values obtained in this series of experiments were:

Time to reach maximal height, 14·2 sec.;
Rate of rise, 13·1/100 per sec.;
Height at first $\frac{1}{2}$ min., 53·8/100.

These figures illustrate the levels of magnitude involved, but absolute values will depend on the conditions of stimulation.

The relationship between the rate of rise of light intensity and the maximal intensity of response was considered in the following manner. Records were obtained of sixty-eight animals which were stimulated at two different frequencies, first at 2 per sec., and again at 25 per sec. The absolute increment (h_1) 5 sec. after the beginning of the response was measured, and this was expressed as a fraction of the maximal response (h_2), viz.

$$\frac{h_1}{5} \times \frac{1}{h_2} = r, \quad \text{the fractional increment per sec.}$$

A mean value for r was obtained by pooling measurements from all records. The absolute rate of increment is sometimes greater at higher frequencies, yet, when expressed as fractional increments of the total response, the rates of rise at low and high frequencies show no significant difference, i.e. $r_2 = r_m = r_{25}$, where r_2 = fractional increment at a frequency of 2 per sec., r_{25} = fractional increment at a frequency of 25 per sec., and r_m = the mean fractional increment of all records irrespective of the frequency of stimulation. This result indicates that the rate of rise of light intensity (i.e. the rate of cellular secretion), and the maximal intensity reached (i.e. the maximal output of secretion) are related to one another.

Effect of Drugs on the Neuro-glandular Junction

The results just described reveal the characteristics of certain excitatory events taking place in the light glands. It has also been shown that acetylcholine is an effective agent in causing luminescence in this animal, and acts at the neuro-glandular boundary (Nicol, 1952*b*). In conventional terminology, the nerve fibres to the light glands are therefore cholinergic, and acetylcholine becomes implicated as a chemical transmitter in the light response. In view of these facts it seemed of value to test the effects of atropine and curare, which separately antagonize the action of acetylcholine in different neuro-effector systems of vertebrates.

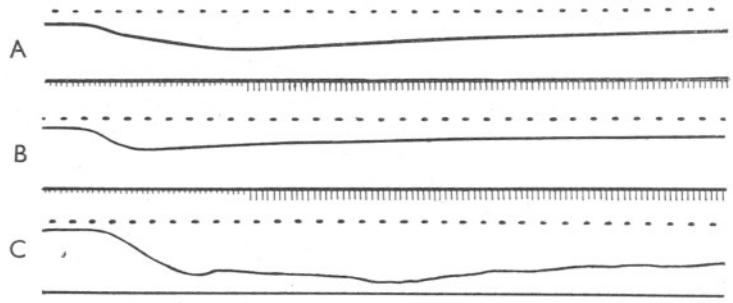


Fig. 21. Records of the light produced by animals treated with atropine or curare and subjected to electrical stimulation (10 sec. burst at 4 per sec.). A, specimen treated with atropine, 1/10,000, for ½ hr., and then stimulated; B, same specimen stimulated after washing out atropine for ½ hr.; C, specimen treated with *d*-tubocurarine, 1/10,000, for ½ hr., and then stimulated. Neither atropine nor *d*-tubocurarine had any effect in abolishing the light response. Time scale, 1 per sec.

Atropine. This drug was applied in concentrations of 1/10,000 and 1/1000 for 30 min., and records of the luminescent response to electrical stimulation were obtained. The drug was then washed out for 30 min., and stimulation was repeated. The drug had no apparent effect in reducing the amplitude of the response (Fig. 21).

d-*tubocurarine.* At a concentration of 1/10,000, *d*-tubocurarine failed to

diminish or abolish the luminescent response evoked by electrical stimulation (Fig. 21).

Eserine. Some previous experiments with eserine failed to reveal any excitatory effect of this drug when used in conjunction with acetylcholine. However, little reliance is placed on this negative result, since the light produced by acetylcholine itself is usually very faint and shows much variation from one specimen to another. The effect of eserine has been further explored in a quantitative manner by stimulating specimens electrically to determine the normal level of response, and then stimulating again after the application of eserine to discover any possible augmentative effect. Such an experiment suffers from the same defects as those mentioned previously in describing the effects of increasing the frequency or duration of stimulation, namely fatigue of the light glands. Despite this latter factor an increase in response after eserinization might still be encountered, and such a result may be considered as positive.

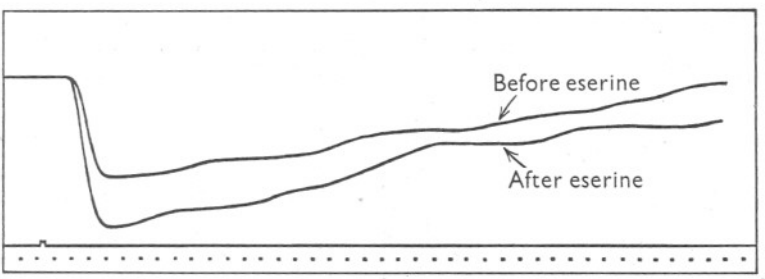


Fig. 22. The effect of eserine on light production in *Chaetopterus*. The upper tracing shows the response to electrical stimulation before eserine, the lower tracing after the application of eserine (1/10,000, 45 min.). Electrical stimulation, in both instances, was a 0·2 sec. burst at 33 per sec. Lower tracing: stimulation, and time scale, 1 per sec.

Specimens of *Chaetopterus* were treated with eserine (physostigmine salicylate) 1/10,000 for 45–60 min., and the light produced before and after eserinization was recorded and compared. Of twelve animals examined, three gave positive results in that a brighter response followed the application of eserine (Fig. 22).

Acetylcholine. It was considered possible that the luminescent responses to electrical stimulation might be affected by acetylcholine, and experiments along this line were carried out as follows. A record was first obtained of the light resulting from a short burst of electrical stimuli. The animals were then immersed in 1/10,000 acetylcholine for 15 min., and stimulation, as before, was repeated. In all instances (five specimens) the light resulting from the second period of stimulation was less than that obtained previously. There was, consequently, no evidence that acetylcholine applied externally can increase the luminescent response that is evoked by stimulation of the nerves.

The negative results with atropine and *d*-tubocurarine do not directly

provide any information about the nature of neuro-glandular transmission occurring in the light glands. However, other cases are known among invertebrates in which cholinergic systems are insensitive to these depressive drugs. Presumably, the receptor surfaces involved in neuro-muscular transmission are not affected by these substances, and the reactant systems consequently do not readily lend themselves to classification as muscarinic or nicotinic. The results obtained with eserine are interesting and point towards acetylcholine being involved as a chemical mediator in the light glands.

Transmission Through the Nerve Cord

In a previous study it has been shown that when the frequency of electrical stimulation is raised above 5 per sec., the luminous response spreads and affects more distant segments. The transmission of excitation involved in this process occurs through the nerve cord (Nicol, 1952*b*). The passage of excitation from one body region to another is rather irregular in occurrence. For example, it is only occasionally that light appears in the middle and posterior regions of the body when the anterior region is stimulated. In order to obtain repeatable quantitative information, therefore, experiments were confined to the posterior region.

The posterior regions of a series of specimens were stimulated at various frequencies in the dark, and the number of posterior segments that lighted was noted. The electrodes were placed on the first few segments (I–III) of the posterior region, and the voltage output of the stimulator was kept constant. The effect of increasing either the number or frequency of stimuli was investigated. A diagrammatic representation of the results is shown in Fig. 23.

Increase in frequency of stimulation. There is no spread of excitation at low frequencies. Stimulation at 1–2 per sec. results in light confined to the notopodia in the neighbourhood of the electrodes. On raising the frequency to higher rates, excitation spreads to involve more distantly located segments, and the whole posterior region finally lightens. The critical frequency is about 6 per sec.

Increase in duration of stimulation. Prolonged stimulation at low frequencies (1–2 per sec.) does not widen the response area, but at higher frequencies (above 5 per sec.) the spatial character of the response depends upon the duration of stimulation as well as the frequency (Fig. 23). Two protocols illustrate the results obtained.

Specimen 1. Electrodes on segments I–II of the posterior region.
Stimulation: 2 per sec.
Time: 10 sec. Segments I–II responded.
22 sec. Segments I–IV responded.
22 sec. Segments I–IV responded.
Stimulation: 25 per sec.
Time: 20 sec. Bright luminescence from all segments of the posterior region.

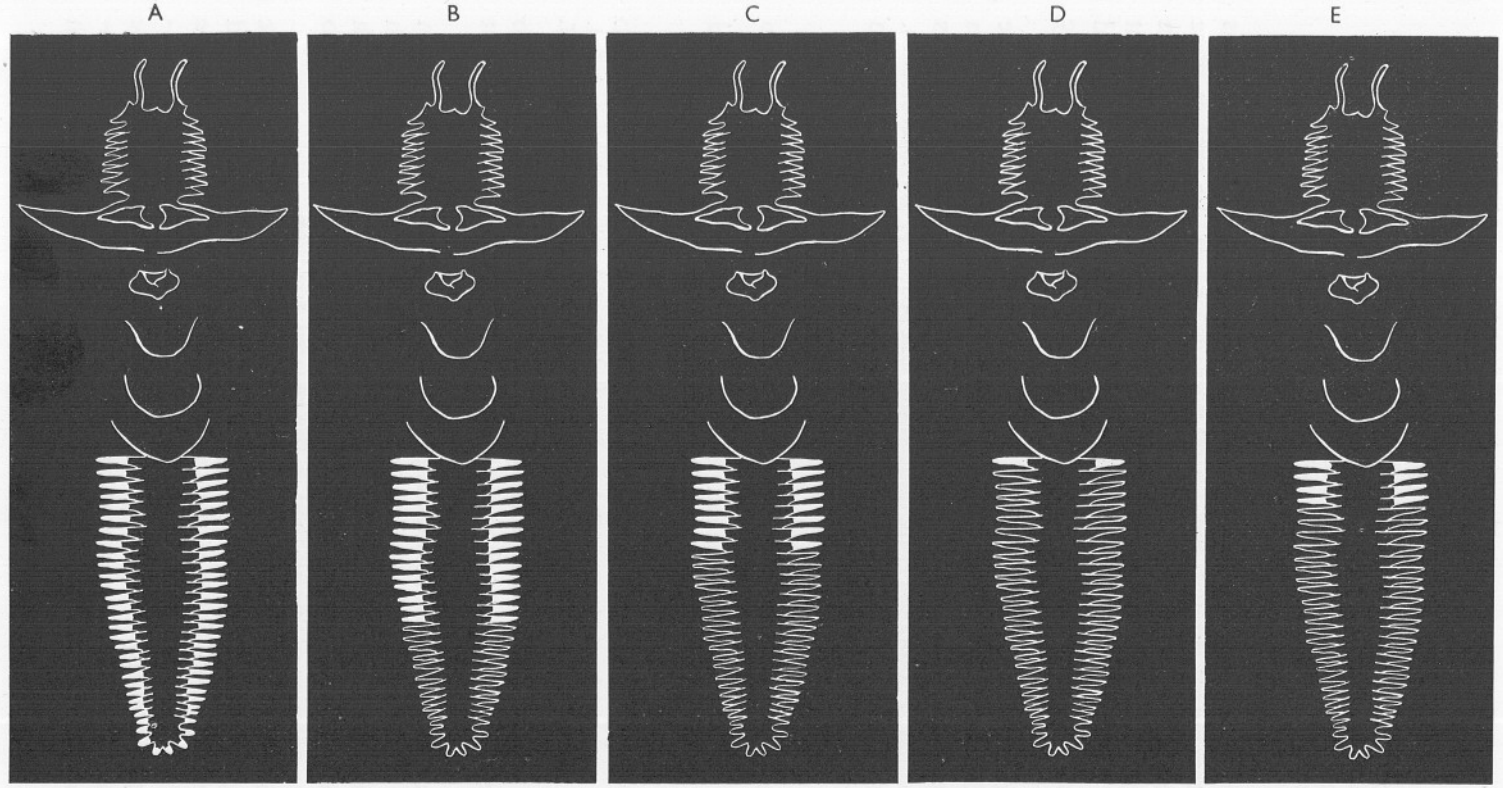


Fig. 23. The effect of altering the frequency and number of impulses on lighting of the posterior region of *Chaetopterus*. A, electrodes on segments I and II. Stimulation, 43 per sec. for 10 sec. Luminescence in all notopodia of the posterior region. B, electrodes on segments II and III. Stimulation, 10 per sec. for 10 sec. Luminescence in segments I–XV. C, electrodes on segments II and III. Stimulation, 10 per sec. for 2 sec. Luminescence in segments I–VIII. D, electrodes on segments I and II. Stimulation, 10 per sec. for 0·2 sec. Luminescence in segment I. E, electrodes on segments I and II of the posterior region. Stimulation, 1·2 per sec. for 40 sec. Luminescence in segments I–IV.

Specimen. 2. Electrodes on segments I–II of the posterior region.
Stimulation: 43 per sec.
Time: 0·2 sec. Segments I–II responded.
0·5 sec. Segments I–II responded.
1 sec. All posterior segments responded, but light weak.
10 sec. All notopodia of the posterior region were briefly luminous.

This spread of excitation is interrupted by cutting the nerve cord, and is therefore transmitted through the central nervous system. To explain these results it has been suggested that some process of internuncial facilitation occurs at higher frequencies (Nicol, 1952*b*). The present results show that the spread of excitation is also dependent upon the number of stimuli, and as the duration of the stimulatory period is increased, the luminous response spreads so as to involve more and more posterior segments.

Slightly different results are obtained by stimulating segments in the middle or hind end of the posterior region. When the middle of the posterior region is stimulated at low frequencies (1–5 per sec.) the appearance of light is usually confined to notopodia lying underneath and posterior to the electrodes. But on raising the frequency and number of impulses the light spreads to involve notopodia lying anterior to the electrodes. Here are two protocols.

Specimen 3. Electrodes on the terminal third of the posterior region.
Stimulation: 1 sec. burst at 5 per sec. Segments from the level of the electrodes to the hind end responded.
22 sec. burst at 5 per sec. All segments of the posterior region lighted up, anterior and posterior to the electrodes.

Specimen 4. Electrodes on the middle of the posterior region.
Stimulation: 3 per sec. for 20 sec. Only notopodia behind the electrodes responded.
5 per sec. for 20 sec. Only notopodia behind the electrodes responded.
10 per sec. for 20 sec. All segments of the posterior region responded.

In most of these animals it was observed that the light appearing anterior to the region stimulated was much weaker than that appearing posterior to that region.

The conclusion drawn from these experiments is that nervous transmission concerned in mediating the luminescent response takes place with much greater facility posteriorly than anteriorly through the nerve cord. Although transmission anteriorly does occur, there is a greater degree of resistance to it in postero-anterior pathways than in the reverse direction.

The build-up of a central excitatory state is presumably a synaptic phenomenon occurring at the junctions between segmentally disposed neurones arranged in linear sequence along the nerve cord. It may be assumed that at low frequencies, below 5 per sec., the excitatory state dissipates in the intervals between stimuli, but that at higher frequencies the rate of build-up of excitation exceeds the rate of decay. The degree of facilitation attained will depend on the frequency and number of impulses, both

factors determining the level of excitation reached. It appears that a single impulse, or a group of impulses at low frequencies, is insufficient to override the synaptic resistance between the segmentally arranged neurones, and that a burst of impulses is necessary to overcome these barriers. The number of segments responding can be interpreted as the external manifestation of facilitatory processes occurring at sequential synapses, each being a barrier in series to the next, and introducing a degree of decrement in stepwise series.

Correlated with this physiological interpretation it has been observed that the nerve cord possesses large tracts of longitudinally directed fibres, coursing from one segment to the next. Besides minute fibres, giant axons have also been noted, up to 7 μ in diameter. These probably only extend short distances, however, since Bullock (1948) could find no electrical evidence for giant fibres in this species.

Discussion

Peripheral augmentation of the luminescent response due to increasing the frequency or duration of stimulation manifests itself in heightened light intensity, often in augmentation of the total amount of light produced, and as an acceleration in the rate of increase of light intensity. The light produced is taken to be an adequate indication of the strength and temporal progress of events in the underlying response mechanisms. The response curve, of course, represents the progress of some chemical reaction, presumably the oxidation of photogenic material, and it only indirectly reveals the course of the physiological processes of secretion. To interpret this curve it is necessary to consider its relationship to the secretory process. Unfortunately, there are no critical biophysical data available for the oxidation of the luminescent secretion of *Chaetopterus*, and experiments with *Cypridina* (Ostracoda) extracts can only be cited for comparison. Harvey and his colleagues (1940) studied the luminescent reaction produced by mixing *Cypridina* extracts, and they found that the light emitted by this material reached its maximum in about 0·03 sec., and the decay time for a fall to half intensity occupied 0·5 sec. These experiments were carried out with refined extracts, and it is obvious that the reaction in *Cypridina* preparations proceeds at a much faster rate than in the luminescent material of *Chaetopterus*, as released by the normal animal (see p. 129). The secretion of *Chaetopterus* does not become luminescent until it is discharged from the cell and this in itself must introduce a time lag so that the luminescent response is not contemporaneous with the secretory process. Under optimal conditions it may be presumed that the chemical reaction will proceed rapidly to completion, but the luminescent secretion of the light glands in the aliform notopodia is actually released into a dense body of mucus that greatly retards gaseous diffusion, and, consequently, the oxidation of the photogenic material.

A study of the latent period is more informative. When the aliform

notopodial light glands are stimulated, the average latent period is 4 sec., as determined by measurements of the response curves. This latent interval of 4 sec. could be occasioned by the latent period of the effector, by the time consumed in the response of the effector, and by delay in the course of the chemi-luminescent reaction. In several experiments in which the effect of raising the frequency was investigated, stimulation consisted of short bursts of 0·5–4 sec. (p. 116). With these short bursts, lying within the latent period, a definite augmentation of the response was obtained on increasing the rate of stimulation. Other evidence indicates that augmentation of the response is due to summation. Since this process can take place well within the latent period of the response, it is clear that the luminescent response first becomes evident several seconds after the sequence of events in the underlying mechanism has taken place. On these premises it is now possible to predicate that most of the interval that elapses between the delivery of the first electrical shock, and the first appearance of light, is due to the slow progress of the chemiluminescent reaction in the material discharged upon the surface of the animal.

Since luminescence becomes evident considerably later than the sequence of events taking place in the underlying mechanism of secretory discharge, the response curve will not reveal detailed information about the temporal course of events in the secretory structures. On the other hand, its height and slope reflect the magnitude of the underlying secretory processes, and give a delayed, smoothed, and aggregate portrayal of the events preceding it.

The cytomorphological appearance of the photogenic glands is that of oval or elongated cells closely packed with eosinophilic granules (see Nicol, 1952*a*, for details and references to literature). These are arranged as a closely packed mass of paraplasm occupying most of the cell interior, and invested by a thin layer of cytoplasm. The mechanics of secretion in such a cell appear to be poorly understood, and present a problem common to many glandular cells of that type, for example, muciparous cells containing a mucous plug. It may be suggested that excitation causes the superficial protoplasm of the cell to contract, and this process leads to the expulsion of the cell contents; or that excitation initiates changes resulting in imbibition of water and a rise in internal pressure, again causing the photogenic mass to be squeezed forth. In either case nervous excitation of the glandular cell would be direct, and would result in a rise in internal pressure. Other possible mechanisms that can be suggested are the existence of myo-epithelial elements about the cell-body, as obtains in the oral glands of mammals, or of muscle elements between the epidermal gland cells as Eisig (1887) has described in the epidermis of capitellids. The mechanism in these instances would be indirect and neuro-muscular in nature.

In conjunction with the physiological investigations a considerable amount of histological work has been done on the photogenic glands and cells of

Chaetopterus. Specimens, fixed in a variety of ways, have been stained and impregnated with silver by silver-on-the-slide techniques. This work is summarized as follows.

The thin layer of protoplasm investing the photogenic cells stains rather feebly, probably owing to its tenuity. In preparations triple-stained with Heidenhain-azan this protoplasmic investment is coloured orange or reddish like the muscle fibres in the same sections. Treatment with silver (Holmes's method after Bouin fixation) sharply delimits this layer as a distinct deeply

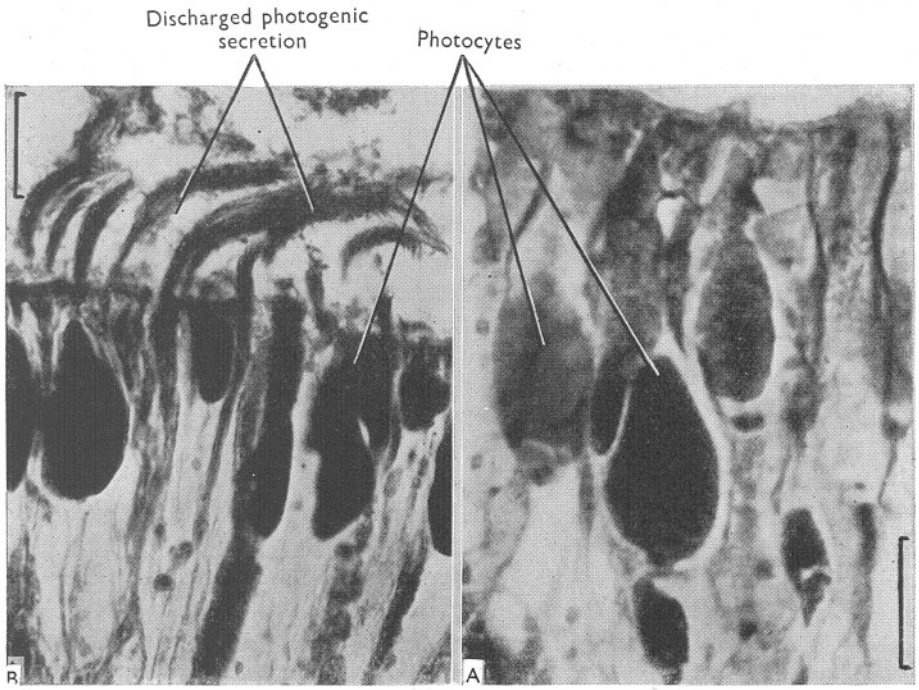


Fig. 24. A, section across an aliform notopodium to show some photogenic cells. Bouin fixation; impregnated with silver by Holmes's method. B, section across a notopodium of the posterior region to show photogenic cells caught in the act of secreting. Fixative, formol-sea water; stain, Heidenhain-azan.
The scale in both sections corresponds to 25 μ.

impregnated sheath about the photogenic material (Fig. 24A). The possibility that the protoplasmic investing layer contains fibrous oriented protein similar to that in muscle was considered, and sections were examined in polarized light. This material had been fixed in formalin or Bouin's fluid and was examined unstained. Under crossed Nicols and with a red gypsum plate the photogenic cells displayed no anisotropy although muscle fibres in the same sections were markedly birefringent. It was noted, however, that the protoplasmic layer about the photogenic cells in these unstained preparations was

strongly refractile. Moreover, careful examination of the epidermis has provided no evidence for the existence of myo-epithelial cells or epidermal muscle fibres.

This study, unfortunately, is inconclusive, but some preparations were obtained in which the photogenic cells were caught in the act of discharging their secretion (Fig. 24B). These sections give the impression that the photogenic material is being forcibly squeezed out of the cell. It is tentatively proposed that the protoplasmic investment of the photogenic cells is a contractile structure, capable of compressing and causing the evacuation of the photogenic cells when excited. The postulation of an organization of this kind permits the physiological results obtained in the present investigation to be interpreted as manifestations of the activity of contractile units, excited by nerve impulses, and such an interpretation permits comparison with results obtained in neuro-muscular physiology.

The augmented luminescent response obtained by increasing the number of stimuli or frequency of stimulation can be explained by a process of summation in the effector organ (light gland). Certain of the experiments were designed to reveal the possible existence of a facilitatory mechanism, with negative results. If facilitation were operating it would be expected that two stimuli would have an appreciably greater effect than one stimulus, even after allowing for partial exhaustion of the luminescent secretion. However, such an effect was not discovered.

The events that are considered to be taking place during stimulation of the photogenic glands on the aliform notopodia of *Chaetopterus* are depicted diagrammatically in Figs. 25 and 26, and are interpreted as follows. An electrical stimulus gives rise to a nerve impulse that activates the photogenic cells, causing them to contract, and results in the expulsion of enough secretion to produce visible luminescence. A single impulse causes a strong response that evacuates a large part of the cell contents. After this response has exhausted itself, a second impulse acting on the cell would initiate changes in the same response-mechanism, but since the paraplasmic contents of the cell are now only a fraction of their former level, the response will also appear as a fraction of the former intensity (Fig. 25). Subsequent stimuli, spaced at intervals so as to allow recovery of the effectors, should evoke responses of geometrically decreasing magnitude. This seems to be realized in actuality, and would explain the progress of fatigue under repetitive stimulation.

A series of impulses gives rise to a series of contractions which summate with one another to produce a heightened response (Fig. 26). A large part of the secretion would be expelled with the first contraction, and each subsequent contraction would have a relatively smaller effect. Two contractions then would produce only a little more secretion than a single contraction, and a whole series of contractions would be necessary to increase greatly the amount of luminescent material. Similarly, raising the frequency would

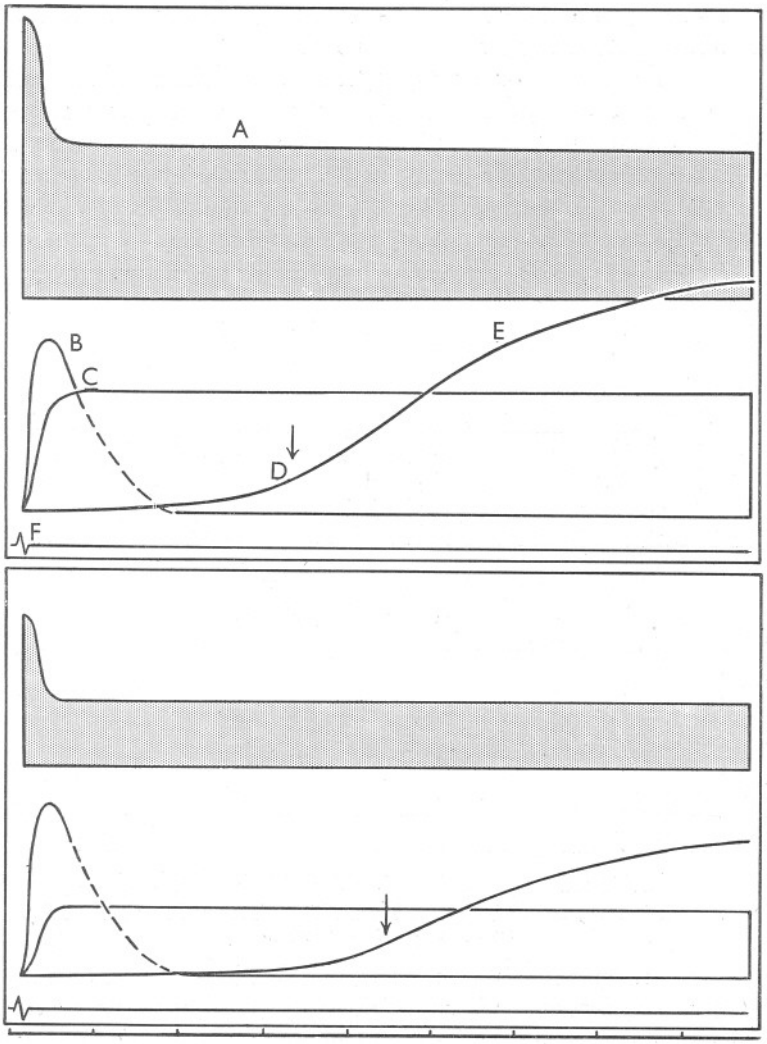


Fig. 25. Diagrams intending to illustrate the possible series of events taking place during the luminescent response of *Chaetopterus*. It is postulated that a stimulus (shown on the bottom line) excites the nerve fibres supplying the light gland and causes the gland cells to contract (illustrated as the first response curve on the left). This brings about the discharge of photogenic material and causes partial evacuation of the glandular cells. Both the secretion of photogenic material, and the residual amount remaining in the cells, are represented. Some time after the secretion of photogenic material, the photochemical reaction attains sufficient velocity to be revealed as a measurable luminescent response (threshold indicated by vertical arrow). A second impulse, after the effect of the first has died away, results in less secretion since the secretory content of the glandular cells is less. A, content of photogenic material in gland cells. B, contraction of gland cells. C, amount of photogenic material secreted. D, threshold sensitivity of recording apparatus. E, luminescent response. F, stimulus. Time scale, 1 per sec.

result in greater secretion since the individual contractions could summate before relaxation ensued or became advanced.

The rate of rise of the luminescent response curve remains to be dealt with. The slope of the curve becomes steeper as the frequency and number of impulses are raised, and it is related to the height of the response, i.e. an increase in the rate of rise of light intensity is followed by an increase in the maximal light intensity reached. This is probably due to the fact that as the contractile response becomes stronger, more secretion is poured out, and the

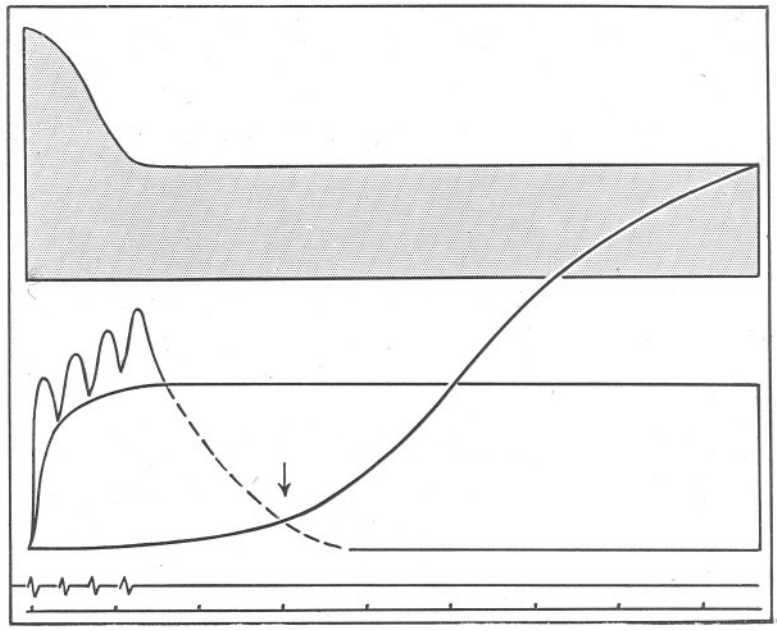


Fig. 26. Diagram, as in Fig. 25. A burst of impulses produces summation and augmented secretion.

velocity of the ensuing chemiluminescent reaction is governed by the initial concentration of the photogenic material. If this is so, it would be expected that the latent period would be reduced when the response becomes greater, and this is borne out by inspection of records of augmented responses. In Fig. 7A, for example, the latent period was reduced from 4·6 to 3·4 sec. when the duration of stimulation was prolonged from 0·2 to 5 sec.

The salient fact that emerges from this investigation is that the luminescent reaction is a triggered response. In the light glands of segment XII, a single impulse as well as a burst of impulses evokes luminescence. It may therefore be concluded that any effective natural stimulus, sufficient to excite sensori-neural pathways, will produce a response, without the necessity of reinforcement by repeated stimulation. In some specimens of *Chaetopterus* it has been possible to produce an enhanced effect with a second period of stimulation

at a higher frequency or duration, but in many of the animals investigated the second response was actually lower than the first, and fatiguing of the light response is an invariable accompaniment of repetitive stimulation. Moreover, recovery from fatigue is extremely slow, a matter of hours rather than minutes. The nature of the effector organ itself, therefore, precludes the possibility of the nervous system exercising any effective control over the levels of response on frequently repeatable occasions.

Although the magnitude of the initial response would depend on the conditions of stimulation, nevertheless, any excitatory stimulus results in the appearance of light. It has been suggested that luminescence in the aliform notopodia of *Chaetopterus* may be in the nature of a sacrifice lure associated with autotomy of the anterior region (cf. Joyeux-Laffuie, 1890), and, under this interpretation, the significance of luminescence as a triggered response becomes intelligible. It would be of value to the animal to produce a maximal amount of light initially, when stimulation reached or exceeded threshold, and actual variations in a few millilamberts at the low levels of brightness involved, would probably have little survival value as long as the response exceeded the visual threshold of the photoreceptor concerned.

In contrast to the events occurring peripherally, where a single impulse produces a pronounced luminescent response, the operation of facilitatory processes can be observed as the result of stimulating the nerve cord. These processes are manifested by an extension of the luminous area as the rate and duration of stimulation are raised. Since the response is observed as an extension of the field of luminescence involving more and more glandular units, it is not obscured by the intervention of glandular fatigue. The underlying mechanism seems to consist of a progressive increment of an excitatory state at synapses in the nerve cord. Such a mechanism could obviously provide a way of regulating the magnitude and extent of luminescence in posterior segments in response to natural stimulation, but the part that it might play in the normal life of the animal still awaits determination.

Summary and Conclusions

The nervous regulation of luminescence in *Chaetopterus* has been investigated by making use of controlled electrical stimulation and photoelectric recording. To achieve the latter a multiplier photocell has been used in conjunction with a galvanometer or oscilloscope, and camera.

A luminescent response is evoked in the aliform notopodia by a single stimulus or by a battery of stimuli. Due to the intervention of fatigue under repetitive stimulation it is difficult to obtain reproducible results, but in favourable preparations it has been possible to secure an enhanced response to increase in frequency and duration of stimulation above a rate of 1 per sec. Similar results were obtained by localized stimulation of the nerve cord, and

by peripheral stimulation of preparations from which the nerve cord had been removed. It is, therefore, concluded that the photogenic glands are being stimulated through their nerve fibres, and that the enhancement or augmentation of the response is not dependent upon the central nervous system, but takes place peripherally in the glandular tissue itself.

In experiments with two closely spaced stimuli no greater response was obtained than with a single stimulus. After making allowance for individual variation in the intensity of the response and partial exhaustion of the light glands following one initial stimulus, these results are interpreted as demonstrating that peripheral facilitation plays no part in the luminescent response. Any augmentation of the response due to prolonged or high frequency stimulation can be explained satisfactorily by summation in the effector organs.

The blocking effect of a number of anaesthetics on the luminescent response was determined. Chloretone, M.S. 222, stovaine, procaine, eucaine, cocaine and isotonic magnesium chloride all greatly diminish the luminescence which can be evoked by peripheral stimulation, and prevent excitation of the photogenic glands when the nerve cord is stimulated. Ether at 0·1 % had little effect. The small response under anaesthesia to peripheral stimulation could be progressively increased by raising the voltage. It is concluded from these results that the anaesthetics block nervous transmission while still allowing the glandular cells to respond directly to electrical stimulation. The assumption is made that the anaesthetics do not materially reduce the responsiveness of the glandular cells. It follows that peripheral stimulation achieves its effect in large part through nervous excitation, but a small amount of direct excitation of the gland cells is also involved.

Augmentation of the luminescent response also was obtained in narcotized specimens by increasing the number of stimuli. This is ascribed to summation in the photogenic cells as the result of direct stimulation.

The temporal characteristics of the response curves and the rate of rise of light intensity have been critically examined. It is found that under a higher frequency of stimulation or more prolonged stimulation there is an increase in the rate of rise of light intensity, as well as an increment of light intensity, and an increase in the total amount of light. The rate of rise is directly related to the intensity of the response. The latent period is also reduced by increasing the period of stimulation. The argument is developed that the response curve reveals the course of oxidation of extruded photogenic material, that light appears subsequent to the series of events involved in photogenic secretion, and that the luminescent curve is only an indirect indication of the magnitude of the events preceding it. The rate of rise of light intensity and the length of latent period are indices of the velocity of the chemiluminescent reaction, which in turn is governed by the initial concentration of the photogenic material.

Besides peripheral augmentation of the luminescent response, a process of central facilitation has been discovered in the nerve cord. This is most apparent in the posterior region of the body, and manifests itself as an increase in the number of segments responding as the duration or frequency of stimulation is raised. The threshold frequency lies at about 5 per sec. In addition, luminescence spreads posteriorly with greater facility than anteriorly, indicating greater resistance to postero-anterior transmission.

The effects of certain drugs on the luminescent response have been investigated. Atropine and *d*-tubocurarine fail to abolish the luminescence evoked by electrical stimulation. Acetylcholine does not appear to affect the magnitude of the response due to electrical stimulation, whereas eserine produces an enhanced response in a minority of specimens. In conjunction with the positive effect of acetylcholine in inducing luminescence in *Chaetopterus*, the augmentative effect of eserine provides suggestive evidence of a transmitter role for acetylcholine.

Histological examination of the photogenic glands has yielded inconclusive results. The epidermis is supplied with abundant nerves lying beneath the photogenic cells, and these fibres are well positioned to mediate secretion. The photogenic cells are surrounded by thin protoplasmic layers which stain like muscle, but lack birefringence which might be expected in fibrous protein. No myo-epithelial elements or muscle fibres were seen in the epidermis. The suggestion is made that the protoplasmic investment of the glandular light cells is contractile, and expresses the cellular contents. A mechanism of this kind explains the physiological results obtained in the present investigation.

The implications of some of these results on the physiology of bioluminescence and the nature of control in the normal life of the animal are discussed. In particular, it is pointed out that the luminous response of the aliform notopodia is a triggered response which can be produced by a single impulse. The spread of the response-area in the posterior region under increased stimulation raises the possibility of normal control of luminescence in that region of the body by alterations in the frequency and number of nerve impulses.

ADDENDUM

Since sending this paper to press I have had the opportunity of reading a paper by Hasama (1941) which describes experiments he has carried out on luminescence in *Chaetopterus variopedatus*. After faradic stimulation Hasama notes that the luminescent response lasts 10–40 sec. and that the duration of luminescence remains constant and independent of the duration of stimulation when the latter exceeds a certain time. No data are offered to support this rough generalization, which is merely a roundabout way of describing fatigue. With the use of non-polarizable electrodes this author has recorded potential

changes on the surface of the light-producing regions during luminescence. The resultant monophasic potentials appear to correspond with the luminescent response and are separable from muscle action potentials. Hasama believes that these potentials are electrical manifestations of the chemiluminescent reaction. Two kinds of cells are described in the epidermis of luminescent regions, viz. type 1 cells which contain granules staining well with toluidine blue, and poorly with eosin; and type 2 cells which stain with eosin and possess cilia. Hasama claims the first type are characteristic of luminescent regions, and are the photocytes, but for reasons which I have presented in earlier papers I believe that the photocytes are peculiar granular eosinophilic cells, lacking cilia. These cells are completely overlooked by Hasama. Harvey's recent book (1952) appeared too late to be consulted in the present work.

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