STUDIES ON *CHAETOPTERUS VARIOPEDATUS* (RENIER).
II. NERVOUS CONTROL OF LIGHT PRODUCTION

By J. A. Colin Nicol

Zoologist at the Plymouth Laboratory

(Text-figs. 1–9)

CONTENTS

The production or emission of light is under nervous control in many marine animals that have a differentiated nervous system. The nature of this control is a field of neuro-effector physiology that has been little explored, but one which could advance considerably our knowledge of the behaviour and functioning of luminescent marine animals. Nervous control of luminescence is achieved in diverse ways in different animals, namely, by squeezing forth a pre-formed secretion through muscular contraction, as appears to take place in *Cypridina*; by exposing a continuously luminescent organ through rotation or by movement of shutters, as in the teleosts *Photoblepharon* and *Anomalops*; by initiating cellular changes that lead to intracellular luminescence, for example in the photophores of the shrimp *Acanthephyra debilis*; and by directly activating light gland cells to secrete. The last method is a special example of nervous regulation of glandular secretion, and is the process occurring in the polychaete *Chaetopterus* (Dahlgren, 1916; Harvey, 1920, 1940).

Chaetopterus has long been known as one of the more brilliantly luminescent marine invertebrates, and several previous workers have investigated this animal and reported on certain features of light production. This work is summarized to form a background for the original observations that follow.

REVIEW OF PREVIOUS WORK

The luminous areas have been described and figured in a previous paper (Nicol, 1952). These are the peristomial tentacles, the surfaces of the aliform notopodia, a pair of photogenic glands on the dorsal basal surfaces of the aliform notopodia, the dorsal tubercle, fans, and notopodia of the posterior region. The luminous secretion produced by the photogenic glands on the aliform notopodia is suspended in mucus, and is carried away by ciliary currents to be distributed in the surrounding sea water. Luminescence in other regions is more transitory and localized (Bonhomme, 1943; Dahlgren, 1916; Fauvel, 1927; Joyeux-Laffuie, 1890; Lespès, 1872; McIntosh, 1915; Mangold, 1910–14; Panceri, 1878; Trojan, 1913, 1914; Will, 1844).

The light is blue in colour and has been shown by Lankester (1868) to have a continuous spectrum from about 440 to 530 mμ.

Light is produced in *C. variopedatus* only as the result of stimulation. The stimuli used to excite this worm, and indeed most other marine animals investigated experimentally, seem to have been selected randomly, in order to determine whether or not they were effective, rather than to reveal the mechanism of the response. It was observed that mechanical stimulation, such as results from disturbing the animal on removing it from its tube, or pinching or tapping it, causes the production of light. Effective chemical stimuli which have been used include the application of fresh water and strong reagents such as sublimate, formol and other fixing fluids. Raising the temperature of the sea water and applying electrical currents have also been employed to induce luminescence (Dahlgren, 1916; Joyeux-Laffuie, 1890; Panceri, 1878; Trojan, 1913).

There is a general belief that light production in *C. variopedatus* is under nervous control, but a curious absence of agreement about the details of the process. Panceri (1878) observed that the production of light was confined to the region of stimulation, and that there was no transmission of excitation, and this has been confirmed by Trojan (1913). According to Hempelmann (1934), increasing the strength of the stimulus (kind ?) causes the light to spread to other parts of the body and to become more widely distributed, but Dahlgren (1916) ascribes this effect to the spread of secretion through the water. Again, Stephenson (1946) has stated that stroking the animal causes waves of light to pass over the body. Finally, Harvey has noted that the light cells are probably under nervous control 'as a strong stimulus in one part of the body causes luminescence which spreads over the whole surface of the worm' (1920), and 'a wave of light when the head end is stimulated' (1940). The luminous secretion remains bright for about a minute after stimulation ceases, and then gradually diminishes in intensity. It has also been noted that the luminescent cells fatigue rather readily, and after a period of mechanical stimulation a rest of some hours is required

before the animal can be brought to luminesce again (Harvey, 1920; Panceri, 1878; Trojan, 1913).

There is agreement that luminescence is not inhibited by previous exposure to strong light. Panceri (1878) exposed specimens of *Chaetopterus* to sunlight, and then stimulated them in the dark, and he found that the ability to produce light was not suspended, in contrast to *Beroe* and other ctenophores in which such inhibition is quite pronounced (Panceri, 1872). According to Crozier (quoted by Harvey, 1920), luminescence in *Chaetopterus* is not affected by an exposure to 3000 metre-candles for 6 hr. The luminescent substances, luciferin and luciferase, which have been distinguished in marine ostracods, fire-flies (beetles), *Pholas* and *Odontosyllis*, have not been demonstrated in *Chaetopterus* (Dubois, 1887; Harvey, 1920, 1926, 1940, 1948). Neither do dried preparations of the animal luminesce on moistening. A luciferin-luciferase reaction may still be present, but may escape detection by biochemical methods hitherto employed. Harvey (1940) has reviewed this aspect of the subject.

Young free-swimming larvae of *Chaetopterus* are also capable of producing light (Dahlgren, 1916). Enders (1909), describing larvae about 1·5 cm. long and ready to metamorphose, states that luminosity is noticeable in the region of the ciliated rings, and in the mucus which the animals discharge. It is still more apparent in older larvae, where it appears in the anterior region and about the ciliated rings as the result of stimulation.

In another luminous chaetopterid, *Mesochaetopterus japonicus*, Fujiwara (1934, 1935) found that light is produced by the tentacles, by large areas of glandular epithelium on the dorsal surface of the first segment of the middle region, by a paired scyphiform organ on the second segment, by the dorsal ciliated region of the third segment of the middle region and by notopodia of the posterior region. These regions, in a general way, are similar to those of *Chaetopterus variopedatus*. Fujiwara's description (1935) of luminosity in this animal is difficult to follow. He states that brightest light is produced by the glandular epithelium of the first segment of the middle region, as in *Chaetopterus*. A luminescent secretion is also produced by cells in the walls of the dorsal ciliated groove, and the basal regions of the posterior notopodia are also luminous. He appears to imply that an animal removed from its tube shines continuously, but I interpret his account to mean that the luminescent secretion from the middle region of the body shines for some considerable time, but that light from notopodia of the posterior region, in contrast, appears in quick flashes. Moreover, the posterior segments flash alternately. Fujiwara further states that the luminescent activity can be increased by electrical, mechanical, or chemical stimulation. Electrical stimulation of the tentacles causes the appearance of light both in these structures, and in the luminescent organs of the posterior region. In *Mesochaetopterus japonicus*, therefore, it seems that excitation of luminescent

organs, induced electrically, is transmitted by the nervous system throughout the length of the body; sensitivity to stimulation leading to a luminous response appears to be most pronounced in the tentacles.

MATERIAL AND METHODS

Specimens of *Chaetopterus variopedatus* used in this investigation were maintained under circulation in the laboratory. Before use they were removed carefully from their tubes so as not to stimulate them to produce their luminous secretion before being experimented upon. This no doubt occurred on occasion, and any specimens which responded poorly to subsequent stimulation were discarded.
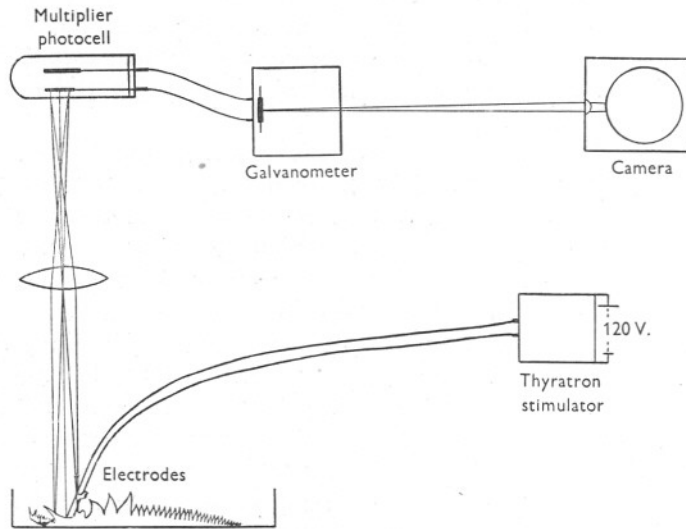

Fig. 1. Arrangement for investigating and recording luminescence, with the aid of a photocell and galvanometer.

Many of the experiments to be described merely depended on observing whether an animal did or did not luminesce, that is, whether the result was positive or negative. When it was necessary to obtain quantitative data, the light produced by the worm was focused on the photocathode of a photo-multiplier cell (RCA 931A). This was usually maintained at 1000 V., 100 V. per stage at which, according to specifications, it has a sensitivity of 3 A. per lumen and a magnification of 2×10^5 with a lamp of colour temperature 2870 K. The arrangement is shown in Fig. 1. As only comparative results within short time-periods were sought, no calibrations of the actual sensitivity of the cell were attempted. In most experiments the light from the basal glandular areas on segment XII only was measured. Photocurrent from the cell activated a moving-coil mirror galvanometer. This was a Tinsley

instrument having a resistance of 337 Ω; deflexion of 130 mm./μA. at 2·16 m.; and a periodic time of 2 sec. Light reflected from the galvanometer mirror fell upon a sheet of moving bromide paper in a camera travelling with a speed of 6 mm./min. Two other spot-lights were thrown on the same paper, one a time marker flashing once per minute, the other a stimulus marker, flashing when the stimulus key was depressed.

There was perceptible dark current in all records, and some drift of dark current base-line. At the beginning and end of each experiment, and at appropriate intervening intervals, the animal was covered so as to record the level of dark current. Some galvanometer records have been replotted to allow for basic level of dark current, drift, and any initial luminescence upon which the effect of a specific stimulus was superimposed.

Success was also attained in recording photocurrent with an oscilloscope, and this method was used when it was desirable to obtain maximal sensitivity, and fast records showing the initial phases of the luminescent reaction. The apparatus employed was a double beam oscilloscope with d.c. amplifier and attached camera.

Other particulars about methods used are described later under appropriate headings.

Observations

Effects of Mechanical Stimulation

Mechanical stimulation, by pulling, pinching, or tapping the animal, causes luminescence. As other observers have noted, light is produced, in consequence, from the peristomial tentacles, edges of the aliform notopodia, cup organ or dorsal tubercle, edges of the fans and notopodia of the posterior region. The response to stimulation tends to be very localized: thus pinching a fan or the cup organ of the middle region results in a faint flash of light in the organ directly stimulated. Similarly, mechanical stimulation of the posterior region causes the appearance of light in a few parapodia in the area directly affected.

It is more difficult to determine the effect of mechanical stimulation on the aliform notopodia of the XIIth segment. Light appears on the borders of the aliform notopodia as the direct result of pinching or touching these structures, and sometimes in the basal glandular areas on the dorsal surface as well. Luminous secretion appears from the latter glands as the direct result of touching them, or indirectly from pulling on the animal so as to produce tension in that region. No luminescent secretion is produced in the XIIth segment as the result of tactile stimulation of other parts of the middle region, and of the anterior and posterior regions, as long as the XIIth segment itself is not disturbed. Strong mechanical stimulation of the glands at the bases of the aliform notopodia causes a profuse discharge of luminous slime. It is probable that actual cellular destruction as well as indirect

nervous stimulation is involved in this display. As in other regions of the body, it appears from these results that the aliform notopodia and basal glands produce light only as the result of stimulation directly impinging on that segment. Light production in the aliform notopodia due to pulling or moving the animal seems to result from mechanical effects transmitted to the XIIth segment. There is, consequently, no definite evidence that mechanical stimulation in one region gives rise to a propagated excitation causing luminescence in more distant regions of the body. It is evident, however, that mechanical stimulation of *Chaetopterus* is very difficult to regulate quantitatively and spatially, and that more exact control can be exercised with electrical stimuli.

Effects of Electrical Stimulation

Direct current, faradic stimulation, and condenser discharges all produce luminescence. Quantitative studies were confined to the use of condenser discharges from a thyratron stimulator, since they allow careful control of strength, duration and frequency. Stimuli were delivered through a pair of platinum electrodes laid on the surface of the animal.
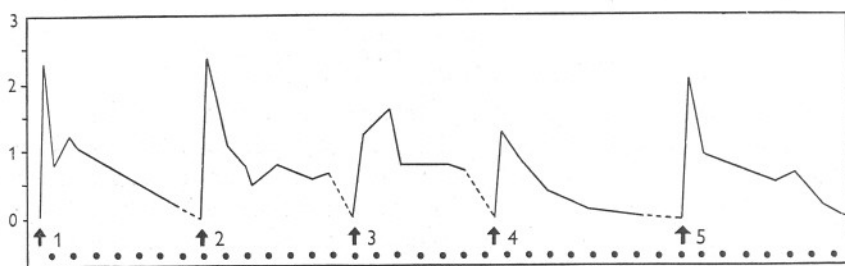

Fig. 2. Record of light produced by the photogenic glands on the aliform notopodia of *Chaetopterus* under electrical stimulation. Vertical arrows indicate application of stimuli. These were: 1 and 2, single stimuli; 3, two stimuli at 2 per sec.; 4, three stimuli at 3 per sec.; 5, one sec. burst at 5 per sec. Light intensity to the left in arbitrary units. Time scale below, 1 per min.

With the electrodes on the dorsal surface of the XIIth segment, a single electrical stimulus, above threshold, causes the production of luminescent secretion from the glands at the bases of the aliform notopodia (Fig. 2). The light appears quickly, within a few seconds of stimulation. Within a minute it begins to fade away, quickly at first, then more slowly, and becomes very dim and almost extinguished in 5 min.

The latent period and the time relations of the first phase of the luminescent response were determined from oscilloscope records (Fig. 3). In one series of experiments twenty animals were stimulated with a 2 sec. burst at 7 stimuli per sec. Latent periods measured from the beginning of stimulation varied from 3·0 to 4·4 sec., with a mean of 4·2 sec. The interval between first

deflexion and maximal height on the records ranged from 3·4 to 24 sec., with a mean value of 10·3 sec. Measurements made by eye (light-adapted) and a stop-watch gave a mean latent period of $2·6 \pm 1·8$ sec. (minimal value 1·5 sec.). The eye is more sensitive than the photocell, and this figure would be reduced further by dark adaptation of the observer. Animals in the dark, therefore, would become visible in about 2 sec., and would attain maximal luminescence in about 12 sec. after stimulation, the exact times depending on the animal.
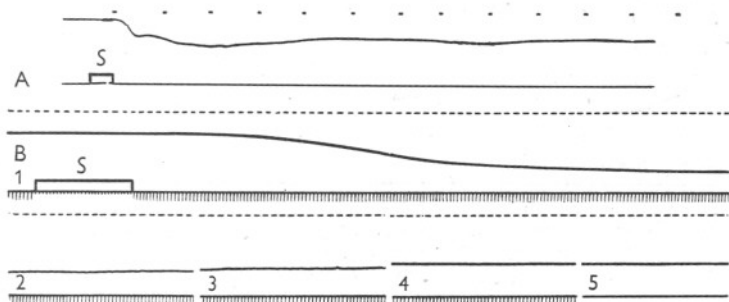

Fig. 3. Oscilloscope records of light produced by glands of aliform notopodia under electrical stimulation. A, 2 sec. burst of stimuli, at 9 per sec., at *S*. Time scale above, 1 per 5 sec. Latent period, 2·7 sec.; time to reach maximal intensity from first deflexion was 9·5 sec. B, 2 sec. burst at 14 per sec. at *S*. Time scale above, 1 per ⅕ sec. There are 5 sec. intervals between 1–2, 2–3, 3–4, 4–5. Latent period, 3·6 sec.; time to reach maximal intensity from first deflexion was 11·4 sec.
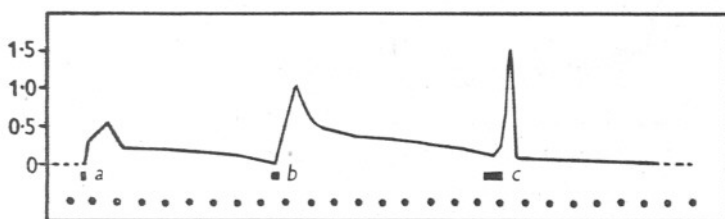

Fig. 4. Records of light produced by the photogenic glands on the aliform notopodia. Bursts of stimuli at 5 per sec. for 5 sec. at *a*, 30 sec. at *b*, and 1 min. at *c*. Stimulation indicated by dashes under record of light intensity. Time scale below, 1 per min.

The production of light by a single shock shows that there is a one-to-one relationship between single impulses and the light response: the arrival of an impulse at the neuro-effector junction excites the cell sufficiently to discharge. By increasing the number of stimuli, other factors, voltage and frequency, remaining constant, the light intensity and the amount of luminescent material secreted become greater. Fig. 4 is a record obtained in investigating this effect. Examination of the record shows that the initial luminescence increased greatly as the stimulation time was prolonged and the number of stimuli increased. With stimulation periods of 1:6:12, the

maximal intensity of luminescence resulting was 1:2:3. The total amount of
light produced lay in the ratio of about 1:2:0·6. Since the responses to the
second and third bursts of stimuli were certainly diminished by a fatigue
effect, the absolute luminescence which would have followed stimulation for
a given period, if diminution due to previous stimulation were excluded,
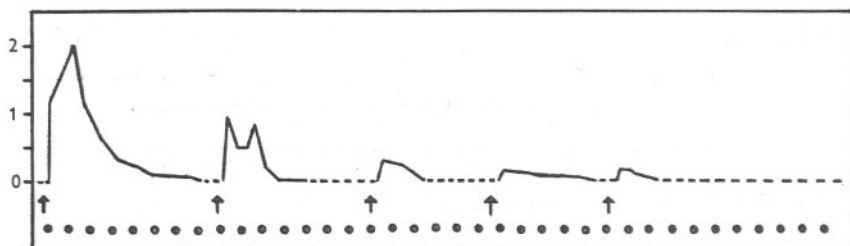probably would have been much greater.

Fig. 5. Record of light produced by the photogenic glands on the aliform notopodia under
repetitive electrical stimulation. Stimulation, indicated by vertical arrows under the
light record, consisted of 5 sec. bursts at 8 per sec. except the last, which had a duration
of 1 min. Time scale below, 1 per min. Intensity, to the left, in arbitrary units.
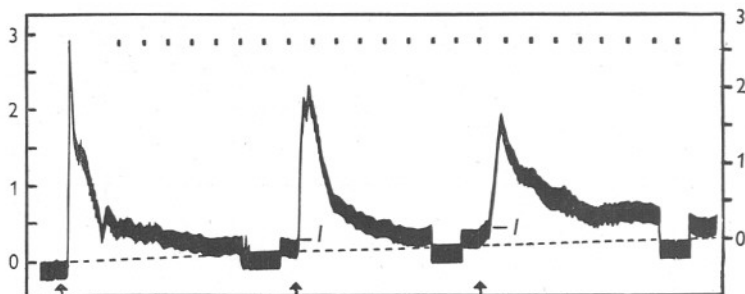

Fig. 6. Fatigue of luminescent power in the photogenic glands on the aliform notopodia.
Stimulation, indicated by triangles below, 5 sec. periods at 4 per sec. The dotted line
represents the level of dark current; I, indicates the initial level of residual luminescence
on which a new response is superimposed. Time scale above, 1 per min. Light intensities
to left and right in arbitrary units.

The light-producing glands soon fatigue on repeated stimulation, and this
makes the interpretation of results more difficult. Information about the onset
and course of fatigue was obtained by subjecting specimens to successive
bursts of electrical stimuli. Figs. 5 and 6 show records obtained from two
animals. In Fig. 5 the animal was stimulated by a succession of 5 sec.
bursts at 8 per sec., except the last which lasted 1 min. An interval of about
6 min. between bursts permitted the luminescence to fade. It will be seen
that each successive burst resulted in a fall in light production as the gland
fatigued. Fig. 6 shows a similar result.

Recovery from fatigue was not observed in experimental animals. Fig. 7
shows the result of an experiment on an animal in which rest periods of

1 and 3 hr. intervened between successive periods of stimulation. In these times the response failed to recover to any extent, and much less luminescent material was secreted than at the beginning of the experiment. Other workers, however, have noted some recovery of luminescent ability after a rest period of several hours (see p. 434), but their observations were subjective. In any event, it is certainly a very slow process, and demonstrates that luminescence cannot function as a frequently repeatable event in the normal economy of this species.
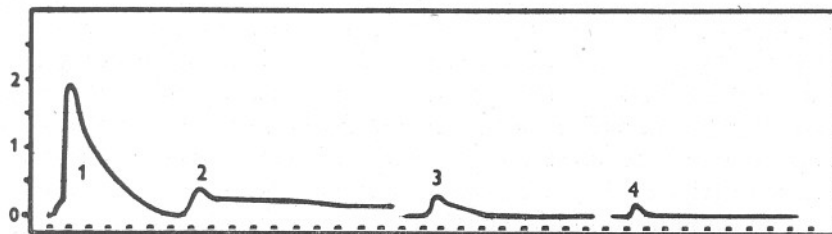

Fig. 7. Record of light produced by photogenic glands on the aliform notopodia. Fatigue in light-producing ability as the result of repeated electrical stimulation is demonstrated. 1, 2, 3, 4, light produced by 30 sec. periods of stimulation at a frequency of 2 per sec. Pause of 7 min. between 1 and 2; 1 hr. between 2 and 3; and 3 hr. between 3 and 4. Time scale below, 1 per min. Intensity, to the left, in arbitrary units.
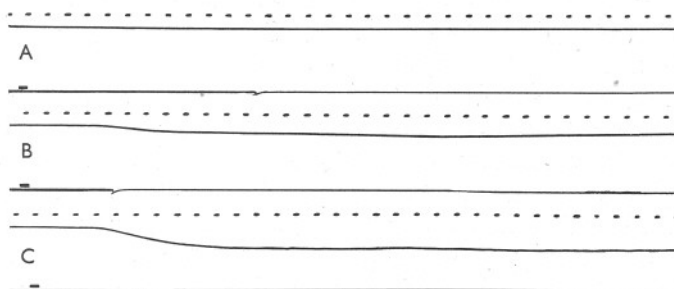

Fig. 8. Effect of voltage increases on the luminescent response. Oscilloscope records of light produced by the aliform notopodia. Stimulation consisted of 0·5 sec. bursts at 25 per sec., A, at 20 V., B, at 40 V., and C, at 60 V. Stimulation indicated below each record by a short horizontal line; time scale above each record, 1 per sec.

Gradually increasing the voltage, other factors remaining constant, increases the light response. Fig. 8 shows the result of an experiment in which the voltage was gradually increased, stepwise. For each rise in stimulus strength above threshold there is a corresponding increase in the resultant light intensity. The effect is probably due to the stimulation of more and more nerve fibres as the electric current becomes more widespread.

At a low frequency of stimulation (1·5 per sec.) light is confined to the region stimulated. Thus, when the stimulating electrodes are applied to the ventral surface of segment XII, against the nerve cord, and low frequency

stimulation is applied, light appears in the aliform notopodia of that segment only. Stimulation of segments XIII, XIV, XV, or XVI, results in luminescence of the dorsal tubercle or fan in the segment stimulated (Fig. 9). Stimulation of the posterior region results in the appearance of light in a few (2–3) pairs of notopodia in the neighbourhood of the electrodes. Stimulation of segments anterior to XII at a low frequency has no effect. At a higher frequency, beginning at about 6 per sec., the excitation spreads to other segments, and the area affected increases as the frequency is raised. Thus, when the ventral surface of the posterior region is stimulated at frequencies of 6–9 per sec., the notopodia of about 10 posterior segments in the region of the electrodes luminesce. Further increase in frequency causes all notopodia of the posterior region to luminesce. Similarly, stimulation of the ventral surface of segments III–V at frequencies of 6 or more per sec. causes the aliform notopodia to luminesce. In some animals stimulation of the anterior region at high frequencies (9 or more per sec.) causes the excitation to be transmitted to middle and posterior regions of the body, as well. This was observed in only certain animals, however, and often excitation was confined to the anterior, middle, or posterior region, whichever was stimulated.

Transmission of excitation in these experiments is through the nerve cord. When the nerve cord is cut, and the animal is stimulated at a high frequency on one side of the incision, the excitation fails to jump the gap.

These observations point to the existence of internuncial facilitation in the nerve cord of *Chaetopterus*. The transmission of excitation from segment to segment presumably depends upon linked neurones arranged in series along the cord, and in connexion with this thesis it may be noted that giant axon systems are wanting in the nerve cord of *Chaetopterus*. In the nervous pathways involved in luminescence, single impulses, or impulses at low frequencies, are not transmitted from one neurone to the next along the cord. When the frequency is raised above a critical level, however, augmentation occurs at intervening synapses, and impulses can sweep through to successive segments.

Effects of Autonomic Drugs

The cholinergic nature of the nerve fibres innervating certain glands in mammals is well established, and acetylcholine has been implicated as a chemical transmitter in the submaxillary and adrenal glands. Chemical transmitters have also been proposed in connexion with certain observations on neuro-muscular functioning of various annelids (Bacq, 1947; Botsford, 1941). It is of interest, therefore, to determine whether similar processes are involved in the nervous control of light production in *Chaetopterus*. Towards this end, the pharmacological effects of several autonomic drugs were tried on this animal.

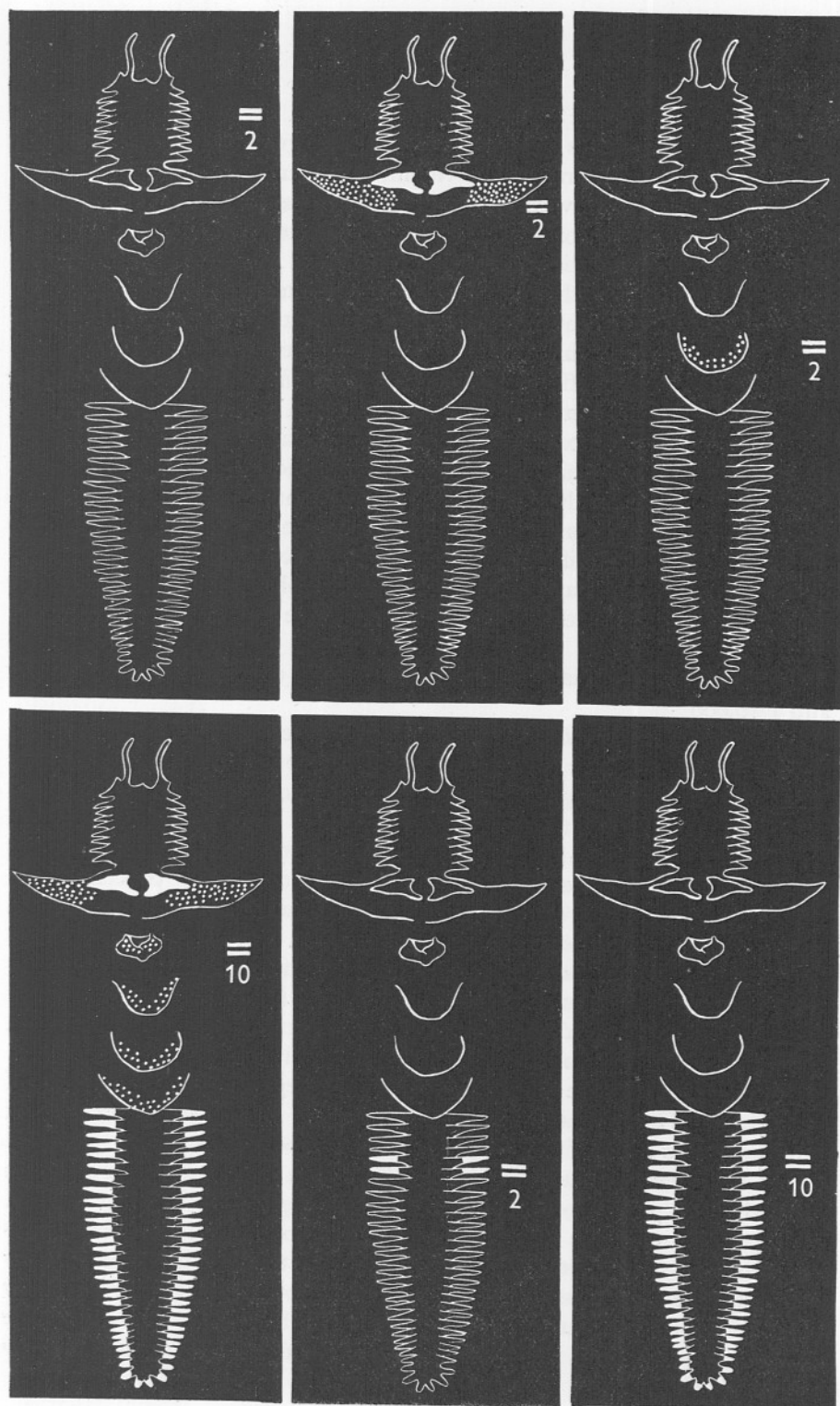The drugs employed were adrenaline, acetylcholine, nicotine, and eserine

Fig. 9. The effects of stimulating the nerve cord at various frequencies. The double bar indicates the position of the stimulating electrodes. The numbers refer to the frequency of stimulation. Note the tendency for the excitation to spread as the frequency is raised.

(physostigmine salicylate). They were dissolved in sea water, in concentrations which are given as weight by volume, except for nicotine where the figures refer to concentrations in volume by volume. The solutions were applied in one of three ways: an intact animal was placed in a solution of the drug to be tested; or excised pieces of potentially luminescent tissues were placed in the solution; or solutions of drugs were injected into intact animals. The results of these experiments are summarized as follows.

Adrenaline. In concentrations of 1/1000 and 1/10,000 this drug failed to induce luminescence. This confirms an earlier observation of Harvey (1931) that adrenaline has no effect on luminescence in *Chaetopterus*.

Acetylcholine. Five intact animals were placed in a solution of acetylcholine (1/10,000), and one responded by luminescing brightly in all regions after intervals of 4–17 min., depending on the region lighting up. Injecting 0·1 or 0·2 c.c. of acetylcholine (1/100 to 1/5000) into the anterior or middle regions caused the appearance of flashes or persistent weak light in the fans, dorsal tubercle, and aliform notopodia. Pieces of the animal containing light-producing tissues and nerve cord shone feebly in solutions containing 1/1000 acetylcholine. Other pieces, from which the nerve cord was removed, behaved similarly.

Eserine. This drug had no apparent effect in concentrations of 1/1000 and 1/10,000.

Eserine and Acetylcholine. These two drugs, used together (both in concentrations of 1/1000 or 1/10,000) failed to evoke luminescence in isolated pieces of light-producing tissues. Injection of 0·2 c.c. of 1/1000 eserine and 1/1000 acetylcholine into the fans and anterior region resulted in some transitory flashes of light from the fans, dorsal tubercle, and aliform notopodia.

Nicotine. When tested on intact animals nicotine, in concentrations 1/1000 and 1/10,000, evoked a faint brief appearance of luminescence in about half the animals tested.

It is apparent from these results that sympathomimetic and parasympathomimetic drugs, which are frequently effective in inducing activity in other animals, have slight and rather inconsistent effects on light production in *Chaetopterus*. Acetylcholine and nicotine definitely induce luminescence in some animals, but the effect is somewhat irregular in occurrence and distribution. There is no evidence that eserine is effective by itself, or that it enhances the effect of acetylcholine in these experiments.

It is interesting that acetylcholine is effective in inducing luminescence in isolated pieces of light-producing tissues lacking central nervous system. This provides evidence that the drug is acting peripherally on the neuroglandular junction, although it does not exclude the possibility that it may be acting at other loci as well. The action of nicotine recalls its stimulatory effect on cholinergic neurones of other animals. The light produced by these drugs is usually rather weak and ephemeral, and probably reflects a slow

rate of diffusion of the acetylcholine or nicotine through the tissues of the body wall. In general, it appears that acetylcholine exerts a nicotine-like action on the nervous system of this worm, and stimulates the neuro-effector junctions in the photogenic tissues.

Autonomic drugs have, of course, been used in the study of the luminescence of other animals. Adrenaline evokes the appearance of light in the photophores of the deep-sea fish *Echiostoma ctenobarba*, and of the midshipman *Porichthys notatus* from shallow waters (both teleosts) (Greene & Greene, 1924; Harvey, 1931, 1940). Adrenaline also produces a constant glow lasting several hours in the lampyrid *Photuris pennsylvanica* (Creighton, 1926; Emerson & Emerson, 1941.)

It is doubtful whether any useful comparisons can be made of the action of drugs on bioluminescence *per se* in such widely unrelated animals as those just mentioned, without first assembling detailed information about the action of those drugs on the nervous system and various effectors in each particular form. Adrenaline, for example, is a normal constituent of fish and is produced by suprarenal tissue. Its effectiveness in producing luminescence in *Porichthys* and *Echiostoma* suggests that the nerve fibres supplying the photophores in teleosts are adrenergic, and that the secretion of adrenaline into the blood stream may also be a factor in evoking luminescence. Chromaffin tissue, on the other hand, is rare in polychaetes (Bacq, 1947; Gaskell, 1914). There is now some evidence that cholinergic fibres are widespread in annelids. Acetylcholine brings about muscular contractions in *Arenicola*, *Branchiomma*, *Hirudo* and *Lumbricus*, and eserine augments and prolongs the muscular response to electrical stimulation in some of these animals (Bacq, 1947; Bacq & Coppée, 1937; Katz, 1949; Wu, 1939). Tonic contracture is induced in the isolated extrovert of *Arenicola* by acetylcholine, presumably through excitation of the oesophageal pace-maker (Wells, 1937), and a cholinesterase has been identified in the body wall of *Myxicola* (Bacq, 1937). The positive response of acetylcholine on bioluminescence in *Chaetopterus* fits into this picture, and suggests that cholinergic neurones are involved in the light response.

Effects of Various Cations and Unbalanced Salt Solutions

The effects of various cations on light production were investigated by immersing whole animals in solutions of the several salts. These were all chlorides of Na, K, Ca and Mg. The salts were employed singly and in different combinations to reveal their balancing or antagonistic effects. The solutions, listed below, were used as made up, and also after readjustment of the pH to 8·2 by the addition of NaOH, KOH, HCl, or MgO, as necessary. Variations in pH from 7 to 9 were found not to be a limiting factor in these experiments. Each cation or combination of cations was tried on at least three animals.

1. 0·54 M-NaCl   2. 0·54 M-KCl
3. 0·36 M-CaCl$_2$ 4. 0·36 M-MgCl$_2$
5. 0·54 M-NaCl, 25 vol. + 0·54 M-KCl, $\frac{1}{2}$ vol.
6. 0·54 M-NaCl, 25 vol. + 0·36 M-CaCl$_2$, $\frac{1}{2}$ vol.
7. 0·54 M-NaCl, 25 vol. + 0·36 M-MgCl$_2$, 2 vol.
8. 0·54 M-KCl, 25 vol. + 0·54 M-NaCl, $\frac{1}{2}$ vol.
9. 0·54 M-KCl, 25 vol. + 0·36 M-CaCl$_2$, $\frac{1}{2}$ vol.
10. 0·54 M-KCl, 25 vol. + 0·36 M-MgCl$_2$, 2 vol.
11. 0·54 M-NaCl, 25 vol. + 0·54 M-KCl, $\frac{1}{2}$ vol. + 0·36 M-CaCl$_2$, $\frac{1}{2}$ vol.
12. 0·54 M-NaCl, 25 vol. + 0·54 M-KCl, $\frac{1}{2}$ vol. + 0·36 M-MgCl$_2$, 2 vol.
13. 0·54 M-NaCl, 25 vol. + 0·54 M-KCl, $\frac{1}{2}$ vol. + 0·36 M-CaCl$_2$, $\frac{1}{2}$ vol. + 0·36 M-MgCl$_2$,
    2 vol.

Certain cations and combinations of cations had marked stimulatory effects, as the following summaries of the experimental protocols show.

1. *Isotonic* NaCl. Addition of this solution caused luminescence in most or all of the notopodia of the posterior region, followed by weak lighting of the fans in the middle region, and in the aliform notopodia and tentacles.

2. *Isotonic* KCl. This solution immediately caused brilliant and lasting luminescence of all areas: posterior notopodia, dorsal tubercle and fans of the middle region, aliform notopodia and peristomial tentacles. A copious secretion was also discharged from the glands at the bases of the aliform notopodia. This brilliant illumination lasted about 15 min., and then faded away.

3. *Isotonic* CaCl$_2$ had no apparent effect.

4. *Isotonic* MgCl$_2$ had no apparent effect.

5. *Isotonic* NaCl + KCl. This solution produced a bright display almost at once from the notopodia of the posterior region, the dorsal tubercle, and the aliform notopodia.

6. *Isotonic* NaCl + CaCl$_2$. In two specimens a few faint pin-points of light appeared momentarily on some notopodia of the posterior region. Two other specimens were negative.

7. *Isotonic* NaCl + MgCl$_2$. This solution caused luminescence of posterior notopodia, fans, dorsal tubercle, aliform notopodia, and the glands at the bases of the aliform notopodia. The light appeared after about 2 min., was usually brief and rather faint, and affected different specimens in varying degree.

8. 9, 10. *Isotonic* KCl + *either* NaCl, or CaCl$_2$, or MgCl$_2$. Each of these solutions caused a bright and lasting glow from all luminescent areas, and a copious amount of luminescent secretion from the glandular areas at the bases of the aliform notopodia.

11. *Isotonic* NaCl + KCl + CaCl$_2$. Addition of this solution produced a few faint points of light in the posterior notopodia of one specimen, but had no effect on three others.

12. *Isotonic* NaCl + KCl + MgCl$_2$. This solution caused some notopodia in the posterior region of five specimens to glow brightly, and in two animals evoked brief bright flashes in the alar notopodia, and a brief glow from the dorsal tubercle and glands at the bases of the aliform notopodia as well.

13. *Isotonic* NaCl + KCl + CaCl$_2$ + MgCl$_2$. This solution had no visible effect.

The stimulatory effect of both sodium and potassium on nerve and muscle is well known, and has been reviewed, among others, by Heilbrunn (1943). Sodium stimulates the cardiac ganglion of *Limulus*, and renews rhythmic pulsations in Scyphomedusae on removal of the marginal sense-organs. Potassium stimulates the marginal sense-organs of Scyphomedusae, excites

the isolated extrovert of *Arenicola*, and generally stimulates nerve cells and nerve fibres. Magnesium usually exerts a depressive effect and is a general anaesthetic for invertebrates, and calcium, in small amounts, offsets the toxic properties of sodium and potassium (Brown & MacIntosh, 1939; Hodgkin & Huxley, 1945; Rosenblueth, 1950; Wells & Ledingham, 1942).

Studies dealing with the effects of ions on the luminescent response of Metazoa have been confined, hitherto, to coelenterates and ctenophores. There is general agreement that in Scyphomedusae and in ctenophores potassium and calcium evoke and augment luminescence, which fails when these ions are omitted. Sodium, by itself, has little effect. Magnesium reduces luminescence, but when this ion is absent the ability to luminesce is increased (Hykes, 1928; Heymans & Moore, 1924, 1925; Moore, 1926). In general, potassium and calcium are stimulatory in these animals, and the effect is offset by magnesium. These results are somewhat divergent from those obtained with *Chaetopterus*. In this animal potassium has the greatest stimulatory effect, evoking bright luminescence of all regions, and it is seconded by sodium. By itself calcium evokes no luminescence and it fails to suppress the luminescence evoked by potassium. The addition of calcium to a solution of potassium and sodium, however, results in a more balanced physiological solution that fails to stimulate. Magnesium also reduces the stimulatory powers of sodium and potassium. These effects are referred to stimulation of the nerve cells and nerve fibres supplying the luminescent gland cells by sodium and potassium, rather than to any action of these ions directly on the glandular cells. Even after a period of maximal glow evoked by the application of KCl it is still possible to produce the release of further luminescent secretion by direct mechanical stimulation of the photogenic cells in the aliform notopodia. This shows that the glandular cells are still intact and contain a considerable residual amount of photogenic material. Calcium and magnesium, in turn, oppose the stimulatory action of potassium and sodium on the nervous elements.

Effects of Hypotonic and Hypertonic Solutions

Many of the older accounts dealing with bioluminescence refer to the stimulatory effect of fresh water in evoking a luminous response. Panceri (1872, 1878), for example, in studies dealing with the luminescence of marine animals, consistently made use of fresh water to bring about the production of light. This earlier work has been followed up by investigating the effects of various hypotonic and hypertonic solutions of sea water on light production in *Chaetopterus*. To test the effect of a solution, an animal was placed in a dish, dorsal side uppermost, and all sea water was drained off; the solution in question was then added to the dish in sufficient quantity to submerge the animal. The effect of each solution was tested on four or more animals.

Hypotonic Solutions

(1) Distilled water evoked luminescence in about 1 min. Light appeared at first towards the posterior end of the worm, and spread anteriorly to involve the fans, dorsal tubercle, and aliform notopodia.

(2) 20, 30, 40 % sea water. All these solutions caused luminescence in about 1 min. The posterior notopodia lighted first, and the effect gradually spread orally to involve more anterior luminescent regions (fans, dorsal tubercle, aliform notopodia), in varying degree, depending on the animal.

(3) 50 % sea water. This solution evoked luminescence in the posterior notopodia of two out of four animals examined.

(4) 60 % sea water. Three animals gave no response. In a fourth animal there were a few weak flashes from notopodia of the posterior region and from the aliform notopodia after about 1 min.

(5) 70 and 90 % solutions of sea water were without effect.

Hypertonic Solutions

Hypertonic sea water, at strength 150 %, was tried on six specimens. The concentrated sea water was prepared by boiling, and the pH was returned to 8·2 with sodium bicarbonate. It failed to induce luminescence.

These experiments reveal that diluted sea water first evokes luminescence at a strength of about 60 %. The effect increases with greater dilutions, and reaches a maximum in distilled water. Hypertonic sea water (150 %) has no effect.

The mode of action of hypotonic solutions is not clear. They may excite luminescence by a direct osmotic effect, causing cytolysis of the glandular cells; or they may act by differential leaching of ions from nervous elements, giving rise to nervous excitation. *Chaetopterus* is not an estuarine form, and would not be expected to possess any power of osmotic regulation. A tendency is noted for the posterior notopodia to lighten first in hypotonic solution, showing that the profuse mucous secretion of the anterior and middle regions exerts some protective effect.

DISCUSSION AND CONCLUSIONS

The present investigation demonstrates that luminescence in *Chaetopterus* is under nervous control. Under localized tactile stimulation light appears only in a restricted area, and even severe injury, amounting to mutilation, fails to evoke widespread luminescence.

Information about the transmission processes involved in the luminous responses is revealed by electrical stimulation of the animal. It is found that low frequencies give a very localized response, confined to a segment or a few segments stimulated, but as the frequency is raised, excitation is transmitted to other regions, until finally the whole animal may lighten. It is

concluded that the spread of excitation is a process depending upon the existence of internuncial facilitation in the nerve cord. Through pathways for the luminescent response appear to be lacking, and transmission through the central nervous system involves the build up of an excitatory state at synapses with the arrival of successive stimuli.

The results obtained by electrical stimulation suggest certain ways in which the luminescent response can be regulated. The latter is in some way dependent on the degree of peripheral stimulation. Increased tactile stimulation, presumably, will evoke a higher frequency of discharge, and maintained stimulation a prolonged volley in sensory pathways. At least two factors are operating on the efferent side which determine the magnitude of the response, namely number of neuro-effector units brought into activity and number of nervous impulses discharged. Increase in either the number of efferent stimuli or the number of gland cells excited will result in more light. The significance of the high frequency effect on central nervous transmission in the normal functioning of the animal is more doubtful, but it may represent a mechanism whereby prolonged and vigorous tactile stimulation will bring glandular cells of more distant regions into activity.

There are few comparable studies on other species for comparison. The interesting physiological investigations on nervous control of luminescence in coelenterates and ctenophores show features peculiar to those groups, and necessitate interpretation in terms of the nerve-net existing in those forms. Fujiwara (1935) has shown in *Mesochaetopterus* that electrical stimulation causes the transmission of an excitatory process along the body, resulting in luminescence. Thus, there are certain physiological features common to two genera of chaetopterids, *Chaetopterus* and *Mesochaetopterus*. Other patterns of nervous control in polychaetes are suggested from studies of luminescence in polynoids. Thus in *Acholoë* it has been found that light tactile stimulation gives rise to a local luminescent response, but strong stimulation evokes more widespread luminescence. Transection of the animal causes the posterior fragment to luminesce, but not the anterior piece (Kutschera, 1909). One explanation advanced is that the excitation can only be transmitted caudally, but the reason for this restriction is not clear, and further physiological studies on polynoids and other luminescent forms would be of great interest.

Summary

The nervous control of luminescence in *Chaetopterus variopedatus* was investigated by various direct and indirect means. Quantitative measurements of the light produced by the animal were obtained by the use of a multiplier photocell and sensitive galvanometer or oscilloscope.

Tactile or mechanical stimulation gives a rather localized luminescent response. The most effective stimuli for evoking luminescence of the conspicuous light glands of the aliform notopodia are tension and pressure acting on segment XII.

Electrical stimulation of segment XII (condenser discharges) causes the appearance of light in the glands on the aliform notopodia. The mean latent period, as determined from oscilloscope records, was 4·2 sec., and the time taken to reach maximal intensity from first deflexion was 10·3 sec.

A single shock causes the appearance of light, and the intensity of the response is increased by increasing the voltage or the number of stimuli. Raising the voltage throws more efferent neurones into activity. Augmenting the number of stimuli results in an enhanced effect, and more luminescent material is secreted.

The preparation fatigues readily, and successive periods of stimulation give greatly decreased responses. No recovery was observed under experimental conditions after a rest period of 3 hr.

At low frequencies of stimulation the response is restricted to the segment or few segments directly stimulated. Raising the frequency brings more segments into luminescence. This effect is ascribed to some process of internuncial facilitation in the nerve cord.

The effects of four autonomic drugs were tried. Adrenaline had no effect. Acetylcholine and nicotine were weakly excitatory. Acetylcholine produced luminescence in fragments lacking central nervous tissue as well as in pieces containing nerve cord; it has an excitatory effect, therefore, on peripheral elements. There was no noticeable augmentatory effect with eserine. The evidence suggests a nicotine-like action of acetylcholine acting peripherally.

Isotonic solutions of NaCl, KCl, $CaCl_2$ and $MgCl_2$ were tried on the preparation. K and Na were excitatory, particularly the former. Addition of Mg reduced the excitatory effect of K and Na, and Ca inhibited the luminescence usually evoked by K and Na salts. A balanced salt solution, containing K, Na, Ca, and Mg, in the proportions in which they occur in sea water, failed to produce luminescence. The excitatory effects of K and Na, and the opposing effects of Ca and Mg, are ascribed to stimulation of nervous elements concerned with luminescence.

Hypotonic solutions and fresh water are stimulatory, luminescence appearing in about 60% sea water. The effect is not clear; it may act through cytolysis of glandular cells, or by differential leaching of ions.

Hypertonic solutions (150% sea water) have no excitatory effect.

A review of the literature dealing with luminescence in *Chaetopterus* is given, and the physiological results obtained in the present investigation are compared with observations on several other forms.

REFERENCES

BACQ, Z. M., 1937. Nouvelles observations sur l'acétylcholine et la choline-estérase chez les Invertébrés. *Arch. Internat. Physiol.*, T. 44, pp. 174–89.

—— 1947. L'acétylcholine et l'adrénaline chez les Invertébrés. *Biol. Rev.*, Vol. 22, pp. 73–91.

BACQ, Z. M. & COPPÉE, G., 1937. Réaction des Vers et des Mollusques à l'éserine. Existence de nerfs cholinergiques chez les Vers. *Arch. Internat. Physiol.*, T. 45, pp. 310–24.

BONHOMME, C., 1943. L'appareil lumineux de *Chaetopterus variopedatus* Clap. Recherches histologiques. *Bull. Inst. Océanogr. Monaco*, No. 843, 7 pp.

BOTSFORD, E. F., 1941. The effect of physostigmine on the responses of earthworm body wall preparations to successive stimuli. *Biol. Bull. Woods Hole*, Vol. 80, pp. 299–313.

BROWN, G. L. & MacINTOSH, F. C., 1939. Discharges in nerve fibres produced by potassium ions. *Journ. Physiol. (Proc.)*, Vol. 96, 10P–11P.

CREIGHTON, W. S., 1926. The effect of adrenalin on the luminescence of fireflies. *Science*, Vol. 63, pp. 600–1.

DAHLGREN, U., 1916. The production of light by animals. *Journ. Frank. Inst.*, Vol. 181, pp. 659–96, 804–43.

DUBOIS, R., 1887. De la fonction photogénique chez le *Pholas dactylus*. *C.R. Acad. Sci. Paris.*, T. 105, pp. 690–2.

EMERSON, G. A. & EMERSON, M. J., 1941. Mechanism of the effect of epinephrine on bioluminescence of the firefly. *Proc. Soc. Exp. Biol.*, N.Y., Vol. 48, pp. 700–3.

ENDERS, H. E., 1909. A study of the life history and habits of *Chaetopterus variopedatus* Renier et Claperède. *Journ. Morph.*, Vol. 20, pp. 479–531.

FAUVEL, P., 1927. *Polychètes sédentaires.* Faune de France, Vol. 16. Paris.

FUJIWARA, T., 1934. On a new chaetopterid, *Mesochaetopterus japonicus* sp.nov. *Journ. Sci. Hiroshima Univ.*, Ser. B, Div. 1, Zool., Vol. 3, pp. 1–14.

—— 1935. On the light production and the luminous organs in a Japanese chaetopterid, *Mesochaetopterus japonicus* Fujiwara. *Journ. Sci. Hiroshima Univ.*, Ser. B, Div. 1, Zool., Vol. 3, pp. 185–92.

GASKELL, J. F., 1914. The chromaffine system of annelids and the relation of this system to the contractile vascular system of the leech, *Hirudo medicinalis*. *Phil. Trans. Roy. Soc.*, B, Vol. 205, pp. 153–211.

GREENE, C. W. & GREENE, H. H., 1924. Phosphorescence of *Porichthys notatus*, the California singing fish. *Amer. Journ. Physiol.*, Vol. 70, pp. 500–6.

HARVEY, E. N., 1920. *The Nature of Animal Light.* Philadelphia and London.

—— 1926. Bioluminescence and fluorescence in the living world. *Amer. Journ. Physiol.*, Vol. 77, pp. 555-61.

—— 1931. Stimulation by adrenalin of the luminescence of deep-sea fish. *Zoologica*, N.Y., Vol. 12, pp. 67–9.

—— 1940. *Living Light.* Princeton, N.J.

—— 1948. Introductory remarks: a general survey of bioluminescence. *Ann. N.Y. Acad. Sci.*, Vol. 49, pp. 329–36.

HEILBRUNN, L. V., 1943. *An Outline of General Physiology.* Philadelphia and London.

HEMPELMANN, F., 1934. Polychaeta. In Kükenthal's *Handbuch der Zoologie*, 2 Bd., 2 Hälfte. Leuchten, S. 131–3. Berlin u. Leipzig.

HEYMANS, C. & MOORE, A. R., 1924. Luminescence in *Pelagia noctiluca. Journ. Gen. Physiol.*, Vol. 6, pp. 273–80.

—— —— 1925. Note on the excitation and inhibition of luminescence in *Beroe. Journ. Gen. Physiol.*, Vol. 7, pp. 345–8.

HODGKIN, A. L. & HUXLEY, A. F., 1945. Resting and action potentials in single nerve fibres. *Journ. Physiol.*, Vol. 104, pp. 176–95.

HYKES, O. V., 1928. Contribution à la physiologie de la luminescence et de la motilité des Coelentérés. *C.R. Soc. Biol.*, T. 98, pp. 259–61.

JOYEUX-LAFFUIE, J., 1890. Étude monographique du Chétoptère (*Chaetopterus variopedatus*, Renier) suivie d'une revision des espèces du genre *Chaetopterus. Arch. Zool. Exp. Gén.*, T. 8, pp. 245–360.

KATZ, B., 1949. Neuro-muscular transmission in invertebrates. *Biol. Rev.*, Vol. 24, pp. 1–20.

KREKEL, A., 1920. Das Leuchtorgane von *Chaetopterus variopedatus* Clap. *Zeit. wiss. Zool.*, Bd. 118, pp. 480–509.

KUTSCHERA, F., 1909. Die Leuchtorgane von *Acholoe astericola* Clprd. *Zeit. wiss. Zool.*, Bd. 92, pp. 75–102.

LANKESTER, E. R., 1868. Preliminary notice of some observations with the spectroscope on animal substances. *Journ. Anat. Physiol.*, Vol. 2, pp. 114–16.

LESPÈS, C., 1872. Étude anatomique sur un Chétoptère. *Ann. Sci. Nat. Zool.*, T.15, No. 14, 17 pp.

MCINTOSH, W. C., 1915. *A Monograph of the British Marine Annelids*, Vol. 3. London: Ray Society.

MANGOLD, E., 1910–14. Die Produktion von Licht. In Winterstein's *Handbuch der vergleichenden Physiologie*, 3 Bd., 2 Hälfte, S. 225–392.

MOORE, A. R., 1926. Galvanic stimulation of luminescence in *Pelagia noctiluca. Journ. Gen. Physiol.*, Vol. 9, pp. 375–9.

NICOL, J. A. C., 1952. Studies on *Chaetopterus variopedatus* (Renier). I. The light-producing glands. *Journ. Mar. Biol. Assoc.*, Vol. 30, pp. 417–31.

PANCERI, P., 1872. Études sur la phosphorescence des animaux marins. *Ann. Sci. Nat. Zool.*, T. 16, Art. 8, 66 pp.

—— 1878. La luce e gli organi luminosi di alcuni annelidi. *Atti. R. Accad.*, Napoli, Vol. 7, No. 1, 20 pp.

ROSENBLUETH, A., 1950. *The Transmission of Nerve Impulses at Neuroeffector Junctions and Peripheral Synapses*. London.

STEPHENSON, E. M., 1946. *Animal Camouflage*. Harmondsworth, England.

TROJAN, E., 1913. Ueber Hautdrüsen des *Chaetopterus variopedatus* Clap. *S.B. Akad. Wiss. Wien*, Bd. 122, pp. 565–96.

TROJAN, E., 1914. Ueber die Bedeutung der 'follicules bacillipares' Claparède's bei *Chaetopterus variopedatus. Proc. IX Int. Zool. Congr. Monaco*, 1913, pp. 390–5.

WELLS, G. P., 1937. Studies on the physiology of *Arenicola marina* L. I. The pacemaker role of the oesophagus, and the action of adrenaline and acetylcholine. *Journ. Exp. Biol.*, Vol. 14, pp. 117–57.

WELLS, G. P. & LEDINGHAM, I. C., 1942. Studies on the physiology of *Arenicola marina* L. III. The potassium relations of the isolated extrovert. *Journ. Exp. Biol.*, Vol. 19, pp. 176–85.

WILL, F., 1844. In Krekel, 1920. (See above.)

WU, K. S., 1939. The action of drugs, especially acetylcholine, on the annelid body wall (*Lumbricus*, *Arenicola*). *Journ. Exp. Biol.*, Vol. 16, pp. 251–7.