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THE EFFECT OF LIGHT ON THE SHADOW REACTION OF THE SEA URCHIN, *DIADEMA SETOSUM* (LESKE)

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As is mentioned by Mortensen (1940), sea urchins which belong to the genus *Diadema* react to shadow by spine movements. Since the work of Millott (1954) these responses have been extensively studied on an Atlantic species, *D. antillarum*. In preliminary experiments on a Japanese species, *D. setosum*, some of the important findings in the Atlantic species, such as the part played by radial nerves in the reflex (Millott, 1954), the photosensitivity of the nervous elements (Yoshida & Millott, 1959) and the inhibitory effect of light on the reaction (Millott & Yoshida, 1960*a*, *b*), were confirmed. This made it possible to base the work on the Japanese species on what has been found in *D. antillarum*.

In previous papers (Millott & Yoshida, 1960*a*, *b*), though some suggestions were made concerning the mechanism of the shadow reflex, the work was chiefly concerned with the later part of the reaction and little attention was paid to the events occurring between the moment the light was cut off and the first sign of the reaction, the period which must play a decisive role on the events occurring later.

The main object of the present study is to analyse the effect of light on the events occurring before the reaction ensues, in the belief that such studies reveal more of the mechanism involved in eliciting the shadow reaction.

MATERIAL AND METHODS

Design of the experiments

Previously, various aspects of the reaction, such as the reaction time, the amplitude of the spine movement, and the duration of the reaction, were shown to be to a greater or lesser extent a function of the following factors (Fig. 1): intensity (I_1), size (A_1) and

Fig. 1. Schematic representation of factors which affect the shadow reaction. For explanation see text.

duration (T_1) of the preceding (stimulating) light, those (I_2, A_2 and T_2) of the light (inhibiting) which follows, the interval (t) between the extinction of the preceding light and re-illumination and the spatial separation (d) of the two lights.

The aim of the present experiments is not to relate the intensity or the duration of

the above factors with the quantitative aspects of the response as was done previously (Millott & Yoshida, 1960*a*, *b*), but to determine the quantitative relationship between two of the above factors necessary to elicit a constant response, while keeping all the others constant.

Material

Urchins collected at the Tosa Marine Biological Station were brought by train to the Tamano Marine Laboratory where they were kept in running sea water. They fed well and remained quite healthy for many months.

Methods in general

Experiments were performed in an experimental dark room.

Methods used for preparing isolated pieces of test with one or two spines outside and a radial nerve inside and mounting it in an experimental tank were the same as previously reported (Millott & Yoshida, 1960*a*). The tank was also painted matt black both inside and outside except for two narrow slits on either side. In the present experiments, however, the sea water was not aerated but was renewed continually from a reservoir. The spine movements were observed visually, since the principle of the present experiments was simply to determine supra- and sub-threshold light intensities under various conditions.

In addition to the visual observations of spine movements the reaction times were measured with a stop watch to get a rough idea on the effectiveness of the light being tested. Taking into account the human element involved in the measurements, only the reaction times longer than 2 sec. were considered significant.

In all experiments the duration (T_1) of illumination with the preceding light was 5 min. and that (T_2) of the inhibiting light was 1 min. The two light spots produced by the optical system to be described were superimposed ($d = 0$). Special experimental procedures will be described in each section.

The optical system

The optical system (designed and constructed by the Sugiura Optical Laboratory Inc.) was in principle similar to that described previously (Millott & Yoshida, 1960*b*; Yoshida & Millott, 1960), but as it was improved in many respects a detailed description of the optical system may be needed (Fig. 2).

The apparatus was composed of three microscopes (O, O_1 and O_2). Two of them (O_1 and O_2) produced light spots for stimulating the preparation (P) and the one placed at the centre (O) was used for checking the foci and the positions of the light spots. Focusing was achieved by moving the preparation up and down by rack and pinion. To facilitate manipulations the optical axes of O_1 and O_2 were bent at right angles on opposite sides by means of aluminium mirrors (M_1 and M_2) whose reflectance was reasonably constant over the whole range of the visible spectrum. The two stimulating optical axes were inclined at 14·5° to the central one and the three optical axes thus obtained, including the bent parts, were all in the same plane. A coarse adjustment of the position of the spots was made by moving the preparation horizontally and a fine adjustment was made by rotating the optical axes, a in Fig. 2 being the centre of the rotation.

As the two stimulating systems were identical an explanation will be given for one of them only (O_1). It was composed of a light source (S_1), a condensing lens (L_1) 25 mm. in diameter, two neutral wedges (F), a disk with a hole at the centre (D_1) and an objective lens (l_1). The light source was a 6 V. 30 W. tungsten filament lamp and was operated with a car battery to avoid flicker effects.

The focal lengths of L_1 and l_1 were 63 mm. and 33 mm., respectively. The distance in mm. between S_1, L_1, D_1, l_1 and P was as follows: $\overline{S_1L_1} = 82$, $\overline{L_1l_1} = 275$, $\overline{D_1l_1} = 242$ and $\overline{l_1P} = 38{\cdot}2$. Thus the image of the tungsten filament (S_1) was formed on the inner surface of the objective lens (l_1) by means of the condensing lens (L_1). The image

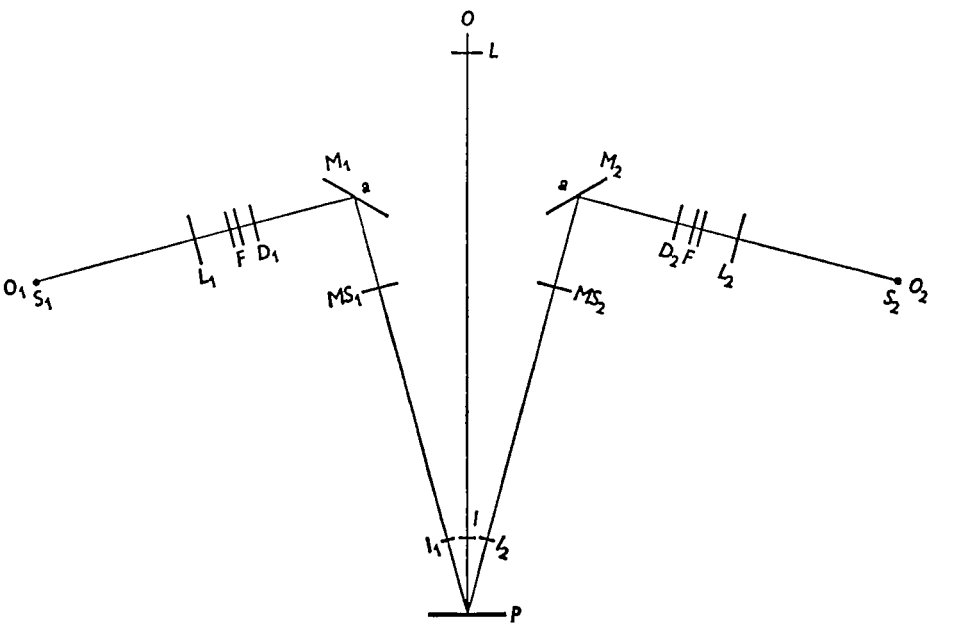

Fig. 2. Diagram of the optical system used. For explanation see text.

of a hole in the disk (D_1), inserted in between L_1 and l_1, may be formed on the preparation placed at 38·2 mm. from l_1. The over-all reduction factor was thus 1/6·3. With this optical system, it was possible to produce a more or less uniform field of illumination at a minimum expense of the brightness.

Six disks (D_1) with different sizes of the hole were used. The size of the spot thus formed at each setting of the disk was measured with a calibrated ocular micrometer loaded in the central microscope.

The intensity of light spot produced through each optical system was controlled by means of two neutral wedges (F) with practically identical gradient in optical density. The two were moved in opposition so as to maintain a uniform field of illumination. Holders of the wedges were marked at 1 mm. interval, the length corresponding to a change of 0·05 in optical density.

Measurements of the light intensity (I_1 and I_2)

The illumination was measured at the site of stimulation by means of a photomultiplier (IP 22) coupled with a d.c. amplifier with seven stages. The sensitivity of the whole unit was calibrated against a standard lamp.

In front of the photomultiplier was fixed an attachment consisting of a lens (25 mm. in focal length and 10 mm. in diameter) and a glass plate with a fine cross marked on its surface. The glass plate was placed in between the lens and the photomultiplier, 80 mm. from the former and 12 mm. from the latter. Thus when the lens came 36·4 mm. from the spot (P) and the optical axis of the attachment coincided with that of either O_1 or O_2, an image of the hole should be formed on the plate. The sharpness and the position of the image were checked with a magnifying lens. This attachment ensured that the light to be measured always took the same path with respect to the receptive surface of the photomultiplier. A possible error due to regional difference in sensitivity of the photomultiplier was minimized by placing it 12 mm. behind the conjugate focus (= position of the plate) so that the light was made to shine on a larger area of the receptive surface.

When the appropriate positioning was ensured the glass plate was removed and measurements were made for each setting of the disks and the neutral wedges. The light intensity of the spot will be expressed in arbitrary units, either logarithmic (Figs. 4–8) or linear (Table 1).

Operation of the shutters (Control of T_1, t and T_2)

The shutters, MS_1 and MS_2 in Fig. 2, which interrupted the beams of O_1 and O_2, were fixed on to relays, Ry_1 and Ry_2, respectively (Fig. 3). The action of the relays, hence the duration of T_1, t and T_2, was controlled by electrical means. The circuit diagram, designed by the Sugiura Optical Laboratory Inc., is shown in Fig. 3. With this it was possible to conduct experiments with any combination of the following durations of T_1, t and T_2 in one action by simply operating an 8-pole-3-way switch (S_1–S_6). The durations available were 1, 2, 3, 4 and 5 min. for both T_1 and T_2 and 25, 40, 50, 80, 130, 230, 520, 1000 and 1200 msec. for t.

When the main and the load switches were closed, the relay Ry_1 became energized but another relay, Ry_2, was not energized and therefore both shutters remained closed. To start an experiment the switches (S_1–S_6) were moved upwards as shown by arrows. Since this shunted a thyratron relay (V_1) the relay Ry_1 returned to the resting state, opening the shutter MS_1. The time of discharge through V_1 to energize Ry_1, hence to close MS_1, was determined by the product of R_1C_1. By closing the points 4 and 6 the new position of Ry_1 connected the grid of V_2 and a condenser C_2 with the resistor R_2. Again, the product of R_2C_2 determined the time of discharge through V_2 to energize the second relay, Ry_2. The shutter MS_2 attached to it would then be opened and the points 4 and 6 closed. The third stage (V_3, R_3 and C_3) was operated in the same way as above and the discharge through it cut the current supply to Ry_2 by means of the third relay, Ry_3. Occasionally it was necessary to keep both shutters opened at the same time for checking the foci and the positions of the light spots. This was achieved by pushing the switches (S_1–S_6) downwards as shown by arrows, for this made the current supply to Ry_1 cut off at S_4 and also allowed V_2 to conduct through S_2.

Theoretically, the values for t can be calculated by using the characteristics of the thyratron and the product of RC. For small values, however, mechanical delays in action of the relays may become serious. The actual value for each setting of the resistors (R_2) was calibrated on a cathode-ray oscilloscope, by using 60 cyc./sec. as reference. Sweeping of the oscilloscope was triggered by means of Ry_1 and the signals of 'off' of O_1 and 'on' of O_2 were sent to the scope through the photomultiplier, the response time of which was less than 1 msec.

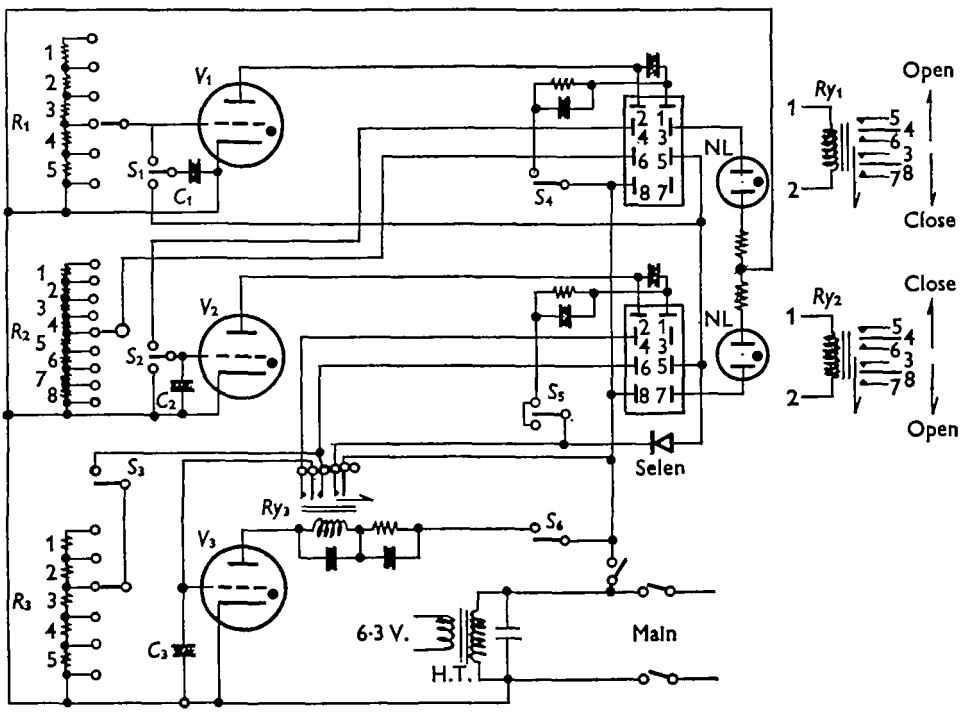

Fig. 3. Circuit diagram to operate shutters attached to relays Ry_1 and Ry_2. V_1–V_3: T66G–GT. C_1–C_3: 5·5 μF. R_1 and R_3: 5 × 3M. R_2: 1 = 50k, 2 = 25k, 3 = 15k, 4 = 5k, 5 = 3k, 6 = 1k, 7 and 8 = 500 Ω. For detailed explanation see text.

Temperature control

Most of the experiments to be described below were carried out at room temperature (25–33° C.) during summer and autumn seasons. In any series of such experiments the temperature of the sea water did not vary by more than 1·4° C.

In winter, when the room temperature became low, the effect of temperature was studied. To this end two 100 W. electric heaters coupled with a bi-metallic thermo-regulator, each being insulated with a glass tube, were immersed in the reservoir which supplied sea water to the experimental tank. Prior to each period of illumination with the preceding light the sea water in the tank was stirred gently. Though a thermometer was placed as near to the preparation as possible and was read a few seconds after the beginning of the inhibitory light, a high accuracy could not be expected, for the temperature distribution in the tank could have been altered during the period of preceding illumination, during which time the sea water was left still.

RESULTS

(1) *Changes in effectiveness of the inhibitory light (I_2) with time of re-admission (t)*

It has been suggested previously (Millott & Yoshida, 1960*a*, *b*), that the shadow reaction is the result of release from inhibition produced by the preceding light and a light coming in later inhibits the reaction to a greater or lesser extent according to the time at which it is admitted. Since a process leading to a final result, the spine jerk, is set in train when a light is cut off, the time course of the process must be identical under identical conditions of the preceding light. Then any change in effectiveness of the inhibitory light with the time at which it is admitted must be an expression of a change in the process released when the preceding light is cut off.

Experimental procedures were as follows. In any series of experiments the intensity (I_1) and the size (A_1) of the preceding light were kept constant. The light was cut off and then after an interval of t the inhibitory light (O_2) was admitted. Its effectiveness was tested by adjusting neutral wedges, and the minimal intensity required to inhibit a response (supra-threshold) and the maximum intensity at which the reaction was observed (sub-threshold) were determined. These procedures were repeated for different values of t.

Seventeen series of such experiments were repeated at different intensities of the preceding light and a few examples of the results are shown in Fig. 4. Here the duration of total darkness (t) is scaled in logarithmic units to show more clearly the part of the curves for small values of t. It is seen that the curves run at first in parallel with the time axis and then show a sharp rise at a critical point. The length of the flat part of the curve becomes shorter at higher intensities of the preceding light (from curve E to curve A).

The sharp break of the curves strongly suggests the existence of two distinct processes going on during the period of total darkness. In order to reveal more of the nature of the processes, series of experiments were repeated at different temperatures of the sea water in which the preparation was immersed.

Experiments were performed when the room temperature was about 14° C. The first series of experiments was carried out at room temperature. The temperature of the sea water was then raised by 4–5° C. by letting the warm sea water into the experimental tank (see p. 593). The new temperature level was roughly maintained by a slow influx of the warm water. The preparation was left for at least 1 hr. to adapt to the new temperature and then the experiment was repeated. Usually a group of curves was obtained at three levels of temperature in one and the same preparation. An example taken from such experiments is shown in Fig. 5.

It is seen that at higher temperatures, the slope of the rising part of the curve becomes steeper and the length of the flat part becomes shorter. This must indicate that some temperature-dependent processes are involved in the rising part as well as in determining the length of the flat part. On the other hand the rise in temperature affects only slightly the level of the initial part.

The effect of temperature on the initial phase of the processes was studied in more detail. Here the sub- and supra-threshold intensities of inhibition for responses to a standard level of preceding light were determined at about 6 min. intervals while the temperature was raised at a rate of about 1° C. in 20 min. The intensity of the

preceding light was so chosen as to be well above the threshold intensity required to induce a response when used alone.

An example of such experiments is shown in Fig. 6. It is seen that over a wide range of temperature the inhibitory threshold stays constant, though falling sharply

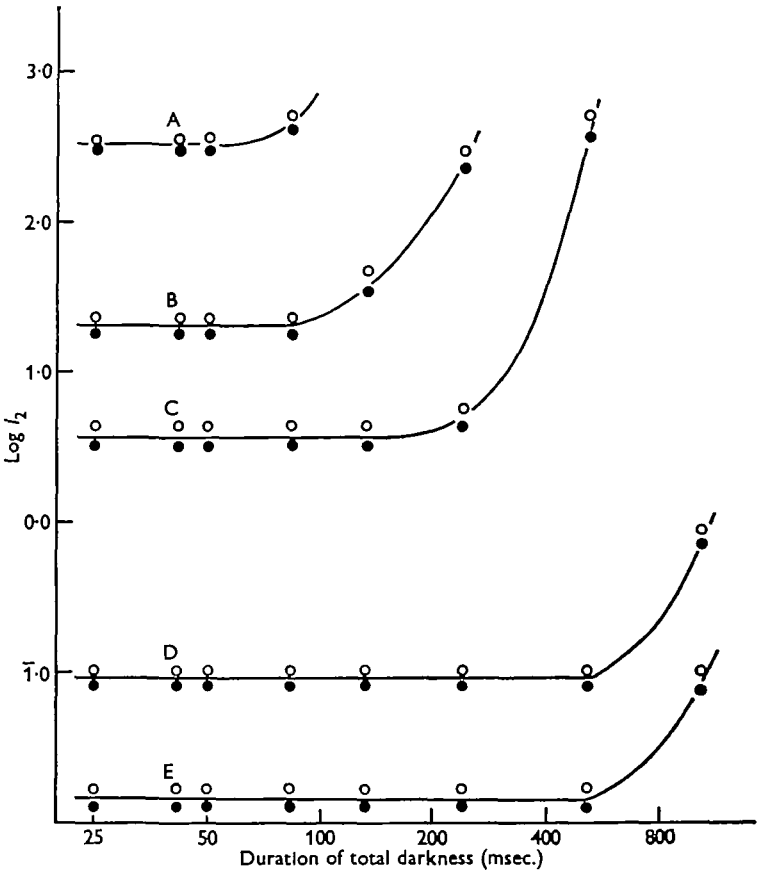

Fig. 4. Relationship between the inhibitory threshold (I_2) and the time of re-admission (t). Five examples at different intensities of the preceding light (I_1) are shown. O, Minimum intensity to inhibit the response; ●, maximum intensity at which the reaction is just induced.

at low temperature range. The temperature at which the curve attains an asymptotic value is here about 17° C. and varies with different individuals, the range observed in six animals being 17–19° C.

(2) *The effect of temperature on the threshold intensity of the preceding light*

In connexion with the experiments described above it is important to study the effect of temperature on the processes occurring during the preceding illumination. Here the preparation was illuminated by the preceding light for 5 min., after cessation of which no light was re-admitted. The temperature was raised in the same manner as described above.

Two examples from six series of such experiments are shown in Fig. 7 A and B. In addition to definite 'positive' (filled circles) and 'negative' (open circles) responses

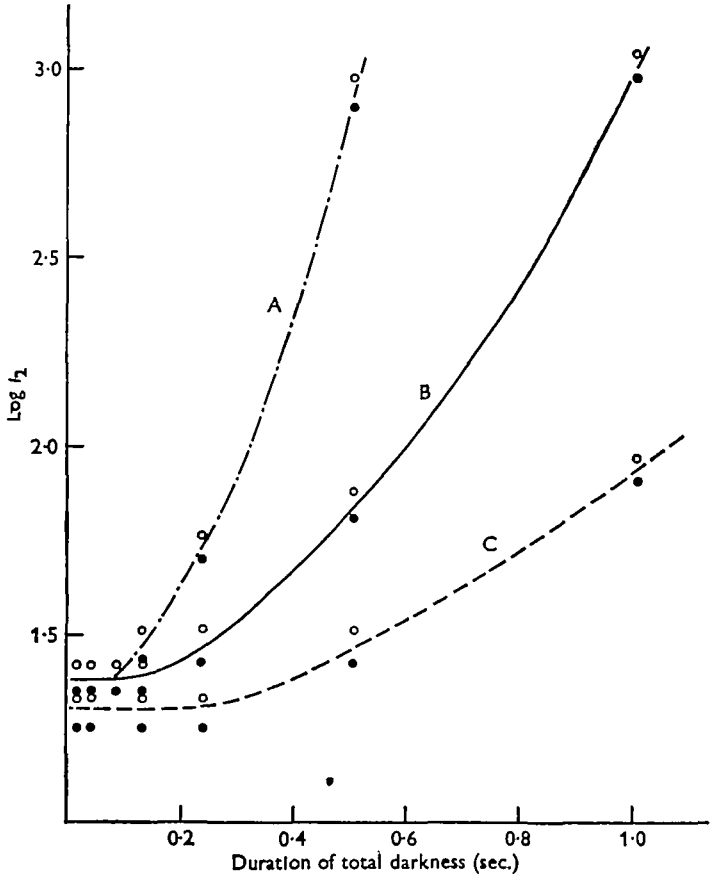

Fig. 5. Relationship between I_2 and t at different temperatures. A, 22·9–23·2° C.; B, 18·5–18·8° C.; C, 14·3–14·7° C. O, ●, as Fig. 4.

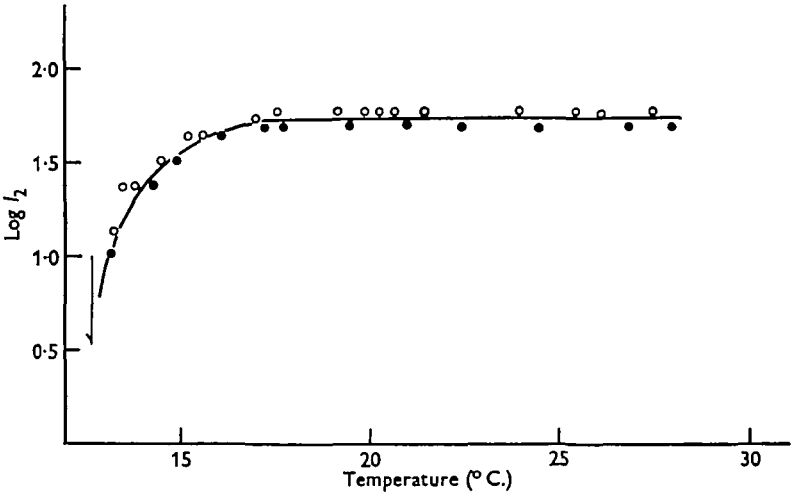

Fig. 6. Effect of temperature on the inhibitory threshold. The inhibitory light was admitted 25 msec. after cessation of the preceding light. Arrow shows the point at which no reaction was observed when the inhibitory light was reduced to zero. O, ●, as Fig. 4.

feeble responses with extremely long reaction times (6 or 7 sec.) were often observed and they are presented in the figure as open circles with cross. A rise in sensitivity with increase in temperature is seen up to 27° C., though the increase is only slight, about 0·2 log unit, between 20° and 27° C.

Towards the lower range of temperature the curve would, if extrapolated, follow the path shown by the broken line. In all experiments, however, the curves showed a break at about 12·5–15° C. (13·5–14° C. in Fig. 7). The possibility that the conduction and the effector systems were impaired at these low temperatures was considered not

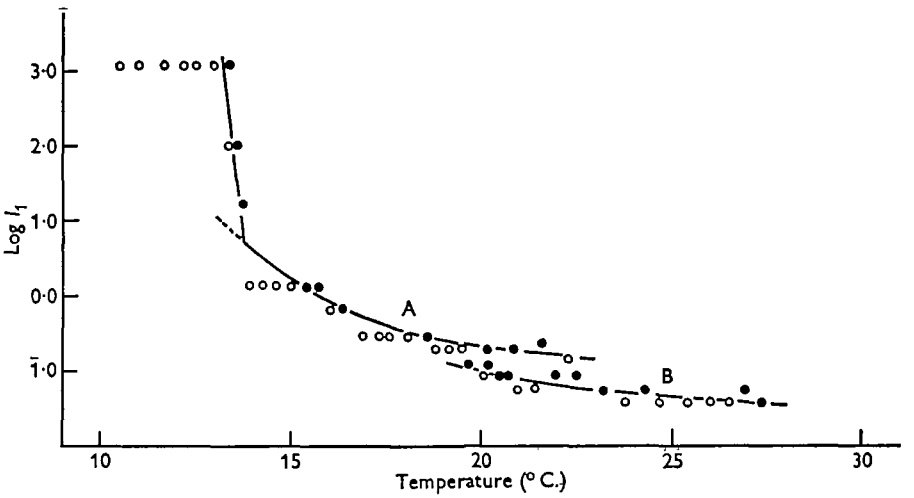

Fig. 7. Effect of temperature on the threshold intensity of the preceding light (I_1) when used alone. Results obtained with two different urchins are shown (A and B). O, Intensity at which reaction was not observed; ⊕, intensity at which only a feeble reaction with extremely long reaction time was observed; ●, intensity at which a clear reaction was observed.

to be the case, since mechanical stimuli to the radial nerve induced clear responses in the spine jerk at still lower temperature such as 10–11° C.

A process which is probably concerned with the initial interaction between excitation and inhibition having been found (see Discussion), the applicability of some of the laws in the field of sensory physiology may now be considered.

In the following two sections the intervals (t) observed between 'off' of the preceding light (O_1) and 'on' of the inhibitory light (O_2) were 25 msec. Since it is far shorter than the shortest length of the flat part, the switch over from O_1 to O_2 may be regarded as instantaneous.

(3) *Interchangeability between intensity and size of spot light (Ricco's law)*

The experimental procedure to test Ricco's law was as follows. In order to study the effect of the inhibitory light (O_2) the intensity (I_1) and the size (A_1) of the preceding light (O_1) were kept constant. After 5 min. illumination with the preceding light the effectiveness of the inhibitory light of different sizes (A_2) was ascertained by determining supra- and sub-threshold for each size.

For testing the effect of the preceding light experiments of the same type were carried out for different sizes (A_1) of the spot, while the intensity (I_2) and the size (A_2)

of the inhibitory light were kept constant. Here the supra-threshold intensity was the minimal intensity to elicit a response and the sub-threshold was the maximal intensity which failed to elicit a response.

Table 1. *Total intensity of light of different sizes, necessary to inhibit* (−) *and elicit* (+) *a response, when the preceding* (O_1) *or the inhibitory* (O_2) *light is kept constant as reference*

Light used as reference ...	O_1				O_1				O_2				O_2			
Its size (mm.) ...	0·03				1·00				0·50				0·50			
	Sub-threshold (+)		Supra-threshold (−)		Sub-threshold (+)		Supra-threshold (−)		Sub-threshold (−)		Supra-threshold (+)		Sub-threshold (−)		Supra-threshold (+)	
Size of light spot to be tested (mm.)	Experimental	Deviation (%)	Experimental	Deviation (%)	Experimental	Deviation (%)	Experimental	Deviation (%)	Experimental	Deviation (%)	Experimental	Deviation (%)	Experimental	Deviation (%)	Experimental	Deviation (%)
1·00	—	—	—	—	8·32	−5·3	12·9	+3·2	6·76	+11·2	7·94	+5·0	11·2	+1·8	15·6	+8·3
0·50	0·562	−2·8	0·692	−1·8	10·0	+13·8	13·0	+4·0	6·03	−0·8	7·59	+0·4	11·8	+7·3	15·9	+10·4
0·25	0·589	+1·9	0·676	−4·1	8·71	−0·9	12·9	+3·2	5·89	−3·1	7·94	+5·0	11·0	±0	12·9	−10·4
0·12	0·575	−0·5	0·692	−1·8	8·13	−7·5	11·2	−10·4	5·63	−7·4	6·76	−10·6	9·87	−10·3	13·2	−8·3
0·06	0·589	+1·9	0·741	+5·1	—	—	—	—	—	—	—	—	—	—	—	—
0·03	0·575	−0·5	0·725	+2·8	—	—	—	—	—	—	—	—	—	—	—	—
Average	0·578	—	0·705	—	8·79	—	12·5	—	6·08	—	7·56	—	11·0	—	14·4	—

Four examples from 25 series of such experiments are shown in Table 1, in which the total intensity as measured photoelectrically is given in terms of arbitrary units. It is seen that, regardless of whether the size of light to be tested is larger or smaller than that used as reference, the total intensity to produce a constant response stays constant over a wide range. The maximum deviation from the average of total intensities obtained with different sizes of spot was 14·2 %, which may be small enough, considering the range tested was 1:1000 between the largest and the smallest spot.

(4) *The intensity relationship between the preceding light and the inhibitory light (Weber–Fechner law)*

Experiments were performed to determine supra- and sub-threshold intensities (I_2) of the inhibitory light under different intensities (I_1) of the preceding light. Results are shown in Fig. 8.

The Weber–Fechner law states that the change in stimulus intensity necessary to produce a critical response is always a constant fraction of the intensity of the stimulus to which the sensitive tissue is already exposed. If the law hold also for the shadow reaction in *D. setosum* the condition required to produce a critical response will be expressed as follows, by using the symbols mentioned above

$$\frac{\Delta I_1}{I_1} = \frac{I_1 - I_2}{I_1} = k.$$

The equation may be re-written,

$$\log I_2 = \log I_1 + \log (1-k).$$

Thus if $\log I_2$ is plotted against $\log I_1$, the curve should become a straight line whose slope is 45°, and if k is very small the line will pass near the origin.

It may be noted here that it is difficult to position the plane of the preparation exactly at right angles to the central optical axis (O) and hence at an identical inclina-

tion to the two stimulating axes (O_1 and O_2). This means that the values of I_1 and those of I_2 measured with the photomultiplier were comparable within each group but not strictly so between the two groups. If, however, the cessation of O_1 and O_2, which had been projected separately on to one and the same receptive site for an equal period, produced an identical (threshold) response, it may be assumed that the two lights were identical in their effectiveness. As the light intensity is expressed in

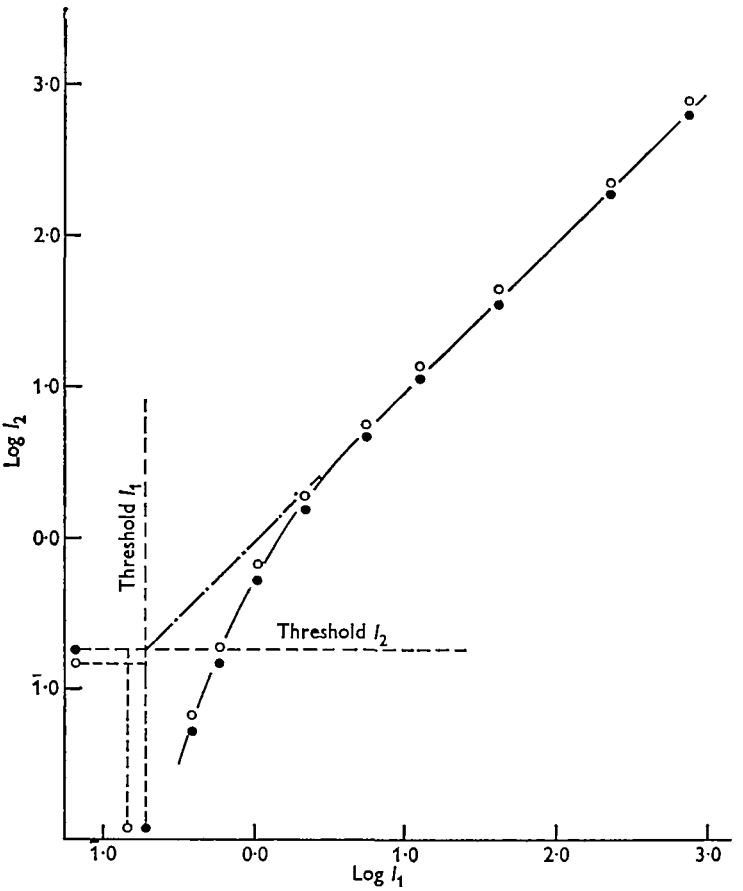

Fig. 8. Relationship between intensities of the preceding (I_1) and the inhibitory (I_2) light. ○, ●, As Fig. 4. Threshold intensity of each light, when used alone as a preceding light, is shown alongside the corresponding axis.

logarithmic units the two groups of values which were measured photoelectrically may be made comparable if the difference between the two ($\log I_{1\,\text{threshold}} - \log I_{2\,\text{threshold}}$) is added to the values of $\log I_2$. Threshold determinations for each light were repeated several times during the course of the experiment. In Fig. 8 this type of correction has been made and the average threshold level is shown by broken lines.

It is seen that under illumination brighter than 1·5 log units above the threshold the curve becomes a straight line with a slope of 45°. When extrapolated it passes close to the point of threshold, and therefore probably passes close to the origin. The deviation from the straight line becomes more and more pronounced as the intensity of I_1 becomes less and finally the curve falls below the threshold level, indicating a large

Weber's fraction. It may be concluded, therefore, that the shadow reaction of *Diadema setosum* obeys the Weber–Fechner law at moderate intensities and deviates from it as the preceding light becomes weak. These phenomena have been observed in a number of photoreceptors.

DISCUSSION

The present study has confirmed in *Diadema setosum* the inhibitory effect of light on the shadow reaction which has been shown previously in *D. antillarum* (Millott & Yoshida, 1960*b*). The work further presents some additional aspects of the action of light on the events occurring between the moment when the light goes off and the beginning of the overt response.

The interpretation put forward in previous papers (Millott & Yoshida, 1960*a*, *b*) is that the shadow reaction may be a rebound from inhibition produced by light, a view which has been advanced for vertebrate and invertebrate retinae by Granit (1933), Hartline (1938) and others. On the other hand Kennedy (1960), working on *Spisula*, succeeded in inducing on-responses by a method of selective adaptation and suggested for this animal a new scheme in which the algebraic sum of the excitatory and inhibitory events, both being caused by light, determines the off-response owing to differences in their time courses. As in *Diadema* photosensitive nerves are involved and the scheme looks applicable to *Diadema* which shows on-responses, the threshold for which is very much higher than that for off-responses (Millott & Yoshida, 1959). However, though the planes of analysis differ greatly, the fact that there is a period during which the inhibitory threshold stays constant (the flat part of the curves in Figs. 4 and 5) is difficult to explain by Kennedy's scheme, for in order to counteract the gradual rise of the summed curve the inhibitory threshold should also be altered with time accordingly. This was not the case. Evidence therefore does not support the view that the off-response in *Diadema* is the result of processes analogous to those proposed by Kennedy to explain the shadow reaction in the lamellibranch *Spisula*.

Though the intimate mechanism is still obscure the results presented above, especially the sharp break of the curves in Figs. 4 and 5, hint at an additional mechanism existing between the receptive system and the effector organ, which has also been suggested from a different point of view (Millott & Yoshida, 1960*a*). The mechanism may be activated as a result of primary interaction between excitation and inhibition and the activity thus ensuing may be inhibited by light through the receptive system. The flat part of the curve may be an expression of the latent period of the primary interacting system, the length of which may be determined by the extent to which the system has been conditioned by the preceding light (effect of intensity, see Fig. 4) or by the potential activity of the system (effect of temperature, see Fig. 5). The suggestion that there are two sites of interaction fits well with the experiments on the inhibitory pathways (Millott & Yoshida, 1960*b*), in which it is suggested that interaction between excitation and inhibition may occur in the radial nerve or at the periphery. It seems possible that the latent period concerns the central inhibition and the rising part concerns the peripheral inhibition, hence the additional mechanism.

The assumption of the additional mechanism would account for the lack of effect of duration of shadow when it is longer than 40–60 msec. (Millott & Yoshida, 1960*a*). Our suggestion that ineffectiveness on the initial part of the reaction is an expressio

of the latency of inhibition seems to be inadequate, because it is possible to inhibit a reaction by admitting a strong light one second or still later after cessation of the preceding light. It seems to be more probable that the durations at which the ineffectiveness was observed correspond with the rising phase, where the peripheral mechanism is supposed to be activated already and whose activity may only be abolished by a stronger inhibitory effect.

The constancy of the inhibitory threshold at temperatures higher than 18° C. (Fig. 6) would suggest that both the inhibitory and excitatory processes are influenced to the same extent by temperature. The excitatory process seems to deteriorate more readily at low temperature so that a weaker light can inhibit a response. This notion is substantiated by the steep rise in the threshold intensity of the preceding light at low temperature (Fig. 7).

A qualitative effect of spot size was shown previously (Millott & Yoshida, 1960*b*). The above quantitative experiments have proved the reciprocity of area and threshold intensity (Ricco's law) for both the preceding and the inhibitory light. The fact that there is a wide range over which constancy of $I \times A$ is observed hints that the extent of the receptive field in the radial nerve may be fairly large.

In a number of receptive systems the so-called Weber's fraction, $\Delta I/I$, has been shown to be constant for a medium range of the adapting stimuli. In the shadow reactions of *Balanus*, *Helix* and *Branchiomma*, von Buddenbrock (1930), Föh (1932) and Nicol (1950), respectively, have claimed that the threshold decrease in intensity is not a constant fraction of the preceding illumination, though their data tend to show that it is so under fairly bright illumination.

Since the work of Hecht (1937) a number of attempts have been made to explain the Weber–Fechner law in terms of photochemistry, but a somewhat different approach has been made in the present study, not only because such theories are now considered untenable (as discussed in detail by Rushton, 1959), but also because the Weber's fraction for shadow reactions is a negative entity. Thus the whole event has been analysed not in terms of a negative Weber's fraction but in terms of the efficacy of the light remaining to inhibit a response. It is shown that the inhibitory threshold is a constant fraction of the preceding light intensity when the latter is fairly bright. This is another way of expressing the Weber–Fechner law (see p. 598) but the new approach has revealed an additional aspect of the action of light.

Near the threshold range of the preceding light the inhibitory threshold is much lower than the threshold intensity necessary to elicit a response (Fig. 8). If we consider the situation in terms of light energy to be supplied to the system ($I \times T$) the difference will become still greater, for the inhibitory light should have accomplished its job within the period of reaction time which is normally less than a few seconds, whilst the preceding light is allowed to shine for 5 min. Since a light which can modify a response in one way or another must affect photoreceptors it is possible that they are activated by light weaker than the threshold intensity of the preceding light and then set in train the inhibitory mechanism.

Lastly, a comment may be made on the methods used here, the principle of which was called 'matching' by Denton (1956) and reminds us of the so-called 'null method' in physics. It has an advantage, as Denton stated, that the accuracy depends only upon the constancy of the differential sensitivity and not upon the constancy of the absolute

sensitivity. In *Diadema setosum* it is further found that the differential sensitivity stays constant over a considerably wide range of temperature (Fig. 6), a fact which gives confidence in the reliability of the methods used here and in previous work (Yoshida & Millott, 1960).

SUMMARY

1. The shadow reaction of the spine jerk in *Diadema setosum* has been studied with an improved optical system and a timing device.

2. Studies on the variation of the inhibitory threshold with time at which light is admitted after cessation of the preceding light, have differentiated two distinct periods. The inhibitory threshold stays constant during the initial period and then rises sharply. The length of the initial period and the rate of rise in the later phase are found to be temperature-dependent, the former being affected, in addition, by the intensity of the preceding light.

3. The reciprocity of area and intensity of light is proved for both the preceding and the inhibitory light.

4. A new approach is made to study the Weber-Fechner law in shadow reactions and it is found that the reaction in *D. setosum* obeys the law. In connexion with this it is found that a light, sub-threshold for eliciting a reaction, can still be effective in inhibiting it.

5. The mechanism involved is discussed in relation to our earlier ideas (Millott & Yoshida, 1960*b*).

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