THE SHADOW REACTION OF *DIADEMA ANTILLARUM* PHILIPPI

III. RE-EXAMINATION OF THE SPECTRAL SENSITIVITY

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Our previous account of the spectral sensitivity (Millott & Yoshida, 1957) was based on a study of the spine response in isolated pieces of test bearing many spines. The areas illuminated and shaded extended over the whole internal or external surface.

Recent work (Millott & Yoshida, 1960), has shown that the shadow response is not a simple reflex and that considerable interaction in the nerve pathways arising from neighbouring receptive areas occurs, so that spatial summation is involved.

Interaction between spines is now known to be significant, so that preparations with several spines behave in a slightly different way from those with only one (Millott & Yoshida, unpublished). Neither of these factors was considered in the preceding study.

The method previously employed had other defects. Thus it was difficult to ensure that the spectral quality of the light transmitted by the interference filters mounted in the apparatus used was identical with that measured when they were mounted in the spectrophotometer. Again, such factors as chromatic aberration, differential absorption, etc., in the lens system used were not taken into account. These factors have been eliminated by the method described below.

METHODS

The general principle of the experiments is to subject preparations to a fall in light intensity by changing instantaneously from white to coloured light, the intensity of which was adjusted by neutral filters so as to be less than that of the white (the intensity of which is kept constant) by an amount just adequate to elicit a shadow response.

Since the most effective wavelengths approach most closely the effect of white light, it will be with these that the most dense neutral filters are required. Less effective colours will require proportionately less adjustment. Thus the relative effectiveness of light of different colours is determined by reference to white light of a constant intensity.

Apparatus

The type of preparation, the method of mounting and the experimental aquarium were the same as previously employed (Millott & Yoshida, 1960). Movements of

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the single spine were observed on a ground glass screen alongside the shadow of a stationary 'hair', to assist detection of small movements.

The optical system is shown in Fig. 1. Two beams Op. 1 and Op. 2, produced from the tungsten filament lamps S_1 and S_2, were focused by L_3 and L_{11} to form identical spots (0·5 mm. in diameter) at the same position on the radial nerve (N), by the means already described (Millott & Yoshida, 1960). Op. 2 was coloured by passing it through one of the same nine Balzer filters (I.F.) previously used and described (Millott & Yoshida, 1957), which pass wave bands 8–11 mμ wide at 50% transmission.

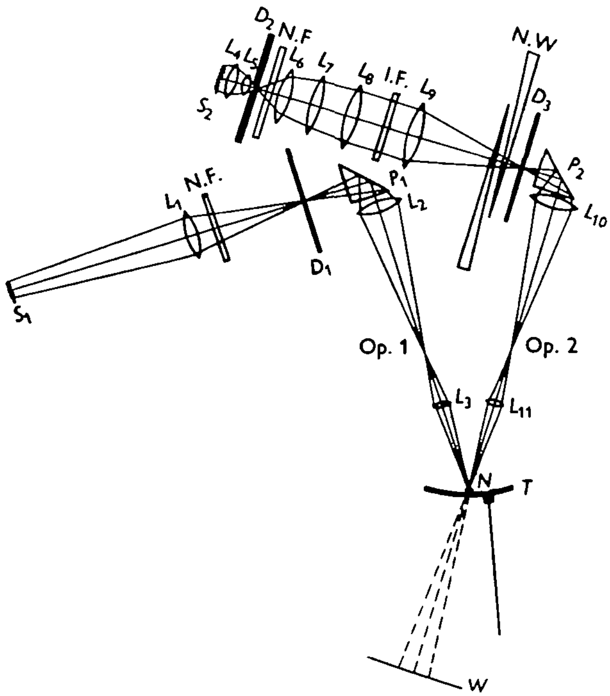

Fig. 1. Diagram of the optical system used (for explanation see text).

Instantaneous (12 msec.) change-over between the beams was achieved by a spring loaded shutter, which admitted one and simultaneously interrupted the other.

The parallel beam necessary for proper operation of the filter was obtained by the lenses L_4 to L_8. L_4 and L_5 (a microscope substage condenser), were placed as close as possible to S_2, so that its reduced image was formed in the plane of the diaphragm D_2 with little loss of intensity. L_6 again reduced loss of light by shortening the focal length of the two succeeding lenses.

To test whether the beam was parallel, a plane mirror was inserted in place of the interference filters, to ensure that the image of S_2, when reflected back, appeared on D_2 at the same place as the focused image, whatever the distance of the mirror from L_8.

The lenses L_1 and L_9 focused the beams on diaphragms D_1 and D_3; the instantaneous shutter operated between these and prisms P_1 and P_2.

Each beam was controlled by the neutral filters (N.F.), and in addition the coloured beam was controlled by two identical neutral wedges (N.W.), moved in opposition so as to maintain a uniform field.

S_1, a 12 V. lamp rated at 100 W., was operated by 5·0–5·8 V. a.c. The 115 V., 100 W. lamp S_2, calibrated by the N.P.L., was run at 89·9 V. to give a colour temperature of 2700° K. The supply to each lamp was controlled separately by means of a voltmeter and a variable resistor.

Procedure

Experiments were performed in a darkroom at 22·0–23·5° C. In any one series of experiments the temperature did not fluctuate by more than 0·5° C.

Each piece of test (T), with its spine, was left to recover and adapt in the dark for 45–60 min., after which the spot of white light was projected on to the radial nerve for 5 min. and then replaced by the coloured. If the reaction was other than threshold the sequence was repeated after making appropriate adjustments to the neutral filters. When a threshold reaction occurred (and with experience this could be obtained quickly) the experiment was repeated several times using the same relative intensities as well as those about 0·1 log units above them, to ensure consistency. By repeating this procedure for each colour filter and comparing its effectiveness with that of the constant intensity of white light, it was possible to obtain values that could be used to calculate the relative sensitivity at each colour (see below).

The sensitivity of each preparation was checked throughout the day by determining the threshold level for the filter with maximum transmission at 465 mμ, which is known from the previous study to be near the point of maximal sensitivity.

Calculation of relative sensitivity

The relative energy (E_λ) of each colour with the same effectiveness as that of the white light is given by

$$\log E_\lambda = \log \eta_\lambda - D_x, \quad (1)$$

where η_λ is the relative total amount of energy falling on the receptive surface and D_x is the optical density of the neutral wedges inserted to elicit a threshold response.

The relative amount of energy (H_λ) radiated from S_2 at each wavelength (λ) can be calculated from Wien's formula, the tungsten filament being regarded as a full radiator and the lamp being run at the prescribed colour temperature. But some of the energy is dissipated by absorption in lenses, prisms, sea water, and by chromatic aberration, etc., so that the relative amount of energy (η_λ) actually reaching the preparation is given by

$$\log \eta_\lambda = \log H_\lambda - T, \quad (2)$$

where T is the transmission coefficient of the interference filter and the total absorbance, etc., of the optical system Op. 2 expressed in terms equivalent to optical density.

Table 1. *Calibration of relative amount of energy*

Wavelength (mμ)	Calculated relative amount of energy = log η_λ	Relative amount of energy required for scotopic vision. log E_λ (see equation (1)) M.A.G.	D.B.	M P.M.S.	Average	Relative energy obtained by Crawford (log S_λ)	Deviation from the standard (log S_λ − log E_λ)	Corrected relative amount of energy (log η'_λ)
410	0·08	1·37	1·42	1·66	1·48	1·56	0·08	0·27
442	0·32	0·75	1·01	0·96	0·91	0·48	−0·43	0·00
465	0·36	0·37	0·48	0·50	0·45	0·21	−0·24	0·23
475	0·36	0·27	0·26	0·30	0·28	0·14	−0·14	0·33
501	0·50	0·00	0·00	0·00	0·00	0·00	±0·00	0·61
534	0·76	0·16	0·28	0·14	0·19	0·14	−0·05	0·82
544	0·69	0·35	0·52	0·26	0·38	0·26	−0·12	0·68
558	0·80	0·69	0·84	0·70	0·74	0·46	−0·28	0·63
594	0·98	1·73	1·96	1·64	1·78	1·27	−0·51	0·58

Table 2. *Spectral sensitivity obtained by matching method*

Wavelength (mμ)	E1 log H	E1 1/H × 100	E2 log H	E2 1/H × 100	E3 log H	E3 1/H × 100	E4 log H	E4 1/H × 100	E5 log H	E5 1/H × 100	E6 log H	E6 1/H × 100	Average log H	Average 1/H × 100
410	—	—	0·86	13·8	0·96	11·0	—	—	0·84	14·5	0·80	15·9	0·869	13·8
442	0·04	90·9	0·01	98·0	0·02	95·2	$\bar{1}$·99	102	$\bar{1}$·98	104	$\bar{1}$·99	102	0·004	98·7
465	0·00	100	0·00	100	0·00	100	0·00	100	0·00	100	0·00	100	0·000	100
475	0·16	69·0	0·16	69·0	0·21	61·7	0·21	61·7	0·20	62·9	0·21	61·7	0·193	64·3
501	—	—	0·42	38·0	0·54	28·8	—	—	0·38	41·7	0·38	41·7	0·436	37·6
534	—	—	—	—	0·76	17·4	—	—	0·69	20·4	0·70	20·0	0·718	19·3
544	—	—	—	—	—	—	—	—	0·87	13·5	0·83	14·8	0·851	14·2
558	—	—	—	—	1·16	6 9	—	—	1·04	9·1	1·16	6·9	1·124	7·6
598	—	—	—	—	2·36	0·4	—	—	2·08	0·8	2·28	0·5	2·255	0·6

The magnitude of T will depend largely on the interference filters, so that any error in the previous determination of their transmission coefficients will seriously affect the sensitivity curve. Other factors may also introduce error.

A relatively simple way of determining the total error is to compare the curve obtained by using the above apparatus and equations (1) and (2) with a standard curve for the same receptor. A suitable standard is the curve for human binocular scotopic sensitivity from which relative energy values can be calculated for each wavelength.

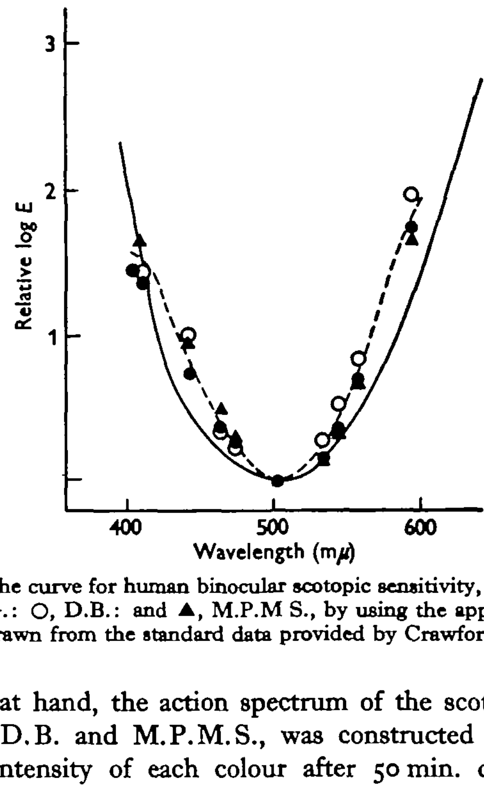

Fig. 2. Comparison of the curve for human binocular scotopic sensitivity, determined for the three subjects, ●, M.A.G.: ○, D.B.: and ▲, M.P.M S., by using the apparatus in Fig. 1 (broken line) and a curve drawn from the standard data provided by Crawford (solid line).

For the purpose at hand, the action spectrum of the scotopic vision of three subjects, M.A.G., D.B. and M.P.M.S., was constructed by determining the relative threshold intensity of each colour after 50 min. dark adaptation. To produce the coloured light the same optical system was used as for the sea urchins, except that the light spot was projected on to white paper (W) to form circles 15 mm. in diameter 30 cm. from the observer. The curve for the average sensitivity is shown in Fig. 2 alongside the curve drawn from data given by Crawford (1949) which form a basis for the C.I.E. standard.

There is a significant difference (Table 1, Fig. 2), and therefore the values of η_λ should be corrected to the values η'_λ, which can be obtained by the equation

$$\log \eta'_\lambda = \log S_\lambda + D_x, \tag{3}$$

where S_λ is the mean relative energy value at a given wavelength calculated from Crawford's data.

These corrected values may now be substituted in equation (1) to obtain the relative effectiveness for each colour.

RESULTS

The action spectrum obtained by matching

The data obtained from six *Diadema* are summarized in Table 2 and Fig. 3. In all cases, the relative amount of light energy (passed by the filter with maximal trasmission at 465 mμ) required to produce a threshold response was taken as unity. In addition, Table 2 shows the relative sensitivity to this filter (expressed as a percentage). In half of the cases this light proved to be most effective, but in the others slightly greater sensitivity was found with a filter transmitting maximally at 442 mμ.

This, and more particularly the shape of the curve of mean values (Fig. 3), suggest that the real maximum is somewhere between 455 and 460 mμ. Unfortunately a filter with a peak transmission in this vicinity was not available, so that we are unable to define the maximum more closely.

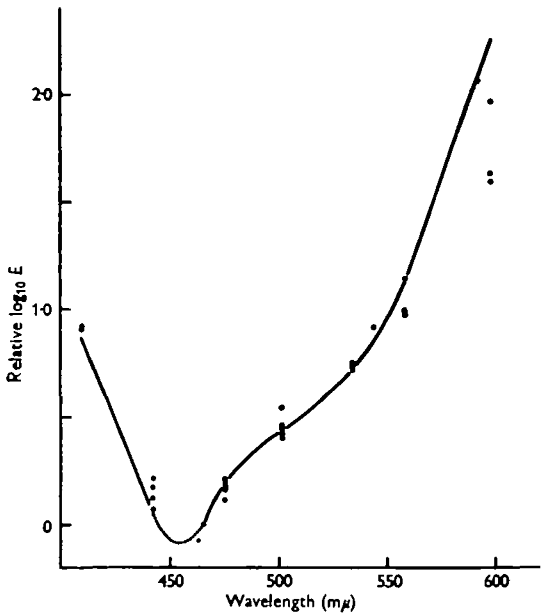

Fig. 3

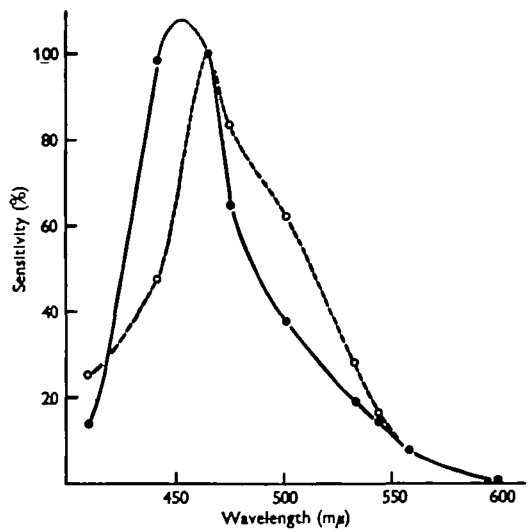

Fig. 4

Fig. 3. *Diadema antillarum*. Comparison of the average data obtained by the matching method (solid line), with the corrected data (filled circles) from Millott & Yoshida (1957).

Fig. 4. Comparison of the action spectra of *Diadema* obtained by the matching method (solid line) with that previously obtained by Millott & Yoshida (broken line).

Comparison with previous results

When compared with the results previously obtained, the present results yield a curve similar in form but with the maximum shifted some 5 to 10 mμ nearer the violet (Fig. 4).

This may be due to any of the sources of error suspected (p. 390), but if we assume that it is mainly due to the errors in the transmission coefficient previously determined for the interference filters (Millott & Yoshida, 1957), we may assess the effect by applying a correction based on the differential between the values for E used in the preceding study and those derived from Crawford's data. When this is done, the filled circles which result from the data previously obtained (Millott & Yoshida, 1957) coincide with the curve obtained by using the present matching method (Fig. 3).

Table 3. *Correction of the data obtained by Millott & Yoshida* (1957), *see p.* 396.

Wavelength (mμ)	Average relative energy (E) necessary to elicit a response	log E	Corrected log E
410	3·93	0·59	0·91
442	2·13	0·33	0·14
465	1·00	0·00	0·00
475	1·21	0·08	0·18
501	1·64	0·21	0·45
534	3·53	0·55	0·74
544	6 12	0·79	0·91
558	12·0	1·08	1·04
598	108·0	2 03	1·76

DISCUSSION

The action spectrum previously published for the shadow response is inadequate. The close similarity between the results now obtained by the matching method and the corrected data from the preceding account, shown in Table 3 and Fig. 3, suggests that of the sources of error originally suspected (p. 390) only those arising in the optical system are significant. No significant difference appears to arise from the stimulation of large areas and from spatial summation, which suggests that the receptive system is homogeneous in its effective absorption.

The possible significance of the abundant pigment resembling echinochrome A (Millott, 1957), discussed in the preceding paper, remains uncertain.

SUMMARY

1. The action spectrum of the shadow reponse of *Diadema* spines was redetermined by a matching method and a procedure which eliminated certain defects of that previously used.

2. Maximal sensitivity occurs between 455 and 460 mμ. When compared with the curve previously obtained the maximum is shifted 5–10 mμ toward the violet, but the form of the curves is similar and when the earlier curve is corrected by a factor obtained in the present study the two coincide.

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