SPECTRAL SENSITIVITY OF CHROMATOPHORES IN *DIADEMA SETOSUM* (LESKE)*

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Classical accounts of the effect of coloured light on retinal pigment migration in frogs and crayfish have been reviewed by Parker (1932). Most of those results agree in indicating violet or blue as the most effective light. Zetter (1956) has shown that the light of wavelengths near to 540 mμ is most effective for the expansion of melanophores in frog's skin. On the other hand, the direct effect of light upon the iris, which responds like melanophores, shows that 486 mμ is the most effective wavelength (Weale, 1956).

In echinoids nothing is known about the relative effectiveness of various spectral regions in producing photic reactions, other than the findings of Millott & Yoshida (1956) in *Psammechinus miliaris*, where the blue-green region appears most effective. Recently, I have shown that chromatophores distributed over the epidermis of *Diadema setosum* respond directly to light (Yoshida, 1956). The present work aims at discovering the relative sensitivity of the chromatophores to light of differing wavelengths.

MATERIAL AND METHODS

The aboral region of the interambulacra of fresh specimens of *Diadema setosum* (Leske) was used in the present experiments, since the colour change was most evident here. Cut pieces of test were examined in running sea water at 25 ± 1° C. in darkness. Observations were not recorded until the pieces had been at least 1 hr. in darkness so as to be sure that the pigment in the chromatophores had concentrated fully. If any chromatophores still appeared stellate, such pieces were discarded.

Chromatophores were then subjected alternately to periods of 20 min. darkness and 15 min. light in the form of a spot projected on to the chromatophores as previously described (Yoshida, 1956). At the end of illumination, a quick observation was made and the stimulated chromatophore photographed. The results of observations as well as the photographs were used to determine the changes in the pigmentary condition of the cell.

The light for stimulating the chromatophores was obtained from a 6 V. 30 W. tungsten filament lamp, the brightness of which was controlled by the voltage applied. A voltage stabilizer was inserted in the circuit of the lamp. The lamp used was calibrated by Dr M. Okamatsu of the Electrotechnical Laboratory, Tokyo, so

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that the colour temperature of the filament was known at 0·4 V. intervals between 2 and 5 V.

The filament was focused by a condenser lens on to the plane of the entrance slit of a double monochromator, the exit slit of which served as the source of monochromatic light for stimulating the chromatophores. The monochromator consisted of two SF 2 prisms of Perrin–Broca type, and the dispersion characteristics in the plane of the exit slit were calculated from the refractive index of the prisms at each wavelength and the focal length of collimator lenses. The width of each slit (entrance, central and exit) was fixed at 0·5 mm.

The stimulating apparatus was the same as that previously described (Yoshida, 1956). Here, however, the half mirror, from which the light beam was reflected so as to enter the optical system of the microscope, was made of Incomel metal evaporated on glass. Both the transmission and reflexion characteristics of this material were found to be fairly constant in the range 420–670 mμ.

The minimum voltage necessary to make any perceptible change in pigment dispersion was measured for each wavelength, and the relative energy at such threshold voltage was obtained as follows.

The colour temperature at a given voltage was substituted in Wien's radiation formula, thus giving the spectral radiance of the filament. This value, divided by the value of spectral dispersion in the plane of the exit slit of the monochromator, gave the energy content of the light falling on the chromatophores, any slight spectral variation of the transmittance of light in the optical system being neglected. The energy was expressed in arbitrary units.

RESULTS AND DISCUSSION

As previously mentioned (Yoshida, 1956), difficulties arise when high intensities are used, because the pigment of the chromatophore may shift bodily with respect to the light source so that, after dispersion, the pigment does not necessarily return to its original position or conform to its original outline when concentrated. In experiments performed immediately after such a mass shift of the pigment had taken place, the minimal amount of light energy required to elicit the response was usually lower than before. In some experiments, the threshold values altered during the course of the experiment. The reason for this is unknown. Such experiments were considered unreliable and the results were not used.

The results shown in Table 1 and Fig. 1 were obtained from nine series of experiments, in which the threshold values for a given wavelength (usually 500 mμ) were the same at the beginning and the end of each series. In order to make values in different series of experiments comparable, the results were recalculated so as to make the threshold unity at 500 mμ. The second column in Table 1 shows the relative threshold energy which is necessary for the response. The reciprocal of the values expresses the relative sensitivity which is shown in the third column, the figures being adjusted to make the value 100 at 468 mμ. Because of the superficial position of the chromatophores it is assumed that differential absorption in the

overlying regions of the skin is not likely to be significant. The chromatophores are sensitive over a broad band (450–500 mμ), and their maximal sensitivity is in the vicinity of 470 mμ.

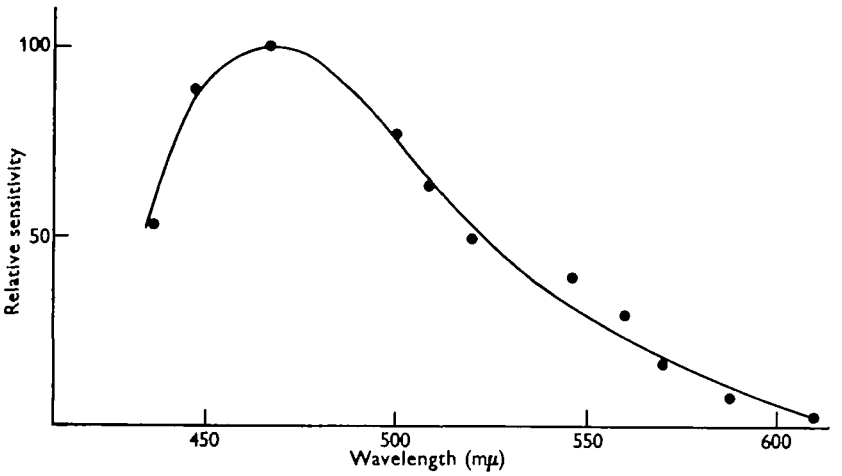

Fig. 1. Spectral sensitivity of chromatophores in *Diadema setosum*.

Table 1. *Spectral sensitivity of chromatophores in* Diadema setosum

Wavelength (mμ)	Relative threshold energy	Relative sensitivity
436	1·47	52·4
447	0·86	88·7
468	0·77	100
500	1·00	77·1
509	1·20	64·1
520	1·56	49·4
546	1·95	39·4
560	2·59	29·7
570	4·79	16·1
588	9·93	7·75
610	37·0	2·08
649	112	0·69

These results agree with the results obtained by Parker (1932), who showed that blue light was most effective in causing retinal pigment migration in frogs and crayfish, but not with Zetter's conclusions concerning the responsiveness of chromatophores in the frog's skin to coloured light. However, it is difficult to understand how Zetter reached his conclusions since the filters he used transmitted relatively broad bands (one from 460 to 600 mμ and the other from 600 to 690 mμ). It would appear that his results are not incompatible with a maximal sensitivity in the blue.

The region of the spectrum in which the chromatophores of *Diadema* will respond corresponds fairly closely with that in which the tube feet of *Psammechinus miliaris* react. It is thus possible that a common pigment may be involved.

There are also considerable differences between the spectral sensitivity of the chromatophores in *Diadema* and that of many complex photoreceptors. The cause of this must remain obscure until much more has been discovered concerning the photosensitive mechanism in *Diadema*.

SUMMARY

1. The spectral sensitivity of the chromatophores of *Diadema setosum* (Leske) was studied, using monochromatic light.

2. The chromatophores are most sensitive in the region of the visible spectrum between 450 and 500 mμ with a maximum at 470 mμ.

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