Spectral Sensitivity of the Scallop Pecten maximus

Abstract. *The spectral sensitivity of the scallop* Pecten maximus *was determined behavorially; the shadow reflex was used as the index of sensitivity. The photopic visibility curve displays two prominent peaks, one at approximately 480 mμ, and the other with wavelength maximum at approximately 540 mμ. Reduction of background illumination results in a great increase in sensitivity at 480 mμ relative to the 540-mμ peak. This suggests that the scallop may possess photoreceptors that behave like rods in vertebrates.*

The common scallop *Pecten maximus* is remarkable in possessing an image-forming eye with a lens and a tapetum which forms the image by reflection within the distal layer of visual cells (*1*). The retina contains two layers of photoreceptor cells (*2*). In studying the electrical responses of this eye, Hartline (*3*) found that the distal layer of the retina mediated strong *off* responses, while impulses would arise from the proximal layer only at the onset of illumination.

Although structurally much simpler, the eye of *Pecten* bears several similarities to the vertebrate eye. For one thing, the retina, like that of vertebrates and unlike that of cephalopods (*4*), is inverted. In addition, careful study with the electron microscope has shown that the visual cells of the distal layer are composed of concentric lamellae and that each lamella is continuous with the stalk and basal body of a cilium (*5*). In this respect, the visual cells of the distal layer are similar to the photoreceptor outer segments of the vertebrate retina which are also derived from cilia (*6*). The photoreceptors of the proximal layer, on the other hand, appear to be derived from microvilli, and in this respect resemble the photoreceptor cells of octopus and insect compound eyes.

Although the development of image-forming eyes in molluscs and chordates seems to have occurred independently, the vitamin A system is used in both in the photoreceptor process. In fact, it would appear from the apparently universal distribution of the carotenoid-protein system in animal photoreceptors that the system was probably inherited from some primitive common ancestor. Likewise, the functional process of lateral inhibition and the *on* and *off* systems which partake in the coding of primary visual information seem also to enjoy a similar widespread distribution throughout the vertebrate and invertebrate taxa (*3, 7*). One possibility is that some of these functional characteristics may be intimately linked to the nature of the photoreceptor process itself and that this might conceivably impose certain functional restrictions on the manner in which visual information is initially coded. Thus the apparently universal distribution of certain aspects of retinal coding may in part derive from the fact that a particular process of photoreception was inherited from some early common predecessor. Alternatively, these systems may have evolved independently.

It is clear from the foregoing that more data are needed on the functional and spectral characteristics of photoreceptors belonging to different phyla.

The work reported here on *Pecten* was conducted as a preliminary study before undertaking a detailed electrophysiological investigation of this retina. The object was to determine behaviorally the spectral sensitivity of this animal in the hope that this might suggest whether or not the eye possessed more than one visual pigment.

The photopic spectral sensitivity curves of vertebrates which have been determined behaviorally have sometimes given varying results. This has usually been attributed to possible interaction between the visual cells or to the presence of screening pigments (*8*). Nevertheless, there is generally good agreement between the peak wavelength values of the behavioral curves (*9*) and corresponding data from electrophysiological experiments (*10*), and spectrophotometric measurements of photopigments *in situ* (*11*).

The spectral sensitivity of a photoreceptor system may generally be obtained by determining the energy of different spectral lights necessary to produce a constant response. In the experiment recorded here, the reflex closing of the shell in response to a sudden diminution in light intensity was used as the index of sensitivity.

The spectral lights were produced with seven interference filters (Balzers, Lichtenstein). During the experiment the scallop was placed in a transparent glass container filled with clear sea water (temperature 18°C), and the position of the container was adjusted so that the eyes of the animal lay in the path of a collimated light beam from a 500-watt projector.

The relative quantum energy of the different spectral lights was calculated from the color temperature of the source (3100°K) and the spectral transmission curves of the interference filters which had previously been determined with the aid of an automatic recording spectrophotometer. From these results the values of compensating neutral density filters required to equate the quantum energies of the different spectral lights were determined. Table 1 gives the wavelength maximum and half-maximum bandwidth of the interference filters, together with the correction factor required to equate the quantum energy of the different spectral lights.

Table 1. Characteristics of the interference filters used in the experiments.

Maximum (mμ)	Half-maximum bandwidth (mμ)	log $1/T_c$ (correction factor)
418	4	0
443	3.5	−0.30
466	4	−0.314
489	4	−0.518
515	3.5	−0.409
538	4.5	−0.479
566	3.5	−0.526

Two groups of four animals were used in the experiment. The spectral sensitivity of animals in group 1 was

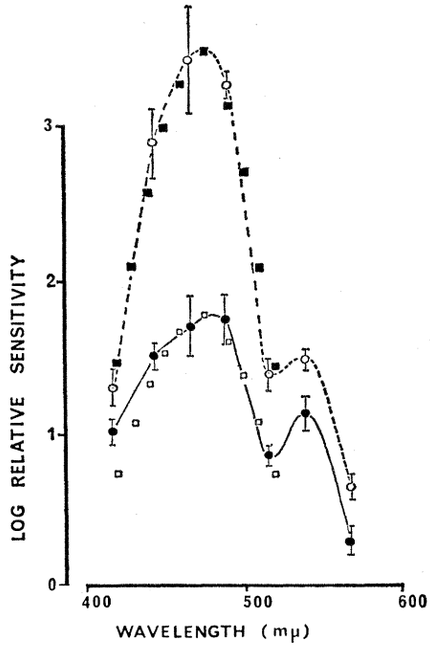

Fig. 1. Spectral sensitivity of *Pecten maximus* under two conditions of background illumination. Group 1: (filled circles) background illumination 0.28 mlam; (open squares) nomogram for visual pigment 475 mμ. Group 2: (open circles) background illumination 0.01 mlam; (filled squares) nomogram for visual pigment 475 mμ. Each experimental point on the curves represents a mean threshold (four animals). Standard deviations are given by the vertical lines in the figure.

determined under conditions of high background illumination (0.28 mlam). Also, these animals were thoroughly light adapted prior to the experiment by exposing them for 2 hours to the light from a 60-watt bulb suspended over their home tank.

The spectral sensitivity of animals in group 2 was carried out under mesopic conditions (0.01 mlam). In each case the background illumination was provided by the room's fluorescent lights and stray light from the projector. The level of background illumination was determined for groups 1 and 2 by measuring with an exposure photometer (Salford Electrical Instruments Ltd., London) the amount of light reflected from the white cardboard base upon which the container was placed.

During a threshold run the stimulus light was left on for 5 minutes before being interrupted by a mechanical shutter. Ten minutes were allowed between trials, and on successive trials the intensity of the stimulus beam was reduced in steps of approximately 0.2 log unit. The animal rarely, if ever, responded to the onset of illumination, and the adequate stimulus appeared always to be the sudden diminution in light intensity. However, once the animal had responded, it seemed to enter into a refractory phase for a period of at least 5 minutes, during which no change in light condition would produce a closure response, although it was still possible to elicit such a response by mechanical stimulation. Because of this, it was necessary to allow at least 10 minutes to elapse between trials. The threshold at a particular wavelength was determined by taking the lowest intensity at which the animal would respond twice in the course of three successive trials.

The spectral sensitivity curves are shown in Fig. 1. Individual results within each group were pooled; each of the experimental points in Fig. 1 represents an average threshold. In each case the appropriate correction factor has been added to compensate for the difference in light energy transmitted by different interference filters. Standard deviations were computed for the data within each group and are represented in Fig. 1 by the vertical lines on the curves.

It is immediately apparent that at least two different types of photoreceptors with different spectral sensitivities contribute to the overall spectral sensitivity of this animal. One of these appears to be maximally sensitive at approximately 475 to 480 mμ, and the other at approximately 540 mμ. In the absence of direct spectrophotometric measurements on the photopigments of this eye we cannot conclude that the two peaks in the behavioral sensitivity curve represent two visual pigments, since the data might equally well be explained by assuming the existence of one receptor pigment and a screening pigment with an appropriate absorption. For the sake of comparison the points of Dartnall's (*12*) nomogram for a visual pigment with absorption maximum at 475 mμ are also displayed in Fig. 1.

Comparing now the spectral sensitivity curves between groups 1 and 2, we see that when the level of background illumination is low the relative increase in log sensitivity of the 475-mμ region is several times greater than the corresponding increase in sensitivity of the 540-mμ region. This suggests that at low background levels of illumination the photoreceptors which mediate sensitivity in the 540-mμ region may have a high-response threshold relative to the 475-mμ photoreceptors. The possibility that under conditions of high background illumination sensitivity in the 475-mμ region may be depressed by active inhibition of other photoreceptors must also be considered. In general, the situation appears to be similar to that which occurs in the vertebrate retina, where it has been shown in goldfish that the rod portion of the photopic visibility curve shows a considerable increase in sensitivity, relative to the cones, when the background illumination is reduced (*9*). These considerations lead one to suspect that the eyes of *Pecten* may possess photoreceptors that are functionally similar to the rods and cones of the vertebrate retina, and that under suitable light conditions the spectral sensitivity of this animal is likely to display a Purkinje shift.

Throughout the preceding discussion it has been assumed that the spectral sensitivity which has been measured represents the sensitivity of the eye. However, a note of caution is required in this interpretation, since the possibility is not excluded that other light-sensitive structures, perhaps located directly within the nervous system itself, may be contributing to the spectral sensitivity of this animal.

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Lipoprotein Patterns in Acrylamide Gel Electrophoresis

Abstract. *Lipoproteins of serum stained before electrophoresis gave distinct patterns in acrylamide gel of 3-percent concentration. Samples compared in parallel on gel slabs showed qualitative differences in migration rates of β-lipoprotein bands.*

Human serum lipoproteins have not been extensively examined by electrophoresis in either starch gel or acrylamide gel, presumably because of inadequate technical methods (*1*). The technique described here provides (i) low gel concentration to permit migration of large lipoprotein molecules, (ii) subsequent stiffening of the gel for further processing of the pattern, (iii) an improved stain for use prior to electrophoresis, (iv) direct comparison of multiple samples on a single gel slab. The technique reveals variations in electrophoretic mobility of β-lipoproteins from different individuals and from the same individual in different clinical conditions.

Serum samples were selected at random from the routine laboratory; they were from healthy individuals and patients with various diseases, some of the patients having hyperlipemic status. Samples stored for several days