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SOME ASPECTS OF VISION IN THE LOBSTER, *HOMARUS VULGARIS*, IN RELATION TO THE STRUCTURE OF ITS EYE[1,2]

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(Plates I–III and Text-figs. 1–7)

Increasing interest and attention are being accorded the visual processes of decapod crustaceans. The electrical responses elicited by exposure of the eye of the crayfish *Procambarus clarkii* (Girard) to light have been described by Naka & Kuwabara (1959). These workers have demonstrated the influence of the degree of penetration of the recording electrode on the shape and polarity of the response. The electroretinogram is negative near the periphery of the eye. When the microelectrode is moved inward the polarity of the electroretinogram is reversed in the region just distal to the basement membrane.

The spectral sensitivity of the eyes of decapods has been estimated in two ways, by prediction from the absorption spectra of photosensitive pigments from the eyes and by electrophysiological measurements. Two photosensitive pigments have been reported, euphausiopsin with a single absorption maximum near 462 mμ (Kampa, 1955; Fisher & Goldie, 1959; Dartnall, personal communication), and lobster rhodopsin with a single maximum at 515 mμ (Wald & Hubbard, 1957). Stieve (1960) has shown by use of electroretinograms that the spectral sensitivity curve of the eye of the hermit-crab *Eupagurus bernhardus* L. has a single peak near 500 mμ. The sensitivity curve is somewhat flatter than the difference spectrum of lobster rhodopsin, and the peak is displaced about 15 mμ toward the blue. Similarly, Kennedy & Bruno (1961) have investigated the effect of colour on the electroretinogram of the lobster *Homarus americanus* Milne-Edwards and the crayfish *P. clarkii*. There is good agreement between the spectral sensitivity curve for the lobster and the difference spectrum of lobster rhodopsin with respect to shape. The sensitivity is greatest near 525 mμ, representing a displacement of 10 mμ

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toward the red from the peak of the difference spectrum of lobster rhodopsin. The spectral sensitivity of the crayfish eye is greatest near 570 mμ, and Kennedy and Bruno conclude that a pigment other than 'a rhodopsin' must be involved.

Boden, Kampa & Abbott (1961) have reported the results of preliminary experiments with the eyes of euphausiids. In this pelagic group there is a great variation from species to species in the structure of the eyes. Concomitantly, differences exist between the electrical responses obtained from different species. In *Euphausia pacifica* Hansen and *Meganyctiphanes norvegica* M. Sars the eye is spherical, and the electroretinogram has both fast and slow components. The spectral sensitivity curve is wide and shows two to three maxima between 460 and 535 mμ. In *Nematoscelis difficilis* Hansen the eye is bilobed, and the electroretinograms from the two lobes differ markedly. That of the upper lobe is a fast response with a single maximum of spectral sensitivity near 465 mμ. The response of the lower lobe is slower and has a broad-band spectral sensitivity. The spectral sensitivity curve for the fast response is nearly identical with the difference spectrum of euphausiopsin, the photosensitive pigment that has been reported from *E. pacifica* and *M. norvegica*.

We have now studied the structure of the eyes of the common lobster, *Homarus vulgaris* Milne-Edwards, and the characteristics of the electroretinograms obtained from them. We have used microelectrodes similar to those employed by Stieve (1960) with *Eupagurus*, rather than wick electrodes (Kennedy & Bruno, 1961), in order to extend the study of the lobster eye to structures underlying the cornea.

The experiments were conducted at the Plymouth Laboratory.

MATERIALS AND METHODS

Animals

Specimens for the experiments were obtained from the lobster fishermen at the commercial fishing wharf in Plymouth, and from the collections of the R.V. 'Sula'. The animals were kept in the outdoor tanks at the Laboratory. Specimens were used within 5 days of capture to obviate any effects of diet on normal visual sensitivity.

Histology

Fresh specimens of *H. vulgaris* were dark-adapted overnight in dark-room tanks at the Laboratory. The eyes were removed in dim red light and preserved in Bouin's solution in light-proof containers. Eyes removed from fresh, light-adapted specimens were similarly preserved in the light, as were all of the eyes from specimens used in the physiological experiments and several of the zoaea larvae.

After fixation the eyes were dehydrated in an ethyl alcohol series and transferred to ethyl alcohol–ethyl ether. The fixed, dehydrated eyes were then embedded in celloidin according to the hot celloidin method devised by Walls (1936). The celloidin blocks

were hardened in chloroform, cleared in terpineol and sectioned dry with a rotary microtome.

A variety of biological stains was used to bring out the various structures in the sections.

Electrophysiology

Light

Experiments were performed in a light-proof box, the inside of which was painted matt black. The box was made of metal and connected to earth. An optical bench extended the length of the box and supported the micromanipulator, the preparation and two lenses. One side of the box was equipped with sliding doors to permit adjustment of the preparation and optical system. In one end of the box was an opening fitted with an iris diaphragm that restricted the diameter of the stimulating light path to 3 cm. Attached to the outside of the box at this level were two 12-place filter carriers and a collimating tube capable of restricting the angle of the stimulating light to a 5° cone at the level of the inner filter carrier. The inner filter carrier held Balzer's B-40 interference filters for which the collimation was necessary. Thirteen filters were used, covering the 421–595 mμ range at intervals of 9–19 mμ. The outer filter carrier held a series of Wratten neutral-density filters mounted in 'B' glass. A camera shutter was attached to the outer end of the collimator. The light source was a tungsten lamp (Osram, 220 V, 500 W) operated at constant voltage from the regulated supply at the Laboratory. It was housed in a standard, fan-cooled 35 mm slide projector from which all focusing lenses had been removed. The projector was levelled until its beam was horizontal and just filled the face of the shutter. When necessary, additional neutral-density filters were placed in the slide holder of the projector.

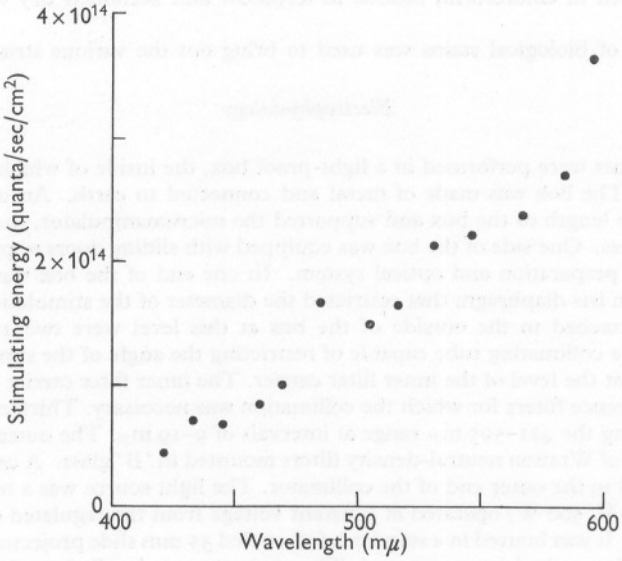
The light passed from the projector, through the shutter, along the collimator, through any desired combination of neutral-density and interference filters, into the box. Inside, it passed through two lenses to a front-surface mirror mounted at 45° directly over the preparation. The positions of the lenses were established by trial and error, such that the light field (2 cm in diameter) at the level of the preparation was uniform in intensity. Its uniformity was verified by scanning the field with a pinhole photometer.

Light intensities through the various interference filters were measured at the level of the preparation with an Eppley thermopile in conjunction with a Leeds and Northrup 2284 galvanometer. Intensity values so obtained were in μW/cm^2. These were converted to quanta/sec/cm^2 (Text-fig. 1); for a given filter the energy of a quantum is taken as equal to $h\nu$ when h is Planck's constant and ν is the frequency of peak transmission of the filter. The thermopile and galvanometer were calibrated with a U.S. Bureau of Standards source.

The actual transmission of each interference filter and each neutral-density filter was determined with a Beckman spectrophotometer. Peak transmissions of the interference filters varied from 40·6 to 49·4%; half-peak band widths varied from 7 to 12 mμ. Actual values of the neutral-density filters at the peak wave-length of each interference filter were used in all plots and calculations.

Preparation

The vessel for holding the preparation was a Perspex dish embedded tightly in an aluminium block. A 'Sno-Gel' cold-storage battery (a commercial colloid with very high thermal capacity) was wedged into a chamber in the block just below the dish. Dish, block and Sno-Gel were assembled and kept in the deep freeze overnight before each experiment. With the pre-cooled block the temperature of the preparation remained at 9° C for as many as 5 h.



Text-fig. 1. Energy of the stimulating light at the 13 wave-lengths used in the experiments. The measurements were made with a thermopile at the level of the preparation.

An excised eye was attached to the indifferent electrode with Plasticine, the aluminium block was placed in position on the optical bench and filled with sea water. The microelectrode was lowered with the micromanipulator until the tip reached the corneal surface. Penetration was facilitated by a slight incision of the cornea or by the use of a solenoid, the movement of which was oriented to coincide with the long axis of the electrode. The range of movement was 0·1 mm, just great enough to thrust the tip of the electrode into the layer of crystalline cones. After the initial penetration the depth of the electrode was again controlled by movement of the micromanipulator.

Recording

Micropipettes were pulled from 2 mm capillary tubing with a Palmer microelectrode puller. The diameters of the tips varied from 0·5 to 1 μ. The pipettes were filled with 3 M-KCl and connected by way of a Ag–AgCl electrode to the G_1 grid of a Grass P-6 preamplifier. The impedance of the recording electrodes ranged from 10 to 30 MΩ and was measured *in situ* at intervals throughout each experiment. A strip of Ag–AgCl gauze in the sea-water bath served as the indifferent electrode. It was connected to the G_2 grid of the preamplifier. The G_2 grid was grounded during the experiments, and recording was single-sided. Responses were displayed simultaneously on a Tektronix 502 oscilloscope and a Sanborn Model 320 recorder.

Procedure

The preparation was left to dark-adapt while adjusting to the experimental temperature. Test flashes were given at intervals. When the amplitude of the response became constant—usually after 30–60 min—the experiment was begun.

(*a*) The relationship between the intensity and the duration of the stimulating energy and the amplitude of the retinal response was determined first with 'white' light. The stimulus intensity was increased in steps from 40·5 μW/cm^2 to 12,800 μW/cm^2 by various combinations of neutral-density filters. The shutter was calibrated to give flash durations of 4, 10, 20, 40, 100 and 200 msec.

(*b*) The effect of depth of penetration of the recording electrode on the shape and polarity of the retinal response was studied from adult animals and from the zoaea larvae still in the eggs. The depth of penetration was determined in three ways. With the first method the depth from which a particular recording was obtained was estimated by reading the verniers on the micromanipulator. With the second method the recording electrode was inserted to a depth at which a particular record was obtained. The electrode was then sprayed *in situ* with a fast-drying opaque lacquer and withdrawn. The length of the unpainted tip was measured with an ocular micrometer on a compound microscope. With the third method lithium carmine was deposited electrophoretically at the site of the recording in a manner similar to that described by Mitarai (1960) for recordings from vertebrate retinas. The micropipettes were filled with 3 % carmine in saturated aqueous lithium carbonate. After a particular response was obtained, a current was passed through the preparation, such that a small amount of the positively charged lithium carmine was deposited at the tip of the microelectrode. The eyes were fixed and sectioned subsequently, and the spot of stain indicated the position of the tip of the recording electrode.

(*c*) The spectral sensitivity of the eye was measured. In all of the spectral-sensitivity work reported here the stimulus duration was 40 msec, and the interval between stimuli was 15 sec. The first interference filter was placed in the light path, and the intensity was increased in steps with neutral-density filters from the lowest value to the highest. This was repeated with each interference filter. In some experiments the wave-lengths were presented in an ascending order from 421 to 595 mμ; in others, in a descending order. The order of presentation did not affect the results perceptibly.

For each set of experiments a stimulus of a particular colour and intensity was selected as a reference. A 460 mμ flash at $8{\cdot}2 \times 10^{13}$ quanta/sec/cm^2 was used in some of the work; in the remainder the reference was a $1{\cdot}5 \times 10^{13}$ quanta/sec/cm^2 flash at 504 mμ. The reference stimulus was presented at the end of each neutral-density series and served to monitor the visual threshold of the eye. The amplitude of the response to the reference was plotted against time. An average value was taken, and the responses to test stimuli were corrected by applying the ratio (amplitude of average reference : apparent amplitude of the reference at that instant) to the amplitude of the test response.

From each experiment a family of curves was obtained relating the amplitude of the retinal potential (ordinate) to the logarithm of the stimulus energy (abscissa) at each of a number of wave-lengths (Text-fig. 5, p. 693). The number of quanta/sec/cm^2 required to elicit a given retinal potential at each wave-length was read directly from the abscissa. Retinal sensitivity was taken as the reciprocal of that number. From the family of reciprocal values a sensitivity spectrum was constructed.

RESULTS

The compound eye of *Homarus vulgaris* has a single lobe. The ommateum (the assemblage of ommatidia) cups the end of the optic stalk. It is indented dorsally and ventrally by protrusions of the stalk integument.

The ommatidia appear uniform except in length (Pl. 1A) and are very

similar to those described by Parker (1890) from the eyes of *H. americanus*. The outer layer of the ommateum, the cornea, is a continuous sheet to which each ommatidium contributes a square facet. In the eye of the adult the facet is about 75 μ thick and is composed of many layers. Presumably they are chitinous. Beneath each facet are the two very thin corneagenous cells which secrete it. Beneath these are the four cone cells which lay down the four segments of the crystalline cone. The cone is square in cross-section (Pl. I B) throughout most of its length. The segments are tapered gradually so that the width of the cone is reduced from about 33 μ at the outer end to about 25 μ at the inner end.

Proximal to the cone in the eye of the adult is another four-part structure, the long (750–950 μ) cone stalk, which extends from the cone layer to the retinula. In the eye of the zoaea larva (Pls. II B, III A) the cone stalk is not elongate. The cone cells fill the entire pyramidal space later occupied by the crystalline cone. Secretion of crystalline material has just begun at the distal end of each cell. In preserved material the cytoplasm contains granules which are stained a brilliant red with acid fuchsin. These resemble the granules of the cone stalk in the adult, and it seems likely that the stubby apex of the cone-cell pyramid in the larva is the rudimentary cone stalk.

At the inner end of the cone stalk in the adult (Pls. I A, II A), and between the apices of the cone-cell pyramids in the zoaea (Pls. II B, III) are the nuclei of the seven retinular cells. These cells surround, and presumably secrete, the rhabdom. The proximal ends of the retinular cells penetrate the basal membrane of the ommateum and proceed to the first optic ganglion, the lamina ganglionaris, in the optic stalk. The retinular cells are densely pigmented,

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EXPLANATION OF PLATES I–III

PLATE I. Sections from the eyes of adult *Homarus vulgaris*

A, Longitudinal section through a number of ommatidia; B, oblique section, showing, from top to bottom: (1) cornea, (2) cone cells, (3) cones, (4) cone stalks. The black frames separating adjacent cones and the dark spots at the intersections of four cone stalks are pigmented cells.

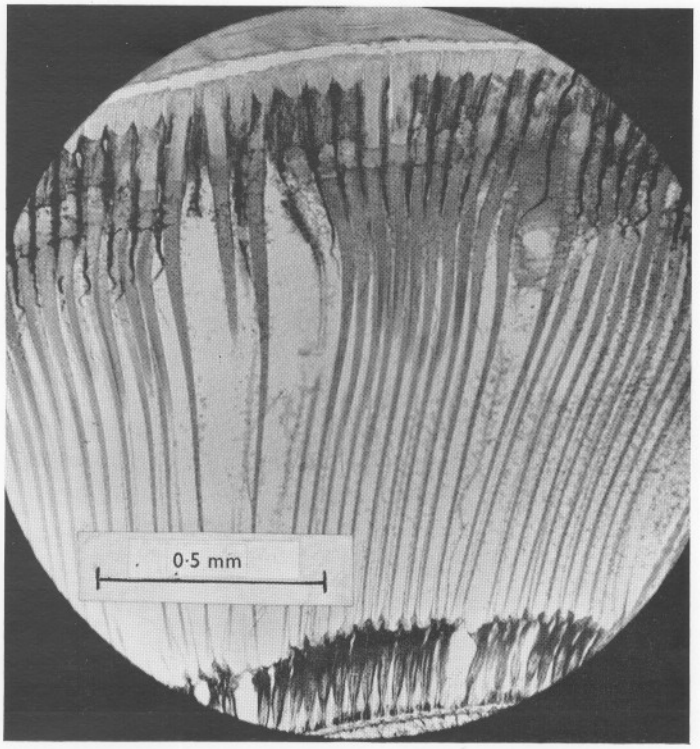
PLATE II

A, Oblique section from the eye of adult *H. vulgaris*, showing, from top to bottom: (1) nuclei of retinular cells, (2) rhabdoms surrounded by seven retinular cells, (3) rosettes of retinular cell axons, and (4) basal membrane. B, Longitudinal section from the eye of a zoaea larva, showing in the clear periphery the cornea, corneagenous cells, cone cells, and nuclei of retinular cells. The lengths of the retinular cells surrounding the rhabdoms are densely pigmented.

PLATE III. Sections from the eyes of zoaea larvae

A, Longitudinal section. The tissue was bleached before staining to show the stubby precursor of the cone stalk. Tapetal cell nuclei are visible between the distal ends of the rhabdoms. B, Oblique section, showing, from top to bottom: (1) cone-cell nuclei, (2) cone cells surrounded by retinular cell nuclei, (3) rudimentary cone stalks and (4) rhabdoms surrounded by the rosettes of seven retinular cells.

A



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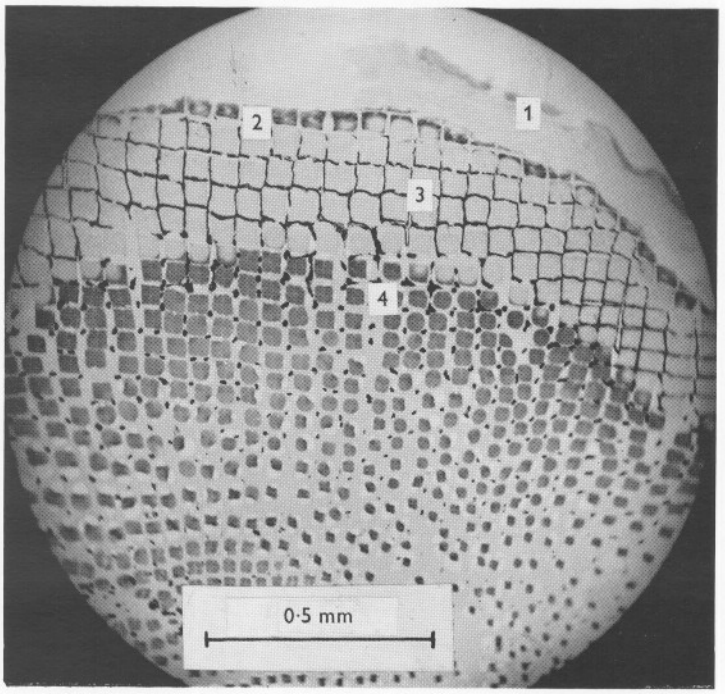

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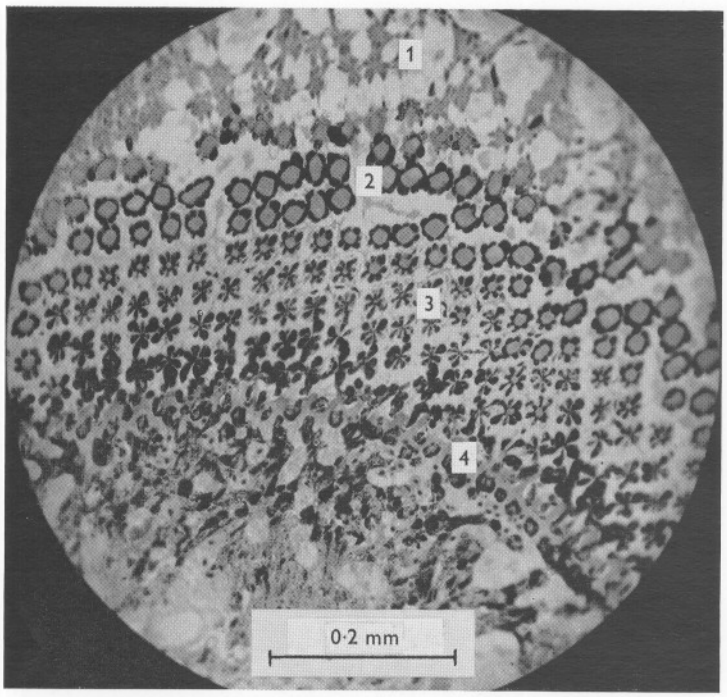

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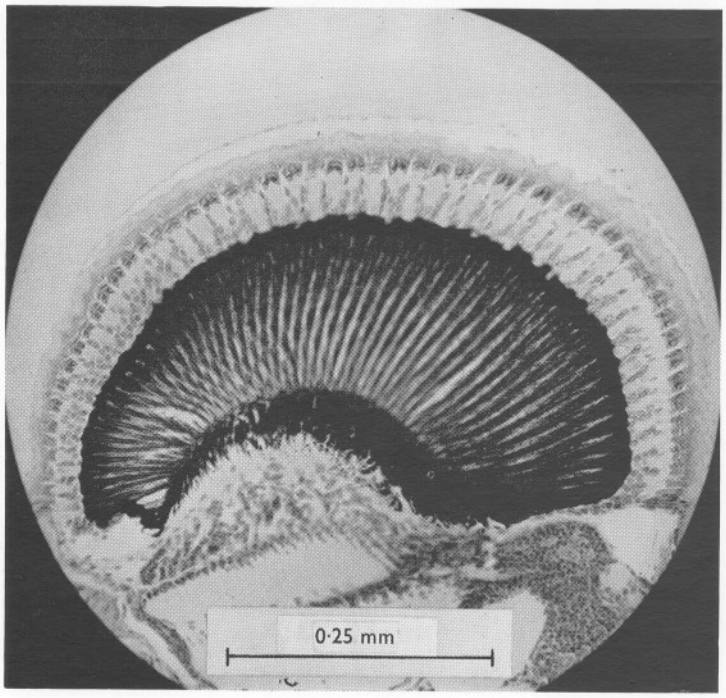

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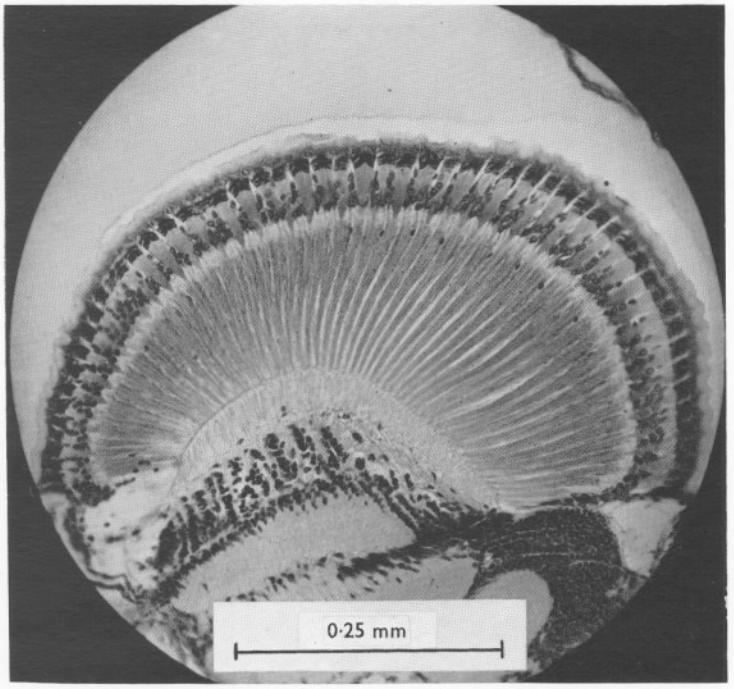

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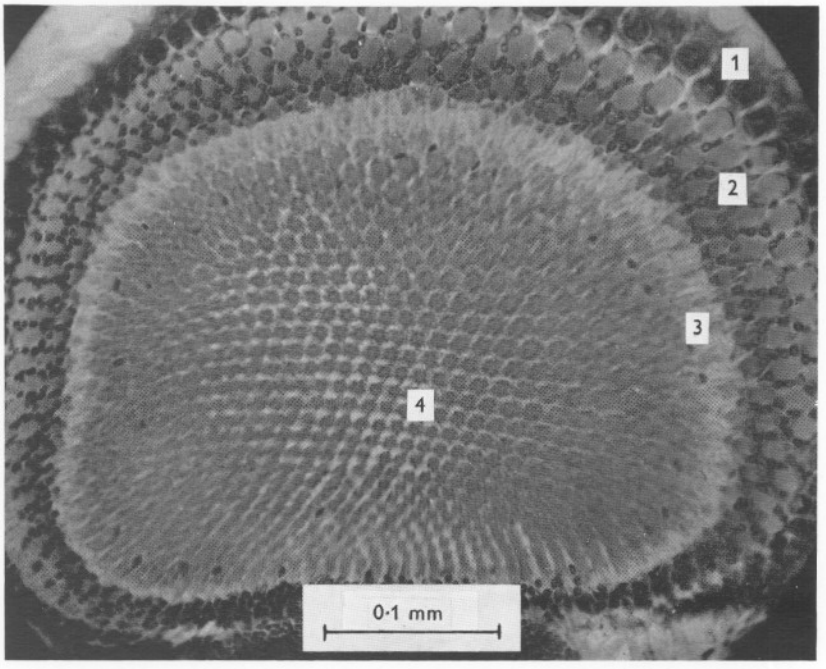


and in the zoaea the pigment is restricted to the length of the rhabdom and the fibrous extensions just proximal to the basement membrane.

The rhabdom in the adult is fusiform in longitudinal section (Pl. IA). It comprises four rhabdomeres which are evident in cross-section (Pl. IIA). The rhabdomeres are apparently composed of stacks of plates. In the zoaea the rhabdoms are very slender, and no plate-like structures were evident in our sections.

In the adult, distal screening pigment cells separate each crystalline cone from its neighbours. The nuclei of these cells are minute, and two of them may be seen at the junction of four crystalline cones. In light adaptation dense pigment extends along the full length of the crystalline cones and inward as slender filaments at the intersection of four cone stalks. In dark adaptation the distal pigment is withdrawn towards the outer ends of the cone stalks, and the pigment of the retinular cells migrates inwards to expose the retinular cell nuclei. Distal pigment cells are not evident in the zoaea.

Between the rhabdoms of bleached sections the nuclei of an additional set of cells, presumably the tapetal cells, may be seen (Pl. IIIA).

Growth and development between the zoaea and the adult of *H. vulgaris* seem to proceed along four lines: the secretion of the crystalline cone, the elaboration of the rhabdomeres, the development of distal pigment cells between the cones and the elongation of the inner ends of the cone cells to form the long cone stalks. Elongation of the ommatidia is effected primarily by the last of these, in distinct contrast to the developmental pattern in another decapod crustacean, *Pleuroncodes planipes* Stimpson (Kampa, in press), in which the cone-stalk length does not change significantly between the late larval and the adult stages. In *P. planipes* the lengthening of the ommatidium depends on outward growth of the retinular cells and deposition of a long hyaline filament which is a modification of the true rhabdom.

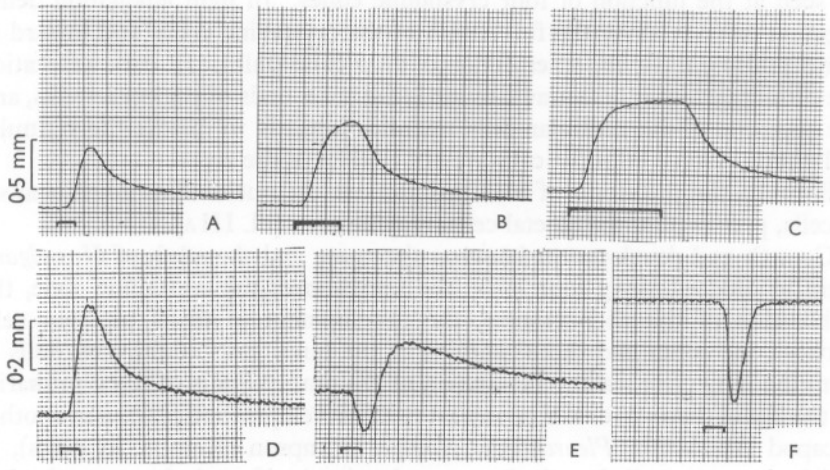
The electroretinogram of *H. vulgaris*, as in other crustaceans, is the sum of a number of electrical potentials arising in various parts of the eye. Its shape and polarity depend on two factors: (1) the response of the various components to stimulus intensity and duration and (2) the proximity of the recording electrode to the site of origin of these various components.

Near the surface of the eye in the adult in the region of the cornea, crystalline cones and cone stalks the response is negative. After a latent period of 20–25 msec the response rises with a time constant of about 20 msec for a duration 50 msec longer than the duration of the flash of light. With brief flashes height increases with duration (Text-fig. 2A, B), but with flashes longer than 100 msec the response reaches a plateau (Text-fig. 2C) with amplitude linearly related to the logarithm of the stimulating intensity over several orders of magnitude. At the end of stimulation the response decays exponentially.

The duration of the test flash used as a stimulus in the remainder of the work

was set at 40 msec, which corresponded to a convenient shutter speed, resulted in a response which was greater than half the maximum for the negative wave, but minimized light adaptation.

As the recording electrode invades the eye more deeply, the amplitude of the negative response increases gradually (Text-figs. 2A, D, 3). At depths of 1·4–1·6 mm, depending on the size of the adult specimen, we obtained responses with amplitudes as great as 2 mV to 'white' light with an intensity of 12,800 μW/cm^2. At this depth the electroretinogram suddenly became

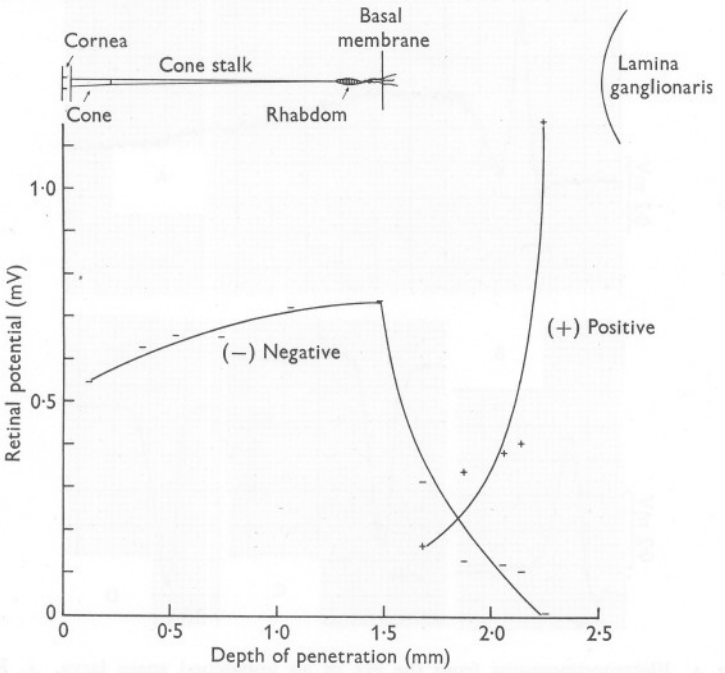


Text-fig. 2. A–C, electroretinograms from the periphery of the eye (blue-green light, $\lambda_{max.}$ 504 mμ, 1·5 × 10^{13} quanta/sec/cm^2). A, negative response to 40 msec flash; B, response to 100 msec flash; C, response to 200 msec flash; D, negative response to 40 msec flash with the electrode at depth of 1 mm; E, diphasic electroretinogram recorded with the electrode at depth of 1·6 mm; F, positive response recorded from apparent depth of 2 mm.

diphasic (Texts-figs. 2E, 3), with an initial positive wave and a second negative peak which decayed with the same time constant as that of the negative wave described above. As the microelectrode was advanced, the positive wave increased in amplitude while the amplitude of the negative wave declined. As suddenly as it had become diphasic the electroretinogram again became monophasic (Text-figs. 2F, 3) and presented a single positive response of 50–60 msec duration, independent of the stimulus duration. The amplitude was linearly related to the logarithm of stimulus intensity with 'white' light over a range from 128 to 12,800 μW/cm^2.

While the diphasic electroretinogram was being recorded the microelectrode often appeared to pass through about 0·5 mm of tissue. However, subsequent histological examination of these eyes showed that the basal membrane had been forced inward about this distance before it was pierced.

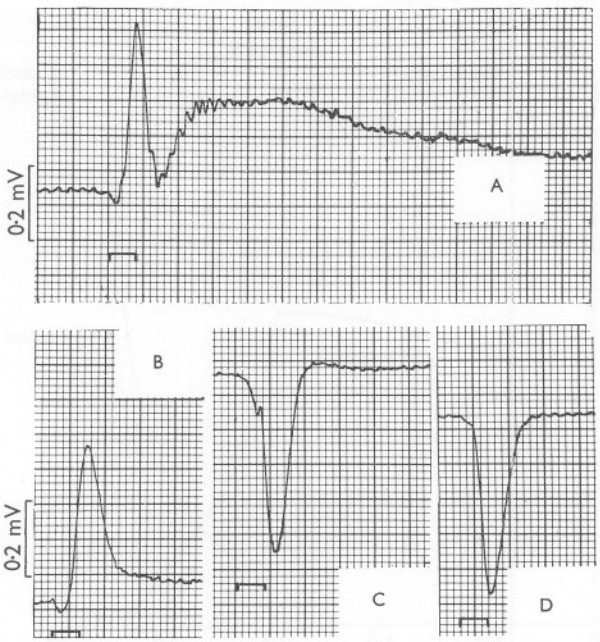
It is also probable that the electrode tip was bent over while in contact with the membrane. While the electroretinogram was diphasic the resting potential drifted steadily 20–50 mV in a positive direction, indicating that the electrode was passing through a membrane. In experiments where the electrode tip penetrated this membrane with no hindrance (probably passing through regions where the retinular cells perforate the membrane) the jump in the resting potential was very rapid.



Text-fig. 3. Relation between the amplitude and polarity of the retinal potential and the depth of the recording electrode tip. In this experiment the electrode depth was determined by readings of the vernier on the micromanipulator.

In experiments where lithium carmine was deposited just distal to the level at which the response became diphasic, a study of serial sections indicated that the electrode had been distal to the basement membrane. In similar experiments where lithium carmine was deposited at the level of the monophasic positive response, the sections showed that the electrode had penetrated the membrane and that the electrode tip had been among the retinular cell axons. In one experiment spraying of the microelectrode with fast-drying opaque lacquer after the first diphasic response had been obtained showed that the tip of the electrode had penetrated 1·6 mm. The average distance from the outer surface of the cornea to the basal membrane in fixed material was 1·5 mm.

From the histological evidence and from the observations of the change in resting potential during the time that the electroretinogram is diphasic, we conclude that the basal membrane offers a degree of mechanical resistance to the electrode tip and that the diphasic electroretinogram is displayed only during the time when the microelectrode is embedded in and deforming the membrane.

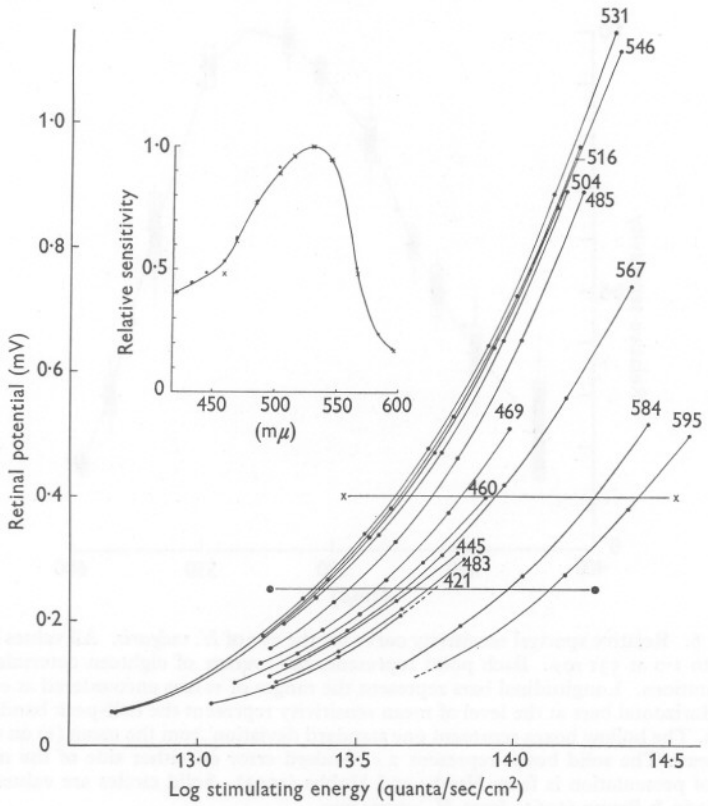


Text-fig. 4. Electroretinograms from the eye of an unhatched zoaea larva. A, Recording from the corneal surface showing well-separated fast and slow negative potentials; B, recording from just below the cornea showing fast negative potential and greatly reduced slow negative response; C, diphasic recording from the region of the basal membrane; D, fast positive potential from below the basal membrane.

The results with the zoaea larvae (Text-fig. 4) were, in many respects, similar to those obtained with adult specimens. There was an initial fast negative wave at the surface not recorded in the adult but similar to that found by Naka & Kuwabara (1959) in *P. clarkii*. The amplitude of this fast negative wave in the periphery of the eye as well as that of the positive response at depth to 'white' light were as great as 1·6 mV. The reversal of polarity of this wave at depth was accompanied by a 20 mV drift toward the positive in the resting potential.

Two sets of observations were made with the larval animals. At the outset it seemed that any incision of the eye might kill such frail creatures, and

several recordings were made with the tip of the electrode resting on the cornea (Text-fig. 4A). In all of these the 40 msec 'white' light stimulus was used. A fast and a slow negative component were apparent in the electroretinogram. The duration of the fast negative response was sufficiently brief for the two waves to be clearly separated in time. The slow wave lasted much longer than the stimulus but decayed at the same rate as in the eye of the adult.

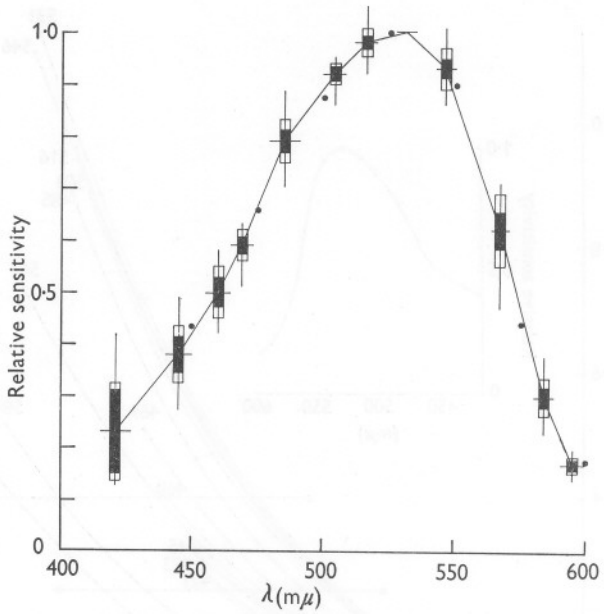


Text-fig. 5. Curves relating retinal potential and log stimulating energy for thirteen wave-lengths. Inset: relative spectral sensitivity curve constructed by equating the reciprocals of the numbers of quanta/sec/cm² required to elicit 0·4 mV (×—×) and 0·25 mV (•—•) responses at each wave-length to 1·0 at 531 mμ.

After it was obvious that the response at the corneal surface was strong, we incised the eye to insert the electrode. From immediately below the surface of the cornea the slow negative response virtually disappeared (Text-fig. 4B), and the reversal to the fast positive wave occurred with a very small penetration.

In records from zoaea there was also an initial, small, fast, positive wave with a latency of only 1 msec. No significance is ascribed to this wave now.

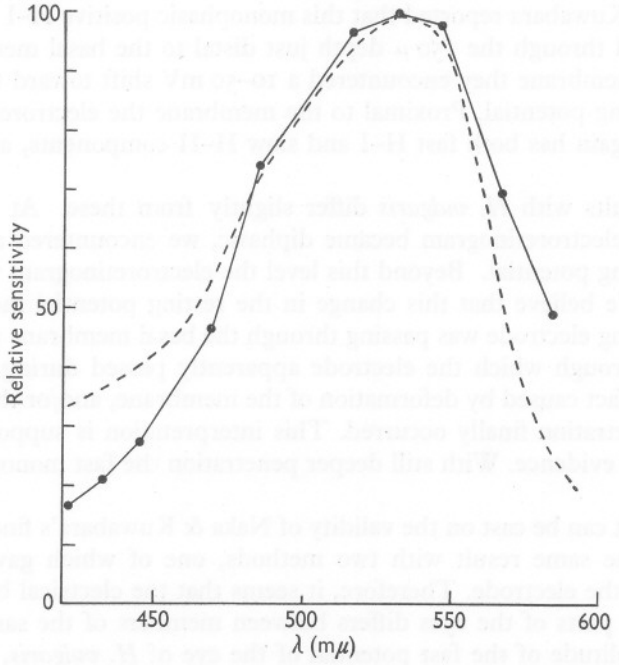
All of the studies of spectral sensitivity of *H. vulgaris* were conducted on adults. The curves relating retinal potential to stimulating energy were closely parallel, and the sensitivity spectra obtained by plotting the reciprocals of the number of quanta/sec/cm^2 against λ for various levels of retinal response were quite uniform (Text-fig. 5). The maximum sensitivity of the negative response in the periphery of the eye as shown by averages of the reciprocals of the number



Text-fig. 6. Relative spectral sensitivity curve for the eye of *H. vulgaris*. All values have been equated to 1·0 at 531 mμ. Each point represents the average of eighteen determinations on six preparations. Longitudinal bars represent the ranges of values encountered at each wave-length. Horizontal bars at the level of mean sensitivity represent the half-peak band widths of the filters. The hollow boxes represent one standard deviation from the mean (σ) on either side of the mean. The solid boxes represent 2 × standard error on either side of the mean. The method of presentation is from Hubbs and Hubbs (1953). Solid circles are values obtained by Kennedy & Bruno (1961) from *H. americanus*.

of quanta/sec/cm^2 required to elicit a given biological response in eighteen experiments with six preparations lies between 516 and 531 mμ (Text-fig. 6). The sensitivity drops sharply between 546 and 595 mμ. At shorter wave-lengths the slope of the sensitivity curve is more gradual. A fair agreement exists between the results of these studies and those of Kennedy & Bruno (1961) with the spectral sensitivity of the New World lobster, *H. americanus* (solid circles, Text-fig.6). As can be seen in this figure, the ranges of the responses to neighbouring wave-lengths overlap. Standard deviation from the mean and standard error are greatest at 421 mμ, where the tungsten source was weakest and the

half-peak band width of the filter was wider than that at other wave-lengths. However, even at this wave-length the standard errors compare favourably with those computed by Kennedy & Bruno (1961) for their measurements of spectral sensitivity in the crayfish; the standard deviations from the mean (σ) in our results at wave-lengths near the peak of the sensitivity curve (Text-fig. 6), are less than Kennedy & Bruno's standard errors.



Text-fig. 7. Spectral sensitivity curve for the positive response (solid circles) of the eye of *H. vulgaris*. The spectrum of the sensitivity of the negative potential from the same preparation is shown by the broken line.

The spectral sensitivity curve of the positive electroretinogram obtained at depth in the eye was similar to that of the negative response near the periphery (Text-fig. 7).

CONCLUSIONS

The negative potentials described here from the peripheral region of the ommateum of *H. vulgaris* are similar in many respects to the H–II potentials of the outer layers of the eye of *P. clarkii* defined by the detailed experiments of Naka & Kuwabara (1959). They reported an early fast negative wave H–I which we have found only in surface records in zoaea larvae of *H. vulgaris*. The slower potential, like the H–II of the eye of *P. clarkii*, increases in amplitude as the duration of the stimulus is increased; it is maintained through-

out stimulation and decays exponentially with time when the stimulus is terminated.

As the recording electrode penetrates the eye more deeply, the electroretinograms in *H. vulgaris* and in *P. clarkii* become diphasic with an early fast positive transient. At still greater depths in both types of eyes, the H–II potential of *P. clarkii* and the negative response of *H. vulgaris* are reduced in amplitude, until the electroretinograms are monophasic and positive.

Naka & Kuwabara reported that this monophasic positive H–I in *P. clarkii* is displayed through the 150 μ depth just distal to the basal membrane. At the basal membrane they encountered a 10–50 mV shift toward the negative in the resting potential. Proximal to the membrane the electroretinogram of *P. clarkii* again has both fast H–I and slow H–II components, and both are positive.

Our results with *H. vulgaris* differ slightly from these. At the level at which the electroretinogram became diphasic, we encountered a large shift in the resting potential. Beyond this level the electroretinogram was entirely positive. We believe that this change in the resting potential indicated that the recording electrode was passing through the basal membrane and that the distance through which the electrode apparently passed during the change was an artifact caused by deformation of the membrane, and/or the electrode, before penetration finally occurred. This interpretation is supported by the histological evidence. With still deeper penetration the fast monophasic wave persisted.

No doubt can be cast on the validity of Naka & Kuwabara's findings. They obtained the same result with two methods, one of which gave a precise location of the electrode. Therefore, it seems that the electrical behaviour of the various parts of the eyes differs between members of the same order.

The amplitude of the fast potential of the eye of *H. vulgaris*, like that of most other animals, varies directly with the logarithm of the stimulating energy. Use is commonly made of this feature to determine the spectral sensitivity of the eye. Like *H. americanus* (Kennedy & Bruno, 1961) *H. vulgaris* is most sensitive to blue-green light at about 525 mμ. The agreement between their measurements and ours is quite close, although the methods of experimentation were significantly different. Kennedy & Bruno concluded that their spectral sensitivity curve for *H. americanus* is sufficiently like the difference spectrum of lobster rhodopsin (Wald & Hubbard, 1957) for this carotenoid-protein to be responsible for lobster vision. The discrepancy between the peaks of the two curves ($\lambda_{max.}$ lobster rhodopsin = 515 mμ; λ_{max} *H. americanus* sensitivity = 525 mμ) is explained by the action of astaxanthin as a screening pigment. Such a conclusion seems justifiable for *H. vulgaris* as well, and it can be anticipated that extraction and analysis of the visual pigment of the Old World species would yield results very similar to those of Wald & Hubbard.

DISCUSSION

It is becoming increasingly apparent that the relationship between scotopic visual spectral sensitivity and the photic environment can supersede familial ties. Within the order Pisces alone, three groups of pigments have been demonstrated—the classic rhodopsin from shallow-water marine fishes ($\lambda_{max.}$ *ca.* 500 mμ), porphyropsin ($\lambda_{max.}$ *ca.* 522 mμ) from fresh-water forms, and most recently chrysopsins ($\lambda_{max.}$ *ca.* 470 mμ) from deep-sea fishes. Little emphasis was placed on differences between the sum of the physical features of the fresh-water environment and that of the shallow marine environment by those working with the rhodopsins and porphyropsins from the time of Kühne's (1877–78) first description of rhodopsin. It has been implied that the two pigments are somehow related to salinity.

When the chrysopsins were discovered almost simultaneously by Denton & Warren (1957) and Munz (1957) the effects of the physical features of the environment, particularly light, on the visual physiological system were first evaluated in terms of a pigment peculiar to deep-sea animals (Denton & Warren, 1957).

Kennedy & Bruno (1961), using data from Hutchinson (1957) and James & Birge (1938), have attempted to equate the spectral sensitivity ($\lambda_{max.}$ 570 mμ) of *P. clarkii* with its photic milieu.

Precise data are available for the photic environment of lobsters. The spectral sensitivity of the homarid eye is remarkably well suited to the spectral composition of its environmental light. Atkins (1945) measured light transmission in the waters near the Eddystone Light and near Plymouth Sound Breakwater. He found that from near the surface to a depth of 50 m the waters were most transparent to light through his 'green' (480–580 mμ; $\lambda_{max.}$ 530 mμ) filter. With interference filters of the sort used in the physiological studies herein reported it has been shown that in continental-shelf waters at depths of 50 m, the peak transparency is near 490 mμ but that the intensity of light at 530 mμ (peak sensitivity for *H. vulgaris*) at 50 m is greater than 100 μW/cm^2, and in many localities as much as 1000 μW/cm^2 (Boden, Kampa & Snodgrass, 1960; Boden, 1961; Kampa, 1961). These intensities are within the range of those used by Kennedy & Bruno (1961) in their measurements of the spectral sensitivity of *H. americanus*, and from 1·2 to 1200 times greater than those used in our studies of *H. vulgaris*.

The eye of the hermit crab, *E. bernhardus*, with maximum sensitivity near 500 mμ (Stieve, 1960) would be similarly efficient in shallow coastal waters.

The visual pigments of pelagic decapod crustaceans have so far been described from only one group, the Euphausiacea. Kampa (1955) reported a light-sensitive pigment, euphausiopsin ($\lambda_{max.}$ 462 mμ), from the eyes of *Euphausia pacifica*.

This pigment has since been extracted from the euphausiid *Meganycti-*

phanes norvegica by Fisher & Goldie (1959) and by Dartnall (personal communication). Kampa showed that the pigment is related to vitamin A_1, and Fisher and Goldie established the occurrence of retinene$_1$ in the bleaching sequence.

The spectral sensitivity curve of the fast response of the upper lobe of the eye of yet another euphausiid, *Thysanoëssa gregaria* G. O. Sars, agrees well with the difference spectrum of euphausiopsin (Boden, Kampa & Abbott, 1961).

As a group, the euphausiids inhabit mid to deep waters where during the day the spectrum of transmitted daylight is very narrow (Tyler, 1958; Boden, Kampa & Snodgrass, 1960; Boden, 1961; Kampa, 1961) with a peak in the region of 478 mμ. Photosensitive pigment has been extracted from the eyes of the pelagic galatheid crab, *Pleuroncodes planipes*, and, although the animal is taxonomically closer to *Eupagurus* than to the euphausiids, the difference spectrum of this pigment and the spectral sensitivity of the electroretinogram are closer to those of the euphausiids than to those of littoral decapods (Kampa, MS. in preparation).

It appears, then, that the relationship between photic environment and spectral sensitivity is as intimate in Decapoda as it is in Pisces.

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SUMMARY

The compound eye of the lobster *H. vulgaris* has a single lobe; its ommatidia are uniform except in length. Each ommatidium consists of a corneal facet, two corneagenous cells, four cone cells, a four-part crystalline cone, an elongate cone stalk, seven retinular cells and a four-part rhabdom. Growth between the zoaeal and adult stages is primarily a lengthening of the cone stalk.

Electroretinograms obtained with electrodes among the ommatidia are negative in sign. In the region of the basal membrane the electroretinogram becomes diphasic; below the basal membrane the potentials are positive. The amplitude of the response varies with the log of the stimulating energy.

The spectral sensitivity of the eye is greatest between 516 and 531 mμ. This range of maximum sensitivity is very similar to the spectral composition of attenuated daylight in the waters and at the depths where lobsters live.

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