The Nature of the Retinal Action Potential, and the Spectral Sensitivities of Ultraviolet and Green Receptor Systems of the Compound Eye of the Worker Honeybee

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ABSTRACT 1. The retinal action potential consists principally of a sustained negative wave which persists for as long as the stimulus. Transitory negative on-effects and off-effects may also be present, particularly at long wave lengths (green, yellow, and red) and in the light-adapted eye.

2. Only the maintained component of the potential can be elicited under CO_2 anesthesia. The transient components are reversibly eliminated from the response at about the same time as the background noise of nerve and muscle spikes. It is suggested that the sustained component arises from the receptor cells, and the other components from second and higher order neurons.

3. The compound eye does not contain a homogeneous population of receptors. A green receptor system (maximum sensitivity at about 535 mμ) determines the response of the dark-adapted eye throughout most of the spectrum; during adaptation to yellow light, however, an ultraviolet receptor system is revealed, with maximum sensitivity at about 345 mμ. The anatomical bases of these receptor systems are unknown; however, they include both retinula cells and neurons in the optic ganglion.

4. There is no change in spectral sensitivity (Purkinje shift) in the first three logarithmic units above the threshold of the retinal action potential.

5. The relatively great effectiveness of near ultraviolet light in stimulating the positive phototaxis of the bee does not depend on excitation of the ultraviolet receptor of the ocellus.

In 1914 von Frisch discovered that the honeybee can be trained to distinguish red, yellow, and green from blue and violet. In 1927 Kühn confirmed the work of von Frisch and showed that two additional regions of the spectrum, blue-green and near ultraviolet, also are distinct colors for the bee. This

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behavioral work has been reconfirmed and extended by Bertholf (1931*a*, *b*), Hertz (1939), Daumer (1956), and Kuwabara (1957); however, virtually no information is available on the neurophysiological and biochemical mechanisms responsible. This paper reports the first electrophysiological observations on peripheral mechanisms of color vision in the compound eye of the worker honeybee (*Apis mellifera*).

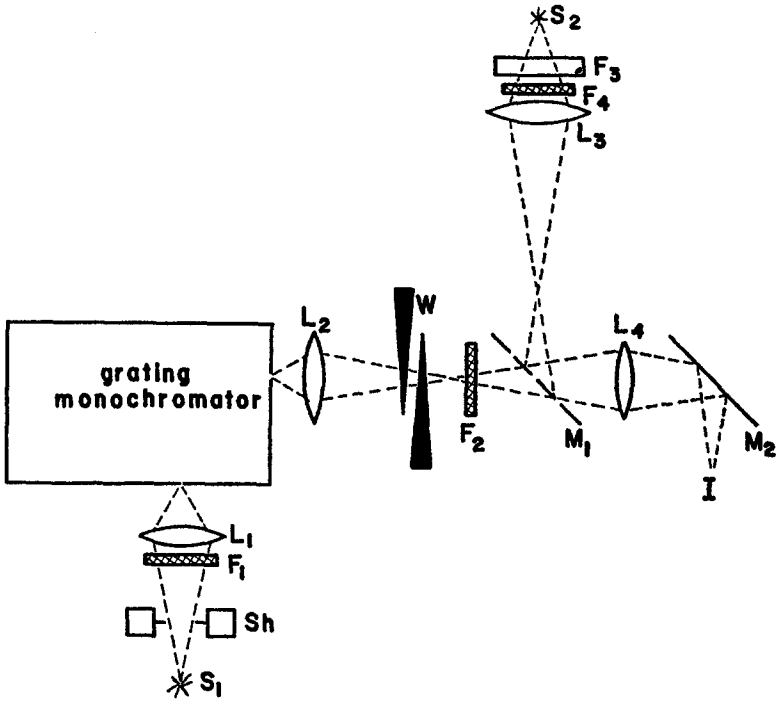

FIGURE 1. Diagram of the optical apparatus. S_1, test source; *Sh*, shutter; F_1, heat filter and filter to remove the second order spectrum; L_1 and L_2, quartz lenses; W, photographic wedges; F_2, chromium film density filters; M_1, polished quartz plate or half-silvered mirror; L_4, quartz lens; M_2, front surface mirror; I, position of insect's eye; S_2, adapting source; F_3, heat filter; F_4, colored filter and density filters; L_3, lens. See the text for details.

Methods

OPTICAL APPARATUS Fig. 1 is a diagram of the optical apparatus. With this equipment a stimulus of known wave length, intensity, and duration could be superimposed on an adapting light which could be varied independently. The stimulating and adapting beams arrived at the eye on the same optical path, thereby assuring that they excited the same population of ommatidia.

As described previously (Goldsmith and Ruck (1957–58)), the test source (S_1) was either a 250 watt mercury arc or a 100 watt zirconium arc. The latter, though

emitting relatively little ultraviolet light, is useful for some purposes because its emission in the visible region of the spectrum is continuous. The monochromator (Bausch and Lomb) employed a 52 mm. square grating with 1200 lines per mm. The monochromator slits were adjusted for half-band widths of 9.9 mμ and 3.3 mμ when using the mercury and zirconium arcs respectively. When using the mercury source, the second order spectrum was cleared with either a Corning No. 774 or a Wratten No. 2 filter (F_1). The former transmits wave lengths longer than 290 mμ and was used when isolating the mercury lines at 365, 405, and 436 mμ; the latter transmits above 390 mμ and was used at 492 mμ and all longer wave lengths. When using the zirconium arc, a water cell served as a heat filter. The glass walls of the cell insured that test stimuli as long as 680 mμ were not overlapped by the second order spectrum.

TABLE I
SPECTRAL CHARACTER OF ADAPTING LIGHTS

Adapting light	Principal wave length	Secondary wave lengths	Lamp	Filter
Red	all λ's > 615 mμ	—	Tungsten	Corning 2404 (transmits λ's > 615 mμ)
Yellow-green	578 mμ 546 mμ	623 mμ	Mercury	Corning 3482 (transmits λ's > 535 mμ)
Near ultra-violet	365 mμ	313 mμ 334 mμ	Mercury	Wratten 18-A (transmits broad band 290 to 400 mμ)

Except in the ultraviolet, the intensity of the test beam was controlled by photographic wedges (Goldsmith and Ruck (1957–58)). At 302, 313, and 334 mμ, intensities were regulated with a series of four density filters consisting of films of chromium on quartz microscope slides.[1] The metallic film was protected by a second quartz slide taped to the first, and the optical densities of the finished "sandwiches" were measured in a Cary recording spectrophotometer. The filters had optical densities of approximately 0.4, 0.6, 1.0, and 2.05 and could be used in any combination. Finer control of intensity, when required, was achieved by narrowing the entrance slit of the monochromator.

The adapting light was either an 85 watt mercury arc or, occasionally, a 100 watt tungsten bulb. Intensities were regulated with standard neutral density filters or with the density filters described above. The spectral content of the adapting light was controlled with glass or gelatin filters; the three combinations of lamp and filter which are referred to later in this paper are listed in Table I.

The method of calibration of the relative intensities of the test lights was described previously (Goldsmith and Ruck (1957–58)).

[1] I am deeply grateful to Mr. E. Barr of Baird-Atomic, Inc., Cambridge, Massachusetts, for producing these metallic films.

ANIMAL PREPARATION AND RECORDING METHODS Intact, living bees were secured with "tackiwax" to a small platform mounted on a ball and socket joint. All appendages were immobilized, and one side of the head was covered with a piece of aluminum foil about 5 mm. square. A small hole in the foil exposed a spot no larger than 1 mm. in diameter over part of the eye. The animal could be positioned so that the test and adapting lights impinged on any part of the eye.

The "active" electrode was a capillary pipette with a tip diameter of 20 to 30 μ filled with insect Ringer (ionic composition of Ephrussi and Beadle (1936)). The tip was inserted just beneath the cornea, through a small hole made previously with an electrolytically tapered steel needle. The reference electrode was a wick of balsa wood immersed in insect Ringer and placed on the surface of the compound eye on the opposite (dark) side of the head. This point was shown to be electrically indifferent to potential changes occurring in the illuminated eye even with the strongest test and adapting lights. That is, no potential difference was recorded on illumination when the capillary electrode was removed from the illuminated eye to, say, a point in the abdomen. Both electrodes employed silver:silver chloride junctions.

The amplifier was a Grass P-6B operated push-pull and direct-coupled. This instrument has an input impedance of 10^{13} ohms; square waves applied in series with the electrodes were amplified and recorded with negligible distortion. The low pass filter was customarily operated at 0.5 kc. (one-half amplitude frequency).

After preparation the animals were dark-adapted for at least 20 minutes before recording began. As will be shown in a following paper, this period allows for complete recovery from the effects of any adapting lights to which the eye was exposed during the time of preparation.

A. THE RETINAL ACTION POTENTIAL

The Form of the Response

On illumination of a compound eye of a worker honeybee, an intraocular electrode becomes negative with respect to a reference electrode on the opposite side of the head.[2] Fig. 2 shows a series of electroretinograms (ERG's) from a single animal in two stages of adaptation in response to stimuli of various duration, intensity, and wave length. The response consists principally of a negative component which persists as long as the light is on. Careful scrutiny of the records reveals, however, that the response is not simple. An off-effect may be present, particularly at long wave lengths and in the light-adapted state (578 mμ, log N_{test} = 1.74). Further, there is an indication in some records (*e.g.*, 334 mμ, log N_{test} = 1.01, 1 sec.) that the initial part of the response may also be the sum of two or more components.

The quantity log N_{test} expresses the relative intensities of the test wave

[2] Actually it is not necessary to pierce the cornea; in a few instances essentially identical records have been obtained with a wick electrode on the surface of the eye.

lengths at the eye. For short durations of stimulus the magnitude of the response depends on both intensity and duration of exposure (Bunsen-Roscoe Law); with long durations, only on intensity. This is shown in Figs. 2 and 3,

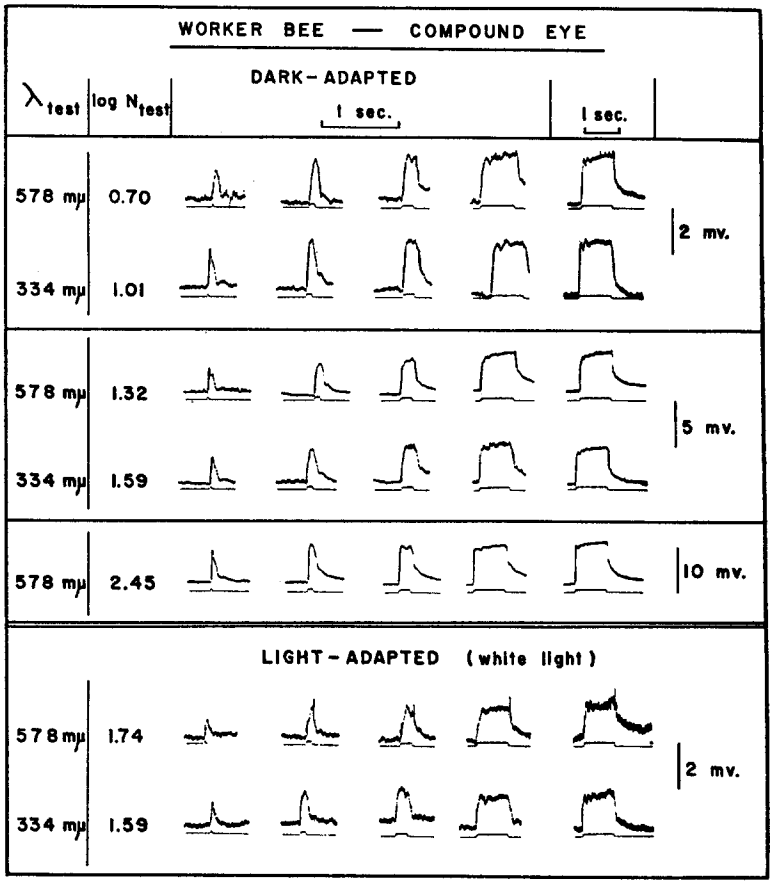

FIGURE 2. Retinal responses from the compound eye of a worker honeybee to flashes of various wave lengths, durations, and intensities. The upper trace of each pair is the response of the eye; upward deflections indicate negativity of the subcorneal electrode. The lower trace of each pair is the response of a photocell monitoring the stimulus. Note that in this preparation the off-effect makes a significant contribution only at long wave lengths and in the light-adapted eye. Log N_{test} is the logarithm of the relative intensity (quantized) of the test flash at the eye. Total energy of the stimulus depends also on the duration of the test flash. Flash durations (left to right): *ca.* one-fiftieth, one-tenth, one-fifth, one-half sec.; 1 sec.

where the second ERG in each horizontal row (stimulus duration $\frac{1}{10}$ sec.) is somewhat larger than the first (stimulus duration *ca.* $\frac{1}{50}$ sec.). Clearly, values of N_{test} should be compared only for stimuli of equal duration.

Fig. 3 is a similar series of responses from another preparation that shows

more clearly the variations in the shape of the retinal action potential. In the dark-adapted state this preparation showed about equal sensitivity at 578 and 365 mμ; that is, equal numbers of quanta (log N_{test} = 0.70) elicited approximately equal sized potentials. Note, however, that the off-effect is seen only in the responses to the yellow light.

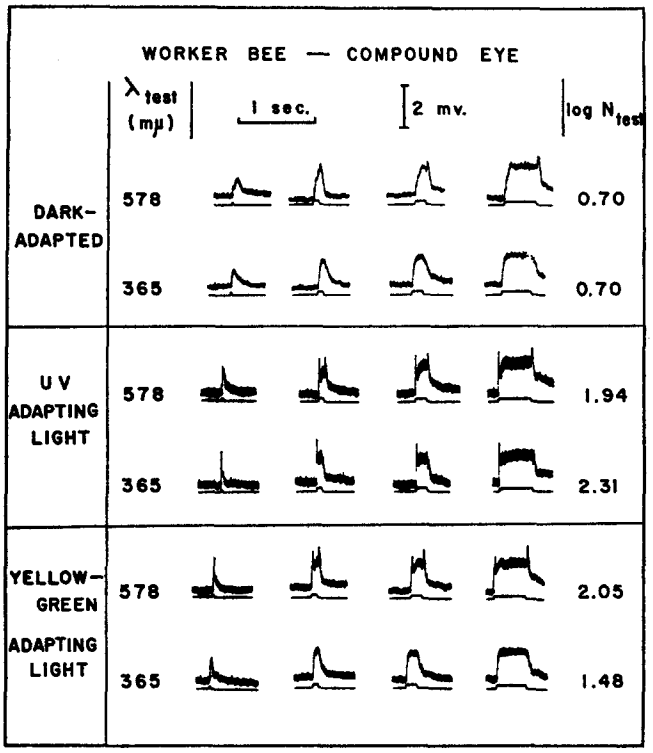

FIGURE 3. ERG's from the compound eye of a worker honeybee. Recording conventions as in Fig. 2. Note that in this preparation there are obvious qualitative differences in the wave form of the response to yellow and near ultraviolet light. Further, in the dark-adapted eye the sensitivities at 578 mμ and 365 mμ are about equal (log N_{578} = log N_{365} = 0.70 for approximately equal responses); however, the ratio of sensitivities at these two wave lengths may be made to vary either way by the proper selection of adapting light.

The middle and bottom series of responses of Fig. 3 were evoked by test stimuli superimposed on an adapting light (Table I).[3] At the time the test

[3] The thickening of the baseline in the light-adapted responses of Figs. 2 and 3 is caused by the fact that the bee's eye was "following" the 120 C.P.S. ripple in the output of the mercury arc serving as adapting light. Though this would hardly be expected in a vertebrate eye, 120 C.P.S. is actually well below the critical fusion frequency of the retinal action potential of the bee (Autrum and Stoecker, 1950).

A similar oscillation in the output of the test light would produce large variations in the intensity

flash was presented, the eye had assumed a level of adaptation characteristic of the intensity and wave length composition of the adapting light. (The rate at which this steady-state condition is reached need not concern us here, but it will be discussed in a following paper (Goldsmith (1960)). It is noteworthy that some light adaptation is produced at both test wave lengths by either adapting light (log N_{test} for an equal response is larger than in the dark-adapted eye); however, the ultraviolet adapting light has relatively more effect at 365 mμ than at 578 mμ, and the yellow-green adapting light has more effect at 578 mμ than at 365 mμ. For example, in the presence of the ultraviolet adapting light more energy was required at 365 mμ (log N_{test} = 2.31) than at 578 mμ (log N_{test} = 1.94) in order to elicit approximately equal sized responses.

As was stated previously, the off-effect, which is associated with long wave

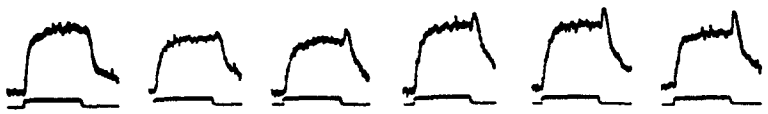

FIGURE 4. The variation of the off-effect with wave length. Dark-adapted eye. Recording conventions as in Figs. 2 and 3. Stimulus duration, one-half sec.

length stimulation, is enhanced by light adaptation. This is shown clearly in Fig. 3. Fig. 3 also shows another component, an on-effect (rows 3, 4, and 5), which is not seen as frequently as the off-effect with this system of recording. Like the latter, it too appears to be elicited more easily by stimulation at the long wave length end of the spectrum and in the light-adapted state.

Fig. 4 is a series of ERG's of about the same size, showing how the off-effect varies in prominence through the spectrum. Clearly, in moving from the ultraviolet to the yellow end of the spectrum, the units responsible for the off-effect are not recruited suddenly at one wave length.

The Effect of CO_2 on the Retinal Action Potential

In several experiments a cup of wax was built around the bee and a small tube inserted through the base of one of the walls of the cup. The light stimulus entered through the open top of the cup. At any time during an experiment CO_2 could be introduced through the tube. The wax cup held the CO_2 concentration fairly high in the immediate region of the bee.

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of very short flashes. However, for the experiments reported in part B of this paper, test flashes of 0.1 sec. were employed.

When CO_2 was applied, there first ensued an irregular but transitory series of events: the baseline drifted erratically; the retinal action potential was greatly reduced and might disappear; the "spontaneous" activity of nerve or muscle fibers, which was often noted in the normal preparation when the amplifier was set for high gain, was greatly increased. Within a minute or so, probably corresponding to the period necessary for the animal to become anesthetized, these events ceased. The baseline became very quiet and stable, but the prolonged, negative component of the retinal action potential reappeared and could be repeatedly elicited (Fig. 5B). This effect is reversible. On turning off the CO_2 the baseline drifted for a while; "spontaneous" activity of nerve and muscle cells returned; and the retinal action

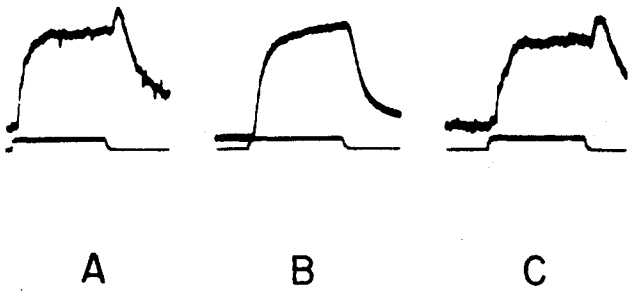

FIGURE 5. The effect of CO_2 on the retinal action potential. A, before, and B, during anesthesia with CO_2. C, after recovery. Recording conventions as in Figs. 2 to 4. Stimulus duration, one-half sec.

potential decreased and then returned to the normal form (Fig. 5C). It is interesting that in the process of anesthetization with CO_2 the off-effect of the retinal action potential disappears at about the same time as the background spike activity.

The differential sensitivity to CO_2 of the sustained negativity and the off-effect shows them to have different origins, and further suggests that the off-effect arises more centrally. In this view, the retinula cells, which are relatively insensitive to CO_2, respond to light by a partial depolarization which is maintained during the period of illumination. The off-effect, as well as other components arising earlier in the response and ordinarily largely masked by the response of the sense cells, depends on the proper functioning of second and higher order neurons in the optic ganglion, on which the effects of CO_2 are pronounced. The complex responses obtained during the initial stages of anesthesia and during recovery have not been studied further.

Discussion

Such experimental observations as are reported in Figs. 2 and 3 indicate that the compound eye of the bee contains the necessary mechanisms for color

vision. Two features of these experiments point to the presence of more than one type of receptor: (*a*) qualitative differences in the shape of the retinal action potential at different wave lengths, differences which cannot be erased by any manipulation of the intensities of the test lights; and (*b*) differential changes in spectral sensitivity induced by adaptation to ultraviolet as compared with yellow-green light.

Previously two authors have published ERG's recorded from the compound eye of the bee in response to white light. Autrum and Stoecker (1950) found a positive transient at "on" and a negative wave at "off," the response returning to the baseline during the duration of the stimulus. Using stimuli from 20 msec. to several minutes duration, I have not observed potentials of this description. Ruck (1958) found that responses to $\frac{1}{8}$ sec. flashes of white light were negative waves of approximately the same duration as the stimulus. Those ERG's of Fig. 2 that were recorded from the dark-adapted eye are similar to Ruck's records.

It is generally agreed that the retinula cells of insects respond to light with a slow negative potential (Bernhard (1942); Jahn and Wulff (1942–43); Autrum and Gallwitz (1951); Hartline *et al.* (1952)). It is not clear, however, that this is the sole contribution of the ommatidial layer to the retinal action potential (Bernhard (1942)) or what role is played by the *lamina ganglionaris* and other layers of the optic lobe. Burtt and Catton (1956) state that the entire retinal action potential originates in the optic lobe; Ruck (1957), however, has suggested that the same data are equally consistent with the conclusion that the potential originates in the ommatidial layer. The present experiments with CO_2 indicate that the retinal action potential of the worker honeybee does not arise from a single site and suggest that a major, negative component is generated in the layer of retinula cells. On the basis of selective poisoning with cocaine Bernhard (1942) reached a similar conclusion for *Dytiscus*.

B. MEASUREMENTS OF SPECTRAL SENSITIVITY

Receptor Systems

The magnitude of the retinal action potential is a useful index of visual excitation. The measurements described in the following paragraphs are based on the heights of retinal action potentials; however, because this potential does not originate entirely in the retinula cells, measurements of its height may not reflect the properties of primary receptors exclusively. The spectral sensitivity functions to be presented below are therefore measurements of what we shall refer to as receptor systems—receptor cells or groups of receptor cells and associated neurons in the optic ganglion. How well the spectral

sensitivities of these receptor systems reflect the properties of primary receptors must await further study. More will be said of this in the discussion.

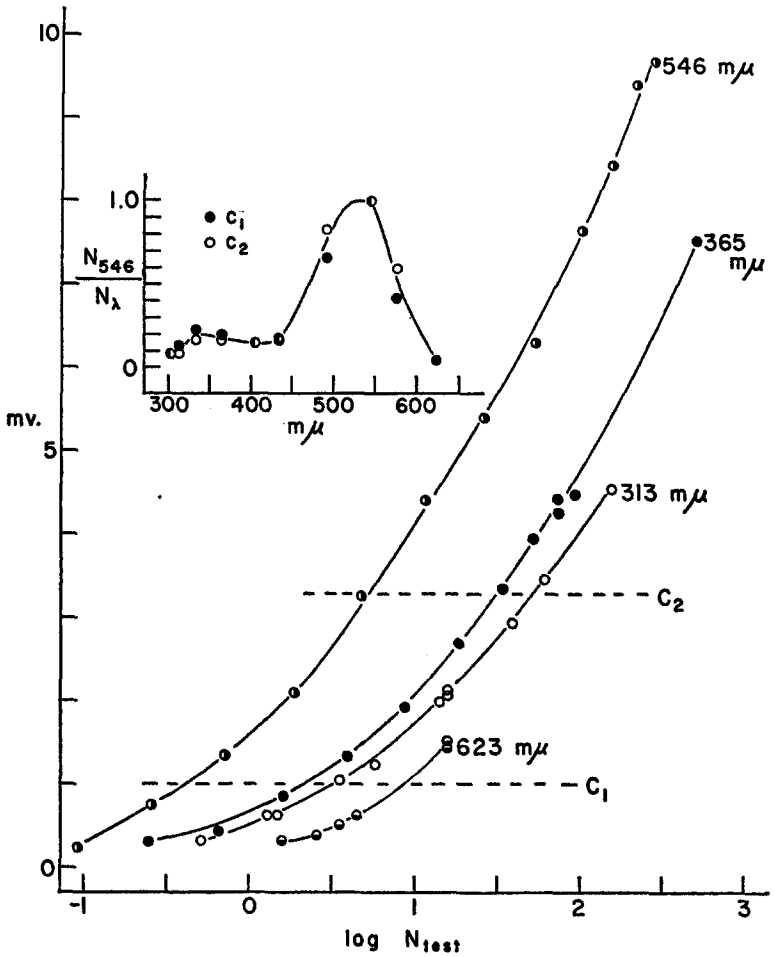

FIGURE 6. Height of retinal action potential as a function of log relative energy (quantized) for several wave lengths. Data from a single preparation. Stimulus duration, 0.1 sec.

FIGURE 7 (insert). Relative spectral sensitivity of the eye of Fig. 6. Sensitivity is defined as the reciprocal of the relative number of quanta required to elicit a constant response. The two responses used (C_1 and C_2) are indicated in Fig. 6 by the horizontal dashed lines. Not all the data required for the curve of Fig. 7 are plotted in Fig. 6.

The Dark-Adapted Eye

The test flashes were 0.1 sec. in duration in all subsequent experiments to be discussed in this paper. With 0.1 sec. stimuli usually little difficulty was experienced in approximately matching responses of the dark-adapted eye at different wave lengths (*cf.* Fig. 2).

The Relationship between Stimulus and Response In many preparations the height of the retinal action potential was determined as a function of the logarithm of the relative number of quanta at a variety of wave lengths. A typical experiment on a single eye is illustrated in Fig. 6. Data were obtained at the ten emission lines available in the mercury arc; however, for clarity, the series of responses at only four of the ten wave lengths are plotted. It is evident that the curves of Fig. 6 are all approximately parallel. The curves at other wave lengths also are parallel. Experiments on other bees usually gave the same result as shown in Fig. 6; the few exceptions will be discussed below. When the zirconium arc was used, more extensive data were obtained at the red end of the spectrum.

The Spectral Sensitivity of the Green Receptor System It is possible to construct a spectral sensitivity curve from the four curves in Fig. 6 plus the six others not shown. The relative number of quanta required to elicit a retinal action potential of any arbitrary constant size can be read from the abscissa for each wave length. The reciprocal of this value, expressed as a fraction of the value at 546 mμ, is a measure of the relative sensitivity, and is plotted in Fig. 7 (insert of Fig. 6) as a function of wave length for two criterion responses (C_1 and C_2) indicated in Fig. 6 by the horizontal, dashed lines. The two sets of points in Fig. 7 can be fitted by a single spectral sensitivity curve; the variation is no greater than that observed when attempting to match responses of a single amplitude from different bees. This spectral sensitivity curve has a single broad peak in the green and a low shoulder extending into the near ultraviolet. It is fairly typical of the dark-adapted eye, although in the near ultraviolet some preparations show a second, small maximum instead of a shoulder (*e.g.* the animals used in the experiments described in Figs. 3 and 10).

The fact that the curves of height of response *vs.* log N are nearly parallel indicates that the shape of the spectral sensitivity curve does not vary greatly with the intensity of the stimulating light. Not enough energy was available at all wave lengths to make a complete spectral sensitivity curve at the highest intensities; however, inspection of the curves at 365 mμ and 546 mμ shows that at three logarithmic units above the threshold of the ERG there is still no indication of a change of spectral sensitivity analogous to the Purkinje shift of the vertebrate eye. As was mentioned above, in some preparations the curves in the near ultraviolet region of the spectrum appeared to have a slightly shallower slope than those at longer wave lengths. At best this was not a large effect, but it suggests that at intensities below the threshold of the retinal action potential, the sensitivity in the near ultraviolet may become somewhat greater relative to the green than indicated by Fig. 7.

Similar measurements of spectral sensitivity were made on a number of other bees. The results are shown in Fig. 8. Standard errors are indicated by

the vertical lines through the experimental points. The broad maximum of sensitivity is centered at about 535 mμ.

Each point in Fig. 8 is the average of from four to fourteen independent experiments on different bees. These experiments were conducted as described above; or, alternatively, the setting of the wedge necessary to produce a constant size response was determined at each wave length, the responses at the different wave lengths

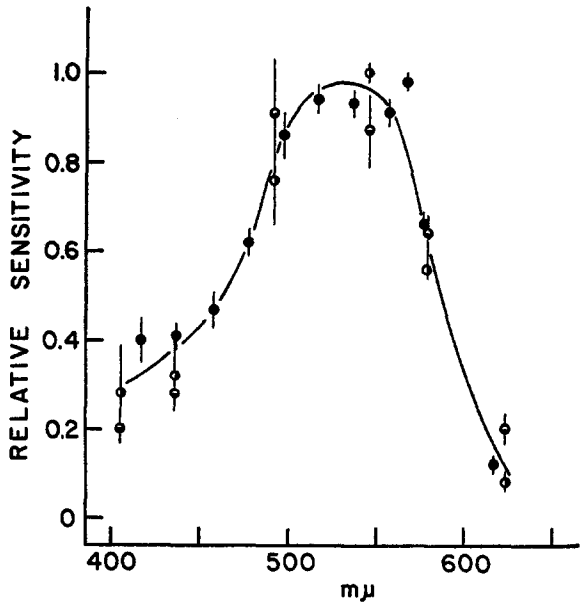

FIGURE 8. Spectral sensitivity of the dark-adapted eye. This curve is thought to represent the spectral sensitivity of a green-sensitive receptor system. Sensitivity is defined as the reciprocal of the relative number of quanta required to produce a constant effect. Maximum sensitivity is at about 535 mμ. Each point represents an average of 4 to 14 measurements on different bees; standard errors are indicated by the vertical lines. Different symbols indicate different sources of stimuli. Further details in the text.

being matched visually on a ruled oscilloscope screen. With the latter method it was possible to guard against drifts in sensitivity by continually checking the setting of the wedge at the first wave length measured. These measurements were made over the course of about a year, and the different symbols used in Fig. 8 correspond to different sources of stimulation; filled circles, zirconium arc and grating monochromator; vertically divided circles, mercury arc and grating monochromator; horizontally divided circles, mercury arc and two prism monochromators (Goldsmith and Ruck (1957–58)). The large standard errors at 492 mμ reflect the difficulty in obtaining accurate measurements of the energy of this weak mercury line. The point at 567 mμ (zirconium arc), which appears to lie rather far off the curve, is based on only four animals, and furthermore is tied to the point at 517 mμ but not to 537 or 557 mμ. Because of the variation from preparation to preparation in

the prominence of the shoulder or small peak in the ultraviolet region of the spectrum, there is greater variation in the values at the violet end of the curve than there is in the red.

The Ultraviolet Receptor System

Figs. 2 to 4 show that different regions of the spectrum exert qualitatively different effects on the compound eye of the worker bee. The spectral sensitivity curve of the dark-adapted eye indicates the presence of a receptor system maximally sensitive at about 535 mμ. Apparently, however, there are other receptor systems functioning at shorter wave lengths. Clearly, such receptor systems do not contribute prominently to the spectral sensitivity of the dark-adapted eye, and other methods must be devised to reveal their properties.

Partial or Selective Adaptation—An Illustrative Experiment Red light is absorbed more efficiently by the green receptor system than by other receptor systems with maximum sensitivities at shorter wave lengths. Therefore, as is shown in Fig. 9, adaptation to red light reduces differentially the sensitivity of the green receptor system. The relative numbers of quanta (N_{test}) required to produce a constant response in a dark-adapted eye were determined at the wave lengths available in the mercury arc. Log relative sensitivity (log N_{546}/N_λ) is plotted vs. wave length in the upper curve (filled circles) of Fig. 9. As expected, the curve is similar to the curve of Fig. 7 except that the ordinate is plotted on a logarithmic scale. The test area of the eye was then irradiated with a red adapting light (Table I), and a second series of spectral sensitivity measurements was made by superimposing the test flashes on the red adapting light. During this second period of measurement the eye was fully adapted to the red light; that is, all changes in sensitivity induced by the red adapting light had been completed, and the sensitivity of the eye was poised in a steady state.[4]

The spectral sensitivity of the eye during adaptation to red light is shown by the lower curve (open circles) of Fig. 9. Note that the two curves share the same ordinate, and although the red adapting light reduced the sensitivity of the eye throughout the spectrum, the depression of sensitivity is rela-

[4] Throughout this paper, responses of the light-adapted eye were obtained by adding the test stimuli to the adapting light, rather than by the usual practice of presenting the test flash during an interruption of the adapting exposure. The rapid rate of dark adaptation of the bee makes this necessary; however, evidence will be assembled in a following paper (Goldsmith, 1960) to show that for the human eye curves of dark adaptation extend back, without discontinuity, to values of sensitivity determined with the adapting light still on. It should also be pointed out that the spectral sensitivity function of the blue-violet receptor of human cone vision is essentially the same, whether the measurements are made by superimposing test flashes on a long wave length adapting field (method of increment-threshold sensitivity of Stiles, *e.g.*, Stiles (1959)) or whether the sensitivity function is obtained from analysis of breaks in the curves of dark adaptation, following adapting exposures to yellow or orange light (Auerbach and Wald (1955)).

tively greater at long wave lengths. In the dark-adapted eye the spectral sensitivity curve displays only a shoulder in the near ultraviolet; in the presence of the red adapting light there is a second maximum.

These curves are based on the maximum heights of response (discounting

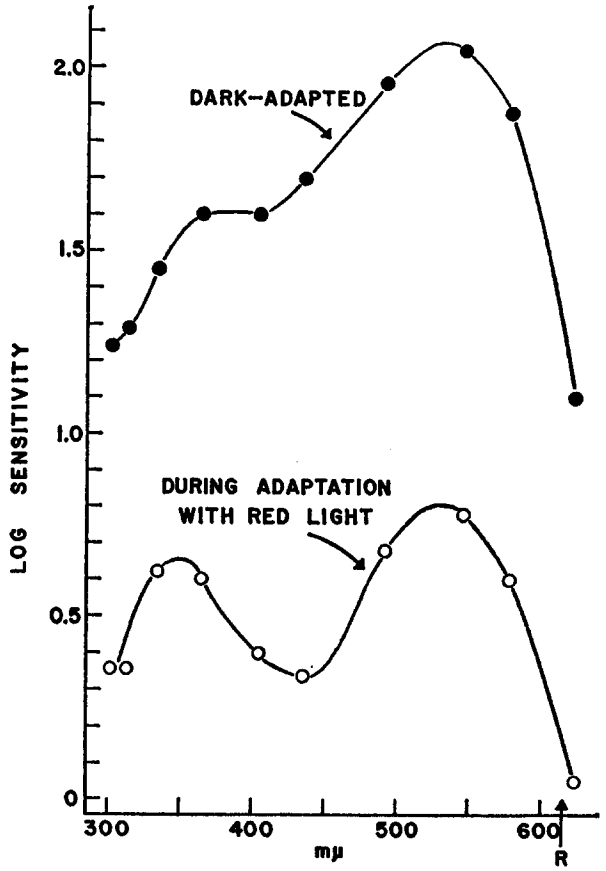

FIGURE 9. The effect of a red adapting light on the sensitivity of the eye. Sensitivity is the reciprocal of the energy (quantized) for a constant effect. Data for the lower curve were obtained by superimposing 0.1 sec. test flashes on a red adapting light. The arrow (R) on the abscissa indicates the shortest wave length present in the adapting beam. Both curves from the same preparation. Note that during adaptation to long wave lengths, a second maximum of sensitivity appears in the near ultraviolet.

the rapid on-effect, if present) to 0.1 sec. flashes of light. It was shown in Figs. 2 and 3 that the shape of the retinal action potential can change with light adaptation, so that strictly speaking it is usually not possible to match the responses of the light- and dark-adapted eye. Although longer test flashes would have made it possible to measure the heights of the sustained negativity (which probably originates in the receptor cells) with a minimum of

interaction with the off-effect, such flashes might themselves have produced a measure of light adaptation. However, it should be kept in mind that had the measurements been based entirely on the sustained negative component, the change in shape of the spectral sensitivity curve would, if anything, have been more pronounced. That this is true is easily seen. The off-effect is prominent at the long wave length end of the spectrum, and particularly in the light-adapted eye. If, from the point of view of spectral sensitivity measurements, the off-effect is an extraneous component of the response, the points at the long wave length end of the spectrum and in the light-adapted eye are in most serious error. Too small a part of the retinal action potential is then contributed by the slow component, and the value of relative sensitivity is accordingly too high.

The fact that the shape of the spectral sensitivity curve can be changed by adaptation to red light confirms our earlier conclusion that the compound eye of the honeybee contains at least two types of receptor which differ in spectral sensitivity, one maximally sensitive at about 535 mμ, and another maximally sensitive in the near ultraviolet. The spectral sensitivity of the ultraviolet receptor system is best revealed when the contribution of the green receptor system is more effectively depressed than in Fig. 9. Before showing the spectral sensitivity of the ultraviolet receptor system, let us first consider in more detail the manner in which sensitivity varies as a function of wave length and intensity of adapting light.

Overlap of Spectral Sensitivity Functions of the Ultraviolet and Green Receptor Systems Since, as shown in Fig. 3, both yellow-green and ultraviolet adapting lights decrease the sensitivity of the eye, albeit not equally, to test flashes at both ends of the spectrum; and since, as just shown, a red adapting light decreases the sensitivity throughout the spectrum, the spectral sensitivities of the two receptor systems overlap. The type of experiment illustrated in Fig. 10 shows that the green receptor system is sensitive throughout the spectrum visible to the bee, whereas the ultraviolet receptor system responds only at the short wave length end.

Fig. 10 shows how the logarithm of the relative number of quanta (log N_{test}) necessary to elicit a constant response varies with the energy of the adapting light. Log N_{test}, or log decrease in sensitivity, was determined at three widely separated wave lengths, 365, 436, and 578 mμ. The experiment was done in two parts with a period of dark adaptation between. Part (*a*) was performed with the yellow-green and part (*b*) with the near ultraviolet adapting lights of Table I. After determining the relative sensitivity of the dark-adapted eye at the three test wave lengths, the adapting light was turned on and the sensitivities were redetermined as soon as the eye had reached a steady level of light adaptation. (In some preparations responses were matched visually on the oscilloscope screen; in others response-energy

curves were made at each intensity of adapting light (Goldsmith (1960).) The intensity of the adapting light was then increased and further sets of measurements made.

First, what are the effects of a yellow-green adapting light (Fig. 10 (*a*))? When the test light is yellow (578 mμ; open circles), the curve of log N_{test} *vs.* log N_{adapt} rises with a continually increasing slope. That is, as the adapting light becomes more intense, it becomes increasingly more effective in depressing the sensitivity of the eye to yellow light. An ultraviolet test light

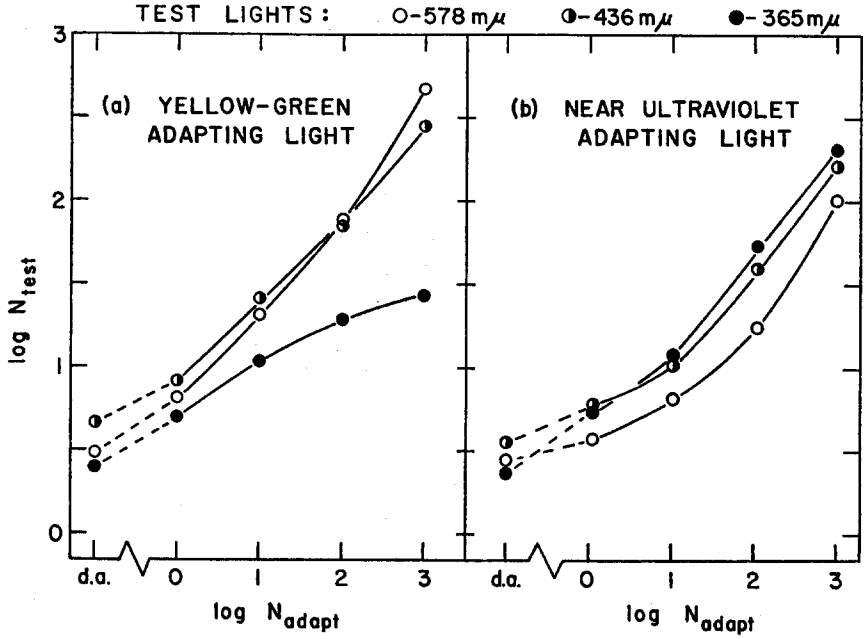

FIGURE 10. Log relative number of quanta required for a constant response (log N_{test}) as a function of log quanta of the adapting light (log N_{adapt}) for several combinatons of wave lengths. Data from a single eye. See the text.

(filled circles) exhibits an entirely opposed relationship; now with higher states of adaptation to yellow-green, the smaller is the increment in the depression of sensitivity. Or in other words, as log N_{adapt} becomes larger, the sensitivity of the eye at 365 mμ is less and less affected by the yellow-green adapting light. Presumably, had it been possible to obtain points at higher values of log N_{adapt}, the curve would have assumed a slope of nearly zero, showing that the sensitivity to near ultraviolet light can be made virtually independent of a yellow-green adapting light. The curve at 436 mμ (half-filled circles) displays intermediate behavior We shall return to this point again.

An ultraviolet adapting light yields very different results (Fig. 10 (*b*)). At low values of log N_{adapt} the sensitivity is depressed most effectively at 365

mμ. However, as the intensity of the adapting light is raised, the curves corresponding to the three test lights become parallel, and with further increments in log N_{adapt} they rise together.

What does this kind of experiment tell us? The sensitivity of the dark-adapted eye to yellow (as well as red and green) light is determined mainly by the green receptor system. Sensitivity to the near ultraviolet, however, involves the excitation of both this and the ultraviolet receptor system. Their thresholds in the near ultraviolet appear to be similar, and both are usually important in determining the sensitivity of the dark-adapted eye to ultraviolet light. The observation that sensitivity to ultraviolet light becomes increasingly independent of the intensity of a yellow-green adapting light means that the ultraviolet receptor system is rather insensitive to long wave lengths. As Fig. 10 (*a*) shows, at log $N_{adapt} = 3$, the sensitivity at 365 mμ is determined by the threshold of the ultraviolet receptor almost alone.

In Fig. 10 (*b*) on the other hand, low intensities of the ultraviolet adapting light selectively depress the sensitivity of the ultraviolet receptor system. This is shown by the fact that the curve for 365 mμ has, over its initial part, a steeper slope than its companion curves. The ultraviolet adapting light does not altogether spare the green receptor system, however, for at higher intensities the sensitivity of the eye to all wave lengths continues to drop. It is noteworthy that over their upper two-thirds the three curves are parallel, for this suggests that a single receptor system determines sensitivity at all three test wave lengths.

It is clear from Fig. 10 (*a*) that sensitivity to blue light does not decrease as much as sensitivity to yellow light. There could be several reasons for this. It is possible that as the threshold of the green receptor system rises, the ultraviolet receptor system may increasingly carry the burden at 436 mμ. Or alternatively, this observation may reflect the presence of a third receptor system maximally sensitive in the blue. This point will be taken up again in the discussion.

The Spectral Sensitivity of the Ultraviolet Receptor System In order to measure the spectral sensitivity of the ultraviolet receptor system with minimum interference from the green receptor system, test flashes were superimposed upon a bright, yellow-green adapting light (Table I, Fig. 10*a*, log $N_{adapt} = 3$). The contribution of the green receptor system to the sensitivity of the eye in the near ultraviolet was therefore negligible; that is, the curve in Fig. 11 reflects the properties of the ultraviolet receptor system virtually alone.

The curve in Fig. 11 is an average of twelve curves from nine animals. Standard errors are indicated by the vertical lines through the experimental points. As before, sensitivity is expressed as the reciprocal of the relative number of quanta necessary to elicit a constant response. As long as measurements did not extend into the green, little difficulty was experienced in match-

ing responses approximately. Because there are gaps between the mercury emission lines, it is not yet possible to determine the position of maximum sensitivity with the same precision as was possible for the green receptor system. The curve in Fig. 12 was drawn free-hand so as to be smooth and approximately symmetrical; it appears to peak at about 345 mμ.

Behavioral Correlates

The Relative Effectiveness of Different Wave Lengths in Directing Behavioral Responses Bertholf (1931*a*, *b*) measured the relative effectiveness of different colored lights in directing the positive phototaxis of the bee. His results are

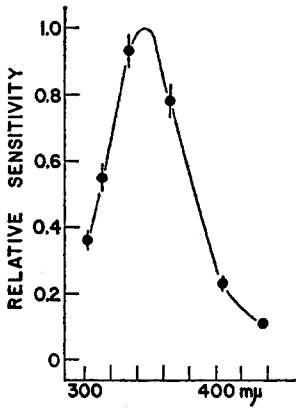

FIGURE 11. The spectral sensitivity of the ultraviolet receptor system as revealed during adaptation with a yellow-green light. Sensitivity is the reciprocal of the relative number of quanta for a constant size retinal action potential. Average of twelve curves from nine animals. Standard errors indicated by the vertical lines through the points. The smooth curve appears to peak at about 345 mμ.

replotted in the upper half of Fig. 12 (filled circles, broken curve). More recently Daumer (1956) has trained bees to feeding dishes illuminated from below with various spectral lights. The relative threshold for this response is also plotted in the upper half of Fig. 12 (open circles, solid curve). Both experimenters found a small peak in the green, but found the major peak of stimulative effectiveness, four to five times higher, in the near ultraviolet.[5]

In view of the large peak obtained in behavioral experiments, it was somewhat surprising to find that the spectral sensitivity function of the dark-adapted eye has only a shoulder or a minor maximum in the near ultraviolet. However, the ocelli of honeybees possess an ultraviolet receptor which appears to have about the same shape of spectral sensitivity as the ultraviolet

[5] This high peak in the ultraviolet is not surprising, for von Hess (1920) showed that normal sunlight is about eight or nine times as effective in stimulating the bee as sunlight with the ultraviolet removed. Sander (1933), however, was unable to confirm Bertholf and reported two peaks of maximum stimulative effectiveness, at 470 mμ and 570 mμ. Nevertheless, I think the weight of evidence supports Bertholf's findings. Both the experiment reported in Table II and the extensive work of Daumer confirm the view that ultraviolet light is particularly effective in evoking certain behavioral responses in the bee.

receptor system of the compound eye (Goldsmith and Ruck (1957–58)). The possibility therefore arose that the behavioral responses to the near ultraviolet might be principally evoked through stimulation of the ocelli rather than the compound eyes. The following simple experiment shows clearly that this is not the case.

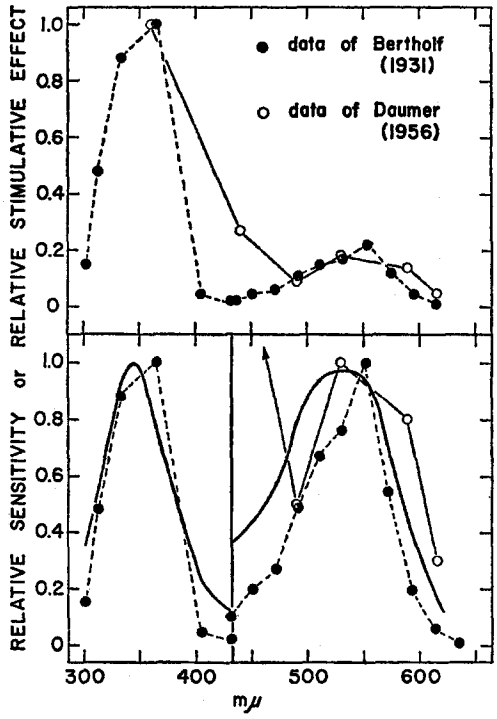

FIGURE 12. Upper half; the relative efficiency of different wave lengths in stimulating the positive phototaxis of the bee (filled circles, broken curve) as measured by Bertholf (1931*a*, *b*). The spectral *Reizwirksamkeit* (open circles, solid curve) as determined by Daumer (1956) in a somewhat different behavioral experiment. Both curves have a maximum in the near ultraviolet and a smaller peak in the green. Lower half; the maxima of the two curves above, appropriately scaled for comparison with the spectral sensitivity functions of Figs. 8 and 11.

Relative Roles of Ocelli and Compound Eyes in Directing Phototaxes The phototactic responses of worker bees before and after painting the ocelli were compared by using the apparatus illustrated in Fig. 13.

Two Gaertner quartz prism monochromators, each used with a mercury arc, were placed so that their exit slits were in positions S_1 and S_2. Two mercury lines in any ratio of intensities could be reflected from the front surface mirrors, M_1 and M_2, so as to illuminate the windows W_1 and W_2. Each window was about three-eighth inch high and one-fourth inch wide, and was covered with ground glass to diffuse

the test light into chamber *C*. Chamber *C* was a shallow box about 5½ inches long by 3¼ inches wide and was covered by a glass lid. Its depth was equal to the height of the windows, three-eighth inch. At the point *E* a piece of glass tubing was inserted into a hole bored in the floor. This tubing projected below the apparatus and provided an entrance into the chamber. The apparatus was constructed of plywood and painted flat black. Chamber *C* could be detached from the rest of the box so that a calibrated photocell could be placed over either of the windows. By

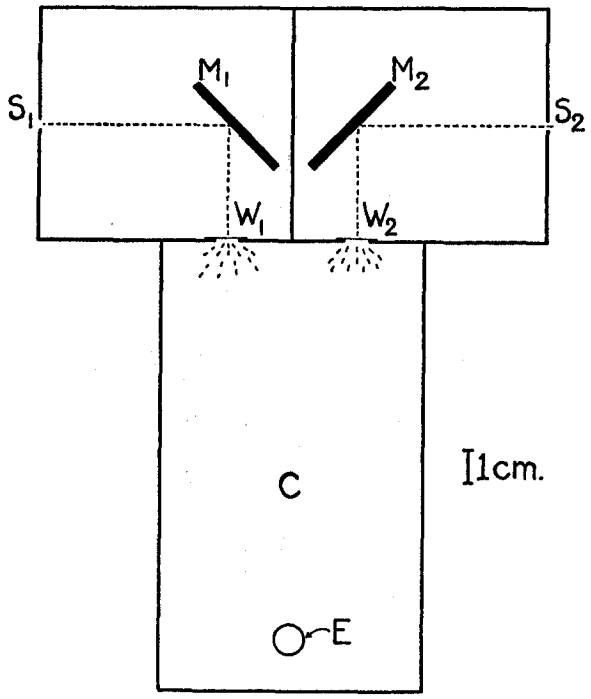

FIGURE 13. Diagram of the apparatus used for testing the phototactic responses of bees with normal and covered ocelli. S_1 and S_2, exit slits of monochromators; M_1 and M_2, front surface mirrors; W_1 and W_2, ground glass windows; *C*, test chamber; *E*, entrance in floor of test chamber.

this means the energies of the lights were measured after they had passed through the ground glass.

In one experiment, one window was illuminated with light at 365 mμ, the other at 546 mμ, and the intensities were adjusted so that equal numbers of quanta entered chamber *C*. Bees, whose wings had been clipped to make handling easier, were introduced through the entrance *E*. They then crawled to the far end of the chamber *C*, toward one of the two windows. Choices were indicated clearly; the animals actually pressed themselves against the ground glass of the window they had selected. As expected, the 365 mμ win-

dow proved the more effective in attracting bees (Table II). Only one insect was in the box at a time; repeated trials were made with each individual. Results did not depend on which window received the green light and which the ultraviolet.

Using a suspension of lamp black in shellac or collodion, the ocelli of several animals were painted over. The responses of these bees were more sluggish than those of the normal controls; as much as a minute was sometimes consumed in making the choice. However, the proportion of the choices in which the ultraviolet window was favored was about the same (Table II). Clearly the ocelli are not necessary for the high preference for ultraviolet

TABLE II
PHOTOTACTIC RESPONSES OF WORKER BEES WITH NORMAL AND BLINDED OCELLI
Lights adjusted for equal numbers of quanta

	Number of choices			
	Ocelli normal		Ocelli painted	
Animal	365 mμ	546 mμ	365 mμ	546 mμ
1	12	3	—	—
2	6	0	—	—
3	—	—	42	12
4	8	2	11	4
5	9	5	12	1
6	24	9	31	8
7	14	2	16	0
	78 per cent	22 per cent	82 per cent	18 per cent

light manifest in phototaxis.[6] For reasons that probably involve some selective activity of the central nervous system, ultraviolet light is much more effective in influencing the behavior of the bee than in evoking retinal action potentials from the compound eye.

Discussion

Behavioral experiments involving various regions of the spectrum fall conveniently into two groups. One type of experiment provides quantitative data on the relative effectiveness of different wave lengths in evoking some characteristic behavioral response such as positive phototaxis. The other kind of experiment gives no quantitative information of this nature but dem-

[6] The point of this experiment is that the relative effectiveness of the two test wave lengths is not altered significantly by covering the ocelli. Whether or not the absolute sensitivity of the bee is changed is another matter. It is the experience of other workers that insects with covered ocelli become sluggish. See Goldsmith and Ruck (1957–58) for discussion and references.

onstrates that different regions of the spectrum can be distinguished on some basis other than intensity. Of the latter group, the experiments of Daumer are the most precise and extensive. According to Daumer (1956), the honeybee possesses a system of color vision which, like that of the human retina, is based on the presence of at least three types of receptor which differ in spectral sensitivity. These receptor mechanisms appear to be maximally sensitive in the near ultraviolet, blue, and green (or yellow) regions of the spectrum.

The present experiments provide electrophysiological evidence for two of the three receptors required—those in the near ultraviolet and green. There is at this time no clear electrophysiological evidence from the compound eye of the worker bee for a receptor maximally sensitive in the blue. This may mean, however, only that the electrophysiological technique has not been suitably refined.

There is, in fact, other evidence to suggest that the compound eye of the worker may possess a receptor maximally sensitive in the blue. The visual pigments of all animals, in so far as they are known, consist of a protein conjugated to a carotenoid, retinene, the aldehyde of vitamin A. The worker honeybee does not seem to be an exception. Retinene is present in the heads but not the bodies, and a large proportion of this retinene is joined to a protein to form a photosensitive pigment with maximal absorption in the blue at about 440 mμ. On exposure to light this pigment bleaches, liberating retinene (Goldsmith (1958*a*)).

There is also electrophysiological evidence from the compound eye of the *drone* bee for a receptor system maximally sensitive in the blue at about 440 mμ (Goldsmith (1958*b*)). This observation lends support to the view that the photosensitive pigment with λ_{max} at 440 mμ is a visual pigment.

Further work is necessary, however, to clarify the role of this pigment in the compound eye of the worker. The quantitative data on the relative stimulative efficiency of different wave lengths neither support nor deny the existence of a blue receptor in this eye. Bertholf has provided the most complete data of this nature, and his curve has a minimum in the blue (Fig. 12, upper half). There is, however, rough agreement between the two peaks observed by Bertholf and the spectral sensitivity functions determined in the present work.

The data of both Bertholf and Daumer have been replotted in the lower half of Fig. 12, appropriately scaled for comparison with the electrophysiological measurements of Figs. 8 and 11. As the retinal action potential is believed to arise in large measure from the receptor cells, measurements of spectral sensitivity based on this response are perhaps more likely to provide information concerning the primary photochemical events than are observations of the behavior of the insect. Nevertheless, such sensitivity curves

should be interpreted with caution. It was pointed out above that the receptor systems of the present study include both receptor cells and higher order neurons. Furthermore, it is quite possible that interactions between receptor systems may exist and be reflected in the ERG. An interpretation which attributes the properties shown in Figs. 8 and 11 to specific receptor mechanisms also assumes more or less complete independence of the two receptor systems, at least as regards their contributions to the retinal action potential. Although such an assumption is not proved, it is made plausible by the observation that the spectral sensitivity of the green receptor system is not changed much under different conditions of selective light adaptation (Figs. 9 and 10*b*).

The ultraviolet and green receptor systems are as yet defined only by their spectral sensitivities; their anatomical basis is unknown. It would be of prime interest to identify the functional unit in the layer of receptor cells.

In the experiments reported above, the electrode was put at various places in the eye, usually near the center. The relative contributions of the ultraviolet and green receptor systems could be altered somewhat by moving the electrode to another part of the eye; for this reason the electrode, once positioned, was not moved during the course of an experiment. Walther and Dodt (1959), using a similar method of selective adaptation, have found that in the compound eye of the cockroach a high sensitivity to ultraviolet light is confined to the dorsal half of the eye. Preliminary experiments on the compound eye of the worker honeybee, however, do not reveal any pattern of distribution of receptor systems as precise as that observed by Walther and Dodt in the cockroach.

The overlap of the regions of the spectrum to which the two receptor systems respond (Figs. 4 and 10) could conceivably arise from neural interaction. The simplest explanation, however, seems to me to be that the various types of receptor system owe their different spectral sensitivities to the presence of different photosensitive pigments. The green receptor system may include a small amount of a second, ultraviolet-absorbing visual pigment, but even this postulate is not necessary. The occurrence of retinene in the heads of bees suggests that the photopigment of the 535 mμ receptor system is a rhodopsin, in which case it should possess, in addition to the major peak in the green, a secondary absorption band in the near ultraviolet, the so called β-band (*cf.* Wald, 1949). Such a molecule could account for the sensitivity of the green receptor system to ultraviolet light.

I should like to thank Professor George Wald for his very helpful criticisms of this manuscript.

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