THE RELATION OF TIME, INTENSITY AND WAVE-LENGTH IN THE PHOTOSENSORY SYSTEM OF PHOLAS.

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I.

A series of studies (Hecht, 1925, 1926–27) of the sensibility to light of a number of animals has brought out the fact that the essentials of the photosensory process are the same in all of them. For example, the reaction time to light of such diverse animals as *Ciona* and *Pholas* and the frog tadpole, is composed of two distinct parts, an exposure period during which the animal must be subjected to the light, and a latent period during which it may be in the dark. Again, the dark adaptation of very different animals presents the same kind of course, and may be regarded as kinetically identical. All such similarities suggest that the organization of the processes which make up the photosensory system is the same in a diversity of animals.

Nevertheless there is evidence to show that although the organization is the same, the materials which compose the reactions, and probably the reactions themselves, are specific. The most quantitative expression of such differences has been found in the effect of temperature on the duration of the latent period. In the three animals in which this has been carefully studied (Hecht, 1927–28) it has been shown that though the relation between temperature and latent period may be expressed accurately by the Arrhenius equation

$$ln\ p = \frac{\mu}{R\,T} - C$$

* The experiments here recorded were made in 1924–25 when, as Fellow of the International Education Board, I occupied the Jacques Loeb Memorial Table at the Zoological Station in Naples.

the value of the temperature characteristic μ is different for each species. Here p is the latent period, R is the gas constant, T the absolute temperature, and C an integration constant of no significance. For *Ciona*, μ = 16,200; for *Mya*, μ = 19,700; and for *Pholas*, μ = 18,300, values for some of which counterparts may be found in certain well known chemical reactions and which may probably represent different chemical materials active in the latent period.

The same kind of difference may be shown to exist in the substances concerned with the part of the photosensory process which occurs in the exposure period. It can be shown that there are differences among species in the degree of sensibility to white light. *Ciona* is much less sensitive to light than is *Mya* or *Pholas*. *Mya* requires about 5 meter-candle-seconds as a minimum amount of energy for stimulation, while *Ciona* needs nearly a thousand times as much (Hecht, 1919–20). This may perhaps be due merely to differences in concentration of the sensitive material. However, it can also be shown that the animals differ strikingly in their sensibility to different parts of the spectrum, facts which are simply explained by assuming differences in the absorption spectra of the sensitive materials concerned with receiving the light energy.

Ciona is comparatively so insensitive to light that it has not been possible to measure the effectiveness of different parts of the spectrum with any greater fineness than will enable one to say that the middle of the visible region is the most effective. *Mya*, however, has been measured in detail (Hecht, 1920–21, *b*), and the data show a well defined maximum of sensibility at 500 mμ, a secondary region of high sensibility at 570 mμ, and a rapid decline of sensibility on either side of the peaks. In the present paper it is proposed to record the measurements made in similar fashion with the Mediterranean lamellibranch *Pholas dactylus*, and to compare them with those of *Mya* and with other animals.

II.

The principle of the measurements is to isolate different portions of the visible spectrum by means of filters, and to determine the amount of energy required for each part in order to elicit the *same sensory effect* in the animal. Technically the experiments involve

three parts. First it is necessary to determine the energy distribution in the spectrum of the lamp and the transmission of the filters in order to know the energy content and the composition of the light transmitted by each filter. Second, it is required to vary this energy in a known manner so as to subject a given animal to different amounts of it. And third, it is necessary to measure the responses of the animal in order to determine when the sensory effects of different portions of the spectrum are identical.

The source of light used in these experiments was a 1000 watt Phillips lamp of the concentrated-filament, half-watt-per-candle variety run at a constant voltage of 200 volts. Its energy distribution

TABLE I.

Energy Distribution in the Spectrum of a 1000 Watt Concentrated Filament Phillips Lamp Running at 200 Volts. The Energy at 550 mμ is Placed at Unity.

λ	Energy
mμ	
450	0.34
500	0.63
550	1.00
600	1.41
650	1.82
700	2.21

was determined at the Physikalisch-Technische Reichsanstalt in Berlin by Dr. W. Dziobek, whom it is a pleasure to thank for his kindness in making these and other measurements for me. The data are given in Table I. The filters were Wratten Filters 72, 73, 74, 75, and 76 of the "monochromatic" series made by the Eastman Kodak Company. The transmissions of these filters are well known (*cf.* Hecht, 1920–21, *b*), and are to be found in detail in a booklet—Wratten Light Filters—published by the Eastman Kodak Company. The energy of the lamp at any wave-length, as taken from Table I, and multiplied by the transmission of a filter at that wave-length gives the amount of energy of that frequency transmitted by the filter. These calculations were made for each filter for every 10mμ of its transmission.

The energy transmission was then plotted on a large scale for each filter, and the total energy transmitted by each filter determined by measuring the area included under the transmission curve. The center of gravity of each area was then found; this corresponds to the wave-length on either side of which an equal amount of energy is transmitted by the filter. Table II gives the resulting information. It contains the number of the filter, its central wave-length, to the nearest 5 mμ, and the total amount of energy transmitted by it in series with the 1000 watt lamp.

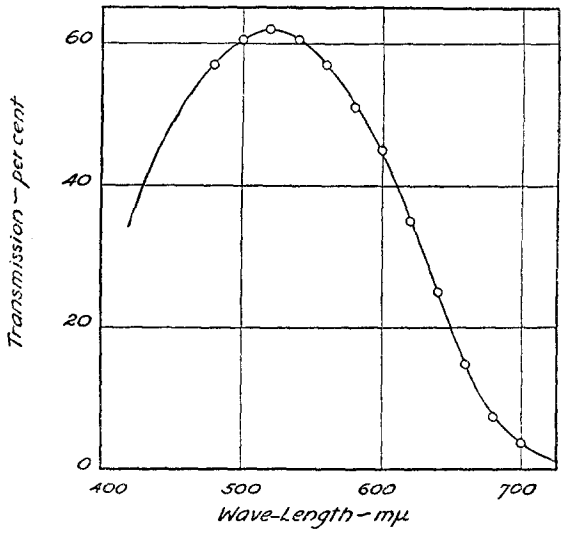

FIG. 1. Transmission spectrum of the copper chloride solution used in Series IV.

In one series of experiments I used an additional filter in conjunction with those already mentioned. This consisted of a layer, 3.7 cm. thick, of 1 per cent aqueous solution of $CuCl_2 \cdot 2H_2O$, whose purpose was to exclude the near infra-red. The transmission of this filter is given in Fig. 1, and is derived from measurements made by Dr. Dziobek for me. This filter was used in Series IV with each of the Wratten filters. Its influence on the composition of the light transmitted is determined by multiplying the energy of the lamp at a given wave-length by the transmission of the copper chloride at that wave-length, and by the transmission of the particular Wratten filter at the same

point. The product gives the energy transmitted at that wave-length. As before, the computations were made at 10 mμ intervals, the curves plotted, the areas under them measured, and the central wave-lengths determined. The results are given in Table II. This table gives the information required by the first part of the procedure, namely, the energy content and composition of the light transmitted by the different portions of the spectrum used for experimentation.

The second part, that of varying the intensity of these parts of the spectrum was accomplished (*a*) by keeping the animals at different distances from the light source and calculating the relative intensities on the inverse square law; and (*b*) by interposing neutral filters, made

TABLE II.

Central Wave-Length and Relative Energy Content of Light from 1000 Watt Lamp Transmitted by Wratten Filters.

Filter No.	Central wave-length	Relative energy content	
		Series I, II, III	Series IV
	mμ		
76	450	100	100
75	485	275	339
74	535	113	156
73	585	260	316
72	615	104	82

of fogged photographic plate, which were calibrated photometrically and spectrophotometrically. In this way an animal could be subjected to any desired intensity of light of known spectral composition.

The third part of the procedure consisted in exposing animals to these various lights, measuring their response, and determining at what intensities the differently colored lights produce the same sensory effect. The reaction time of *Pholas* to light varies inversely with the intensity of the light. With a given part of the spectrum it is thus possible by varying its intensity (I) and measuring the reaction time (r) of an animal to construct a curve relating I and r at that wave-length. Such a curve can be determined for the five different parts of the spectrum isolated by the Wratten filters. From the family of

curves obtained in this manner there can then be found the energy required by each monochromatic patch of the spectrum to cause a response after the same reaction time. The relation among the energies of the different parts of the spectrum gives the relative effectiveness of the various parts in the photosensory stimulation of *Pholas*.

III.

The actual procedure with the animals was as follows. A number of animals were completely dark-adapted by being kept in the dark for about a week. Each animal remained in its own rectangular dish in a light-tight water bath whose average temperature was 16.7°C. An animal was removed from the thermostat, placed on the experimental bench at a definite distance from the light source, exposed to the selected light, its reaction time measured, and at once returned to the thermostat. Half hour later it was again taken out, and placed at another distance, its reaction time measured, and again returned. Half hour later this was repeated at still another distance. At least three intensities were used so as to get the relation between intensity and reaction time for a particular portion of the spectrum. The filter combination was then changed to secure a different part of the spectrum, and the reaction time of the animal was measured at half-hour intervals at three different intensities. This was continued until the five selected parts of the spectrum had been investigated for a given animal.

In this manner I ran four series of experiments. Series I had 4 animals; Series II had 6 animals; Series III had 7 animals. Each of these series was carried through in one day. As a source of light there was used the Phillips lamp and the Wratten filters. Several weeks after these experiments, I ran Series IV which differed in three respects from the other three series. First, it consisted of a larger number of animals, 15 to be precise. Second, it contained the copper chloride filter in addition to the Wratten filters. And third, the measurements were made one day, and on the following they were repeated, but in the inverse order of spectral parts and of intensities.

The data secured in the four series of experiments are presented in Table III. From mere inspection of the table it is apparent that the sensibility distribution along the spectrum is pretty much the same

in all four series, and that the maximum sensibility lies at about 535 mμ. However, a closer examination of the data is necessary in order to determine accurately the relative sensibility of the animals to the different wave-lengths. This treatment of the data will be given in detail for Series I; the method for the other series is identical, and only their results will be given.

TABLE III.

Relation between Intensity of Illumination and Reaction Time of Pholas to Light of Different Wave-Length. Reaction Time, r, in Hundredths of a Minute.

Wave-length	Series I		Series II		Series III		Series IV	
	I	*r*	*I*	*r*	*I*	*r*	*I*	*r*
mμ		*hm.*		*hm.*		*hm.*		*hm.*
	347.0	2.70	141.0	2.90	347.0	2.63	174.0	2.79
	77.6	3.33	77.6	3.37	42.7	3.87	28.2	3.63
450	57.5	3.48	22.9	4.20	5.13	5.91	11.8	4.79
	22.9	4.28	9.55	4.80				
	9.55	6.18						
	63.1	3.68	63.1	3.03	63.1	3.11	95.5	2.94
485	30.2	4.25	26.3	3.52	26.3	4.00	39.8	3.57
	14.1	5.58	14.1	4.88	14.1	4.57	21.4	4.30
	25.7	3.48	87.1	2.60	25.7	3.03	43.7	3.04
535	10.7	4.00	25.7	3.12	10.7	3.59	18.2	3.62
	5.75	5.40	10.7	4.00	5.75	4.21	9.77	4.34
			5.75	4.47	3.16	5.46		
	372.0	3.40	372.0	2.73	204.0	2.96	302.0	3.13
585	151.0	3.95	25.1	4.12	60.3	3.82	89.1	3.83
	60.3	5.20	13.5	6.40	25.1	5.03	37.2	4.87
	912.0	3.58	912.0	3.27	363.0	3.73	347.0	3.63
615	363.0	4.28	363.0	3.67	148.0	4.44	141.0	4.35
	148.0	6.40	148.0	3.95	81.3	4.94	77.6	5.38

Fig. 2 gives the data of Series I in graphic form. It is apparent that the curves describing the relation between log I and the reaction time, r for the different wave-lengths are parallel to one another. In order to find the relative amounts of energy required by the different wave-lengths to produce the same sensory effect, one can read off

from the plot the abscissa values corresponding to a given ordinate value. The reciprocals of these I values will then give the relative stimulating capacities of the different parts of the spectrum. Such measurements of the intensity may be made at convenient values of the ordinates, say at 6.0, 5.0, etc., the results averaged, and the reciprocals taken. Simpler still is to make the measurements for the curve as a whole by determining the logarithmic distance on the abscissas through which the curves have to be moved to the left in order to coincide with the one corresponding to the most effective part of the spectrum. This amounts to finding the factor by which the

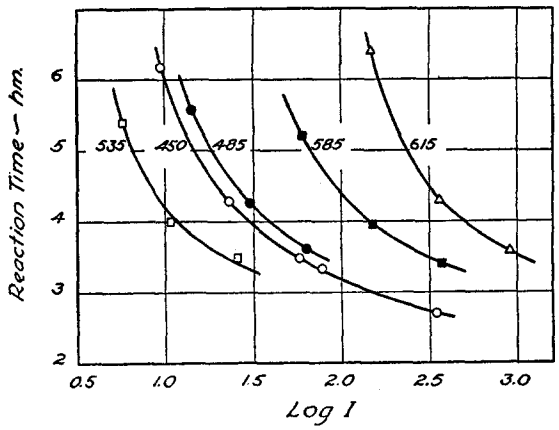

FIG. 2. Relation between log I and the reaction time of *Pholas* to light from the different portions of the spectrum as indicated by their central wave-length. Data of Series I. Reaction time is in hundredths of a minute.

intensity values of the points on a given curve must be multiplied in order to fit on the curve farthest to the left. The factors so found are equal to the ratios of the intensity of the most effective wave-length to each of the intensities of the other wave-lengths required to produce the same sensory effect. If I_{max} is the intensity of light at 535 mμ necessary to produce a given reaction time, and I_λ the intensity necessary to produce the same effect at any other value of λ, then the factor by which the I_λ values of any curve in Fig. 2 must be multiplied in order to coincide with the curve at the extreme left, $\lambda_{max} = 535$, will be equal to I_{max}/I_λ. Obviously the ratio I_{max}/I_λ gives the relative

stimulating capacity of the different parts of the spectrum. Fig. 3 shows the five curves of Fig. 2 whose intensities have been multiplied by the proper factors, and superimposed one on the other. The adequacy of this method may be judged by the way the points for the different wave-lengths lie on the common curve. Similarly in Fig. 3 are given the composite curves for the other three series of experiments.

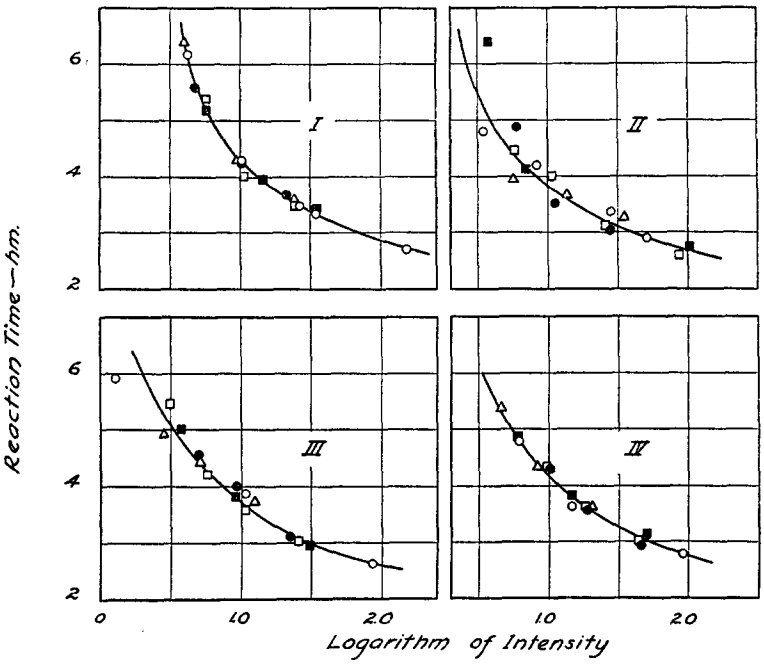

FIG. 3. Data of four series of experiments relating log I and reaction time of *Pholas* to lights of different wave-length. The symbols for the different points are the same as in Fig. 2.

Table IV gives the factors which have been used to superimpose the various curves of the four series of data, and which correspond to the ratio I_{max}/I_λ for each. It is apparent that the relative stimulating capacities of the different parts of the spectrum are similar for the different series. Fig. 4 shows the data of Table IV graphically. From the smooth curves drawn, it is apparent that 550 mμ is the most effective portion of the spectrum. On the red side the effectiveness drops

very rapidly to almost nothing. It is for this reason that it is possible to make all of the manipulations with *Pholas* by the dim light of a 10 watt ruby lamp; the animals are insensitive to such illumination. On the blue side of the maximum the effectiveness drops rapidly, but only to about half the maximum, and then apparently rises again. Series II and III do not actually indicate this rise. But Series I and IV show it clearly. Series IV is undoubtedly the best of the experiments; it is based on about as many animals as all the rest together, and on two readings for each animal for each point. The rise toward the violet end is therefore probably a real phenomenon, and would indicate that the animals may be sensitive to the near ultra-

TABLE IV.

Relative Effectiveness of Different Wave-Lengths in Stimulating Pholas. The Figures in the Table Give the Values of the Factor a × 100 for the Different Wave-Lengths and Different Series of Table III.

Series	Relative effectiveness				
	450 mμ	485 mμ	535 mμ	585 mμ	615 mμ
I	44.7	33.9	100	9.55	2.69
II	36.3	42.7	100	27.5	3.80
III	25.1	35.5	100	15.1	3.47
IV	52.5	47.9	100	16.2	5.89

violet. Unfortunately these computations were made after leaving Naples, and this possibility has not been tested.

IV.

The relative effectiveness of the visible spectrum in stimulating *Pholas* is apparent from Table IV and Fig. 4. It is very unlikely that this variation in effectiveness is due to any differences in the properties of the different parts of the spectrum. The frequencies represented are too near one another to suppose any real differences in photochemical characteristics. The variations shown in Fig. 4 are thus most probably descriptive of something in the organism. The effect produced on *Pholas* by the different parts of the spectrum is a qualitatively invariable reflex. The derivation of the data in

Table IV and Fig. 4 depends on a comparison of quantitatively identical effects in this qualitatively invariable reflex. One may assume that the production of these identical effects depends on the reception of a given amount of energy, regardless of wave-length, by a sensitive material in the sense cells of *Pholas*, and its conversion into the photochemical action which starts the photoreceptor process. If this assumption is correct, then the curves in Fig. 4 represent the absorption spectrum of the photosensitive substance in *Pholas* plus any screening pigments that may be intimately associated with it.

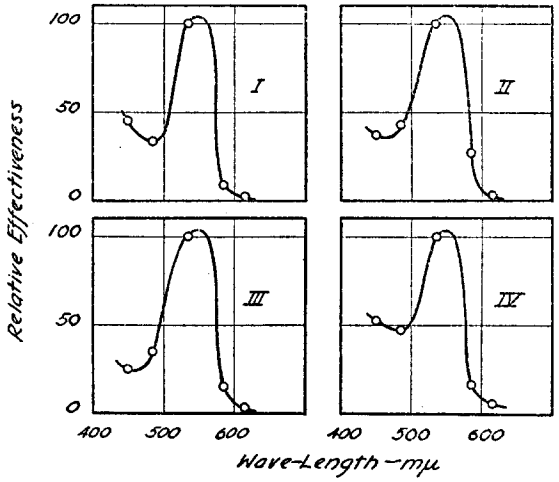

FIG. 4. Relative effectiveness of different parts of the spectrum in eliciting a response to photic stimulation of *Pholas*.

If I_λ is the energy of a portion of the spectrum, which is required to produce a given sensory effect on *Pholas*, and a is the absorption coefficient, as per cent of the maximum absorption, then aI_λ will be the amount of the energy at that wave-length which is absorbed by the photosensitive system. But the absorbed energy necessary to produce a constant sensory effect has been assumed constant. Call this energy K. Then $aI_\lambda = K$, and $a = K/I_\lambda$. In other words the absorption coefficient of the photosensitive system is proportional to the reciprocal of the energy required at a given wave-length to produce a constant sensory effect. These reciprocals are of course

the values in Table IV and Fig. 4. The curves may be then regarded as expressing the absorption spectrum of the photosensitive system in *Pholas*, and as a specific characteristic of this animal.

Fig. 5 shows a comparison between the effectiveness of the spectum for *Mya*[1] and *Pholas*. Considered as indirectly determined absorption spectra, these curves show that the photosensitive systems in these two animals are quite different. Thus although the two animals possess an extraordinary similarity in the general characteristics and organization of their photosensory process, the materials of which these systems are composed are very likely different.

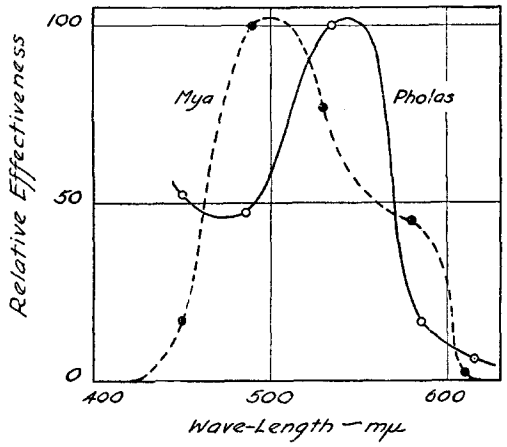

FIG. 5. A comparison of the relative effectiveness of the spectrum in the stimulation of the lamellibranchs, *Pholas* and *Mya*. The curve for *Pholas* is from Series IV.

The conclusion that different animals possess different photosensitive materials would seem too self-evident, were it not for the fact that Hess (1910) has emphasized their similarities to the extent that he supposes the effectiveness of the spectrum to coincide with the luminosity curve of the totally color-blind human eye. The number of

[1] The data are taken from Table II and Fig. 5 of the paper on *Mya* (Hecht, 1920–21, *b*). Due to a typographical error the decimal point in the values for Filters 73 and 74 in Table II of this paper was put one place too far to the right. The correct values are, *e.g.* 2.18 and 1.29 respectively, and are correctly given in Fig. 5 of the same paper.

careful measurements which have appeared in recent years in this connection (Laurens and Hooker (1920), Loeb and Wasteneys (1916), Mast (1917), Hecht (1920–21, *b*), Crozier (1923–24))leave no doubt however that such a generalization is an inadequate expression of the data as known. Special attention is here directed to the differences between *Mya* and *Pholas* because of the very striking similarities shown in the other characteristics of their photosensory processes.

V.

Although these data on the effectiveness of the spectrum show how different are the absorption spectra of the photosensitive systems of *Mya* and *Pholas*, they show, at the same time, how fundamentally similar are the organization and photochemistry of the two systems. This will become evident in the present section.

Examination of the material in Figs. 2 and 3, relating intensity and reaction time, shows that curves have a consistently uniform appearance regardless of wave-length or series. It is significant to inquire into the form of this relation between intensity and reaction time, and to determine its precise meaning in terms of our knowledge of the photosensory process.

It was shown for *Mya* (Hecht, 1920–21, *a*) and for *Ciona* as well (Hecht, 1925–28) that if the intensity, I, of the stimulating light is kept constant, and the time (t) of its action varied, the photochemical effect (E) is very nearly a linear function of the time. In other words

$$E = k_1 t \tag{1}$$

where k_1 is a constant. Similarly if the time of action is constant and the intensity varies, then the effect is proportional to the logarithm of the intensity. This may be written as

$$E = k_2 \log I \tag{2}$$

where k_2 is a constant. It therefore follows from these two equations that

$$E = k\, t \log I \tag{3}$$

and experiments with *Mya* showed that when I and t are both varied, equation (3) holds true experimentally. It is significant that equation (3) is valid for the photosensory responses of *Pholas*.

Perhaps the simplest way of demonstrating this is as follows. The reaction time (r) is composed of two parts, the exposure period (t) and the latent period (p), so that

$$r = t + p$$

and

$$t = r - p. \tag{4}$$

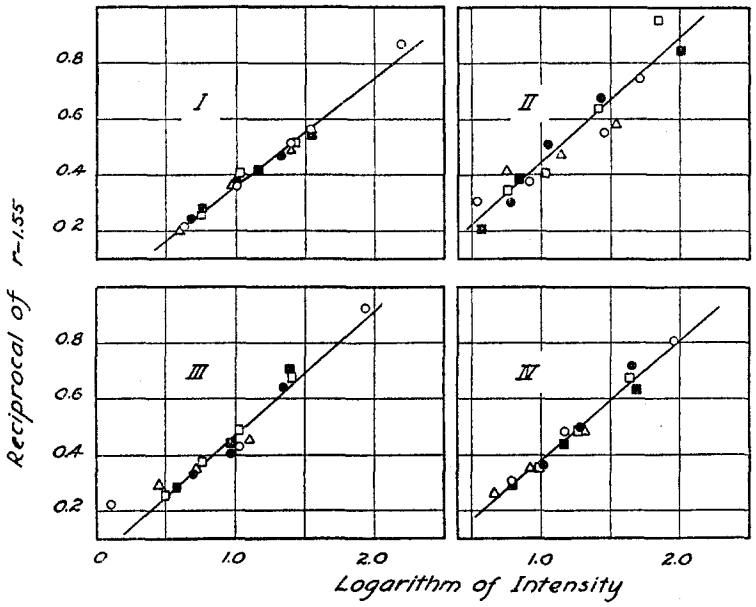

FIG. 6. Relation between log I and the reciprocal of $r - p$, where r is the reaction time and p the latent period.

This value of the time of action of the light may now be substituted in equation (3) which then becomes

$$E = k\,(r - p)\,\log I$$

and

$$\frac{E}{r - p} = k \log I. \tag{5}$$

It is apparent that since E is constant the reciprocal of $(r - p)$ plotted against log I should give a straight line if equation (3) is true.

Fig. 6 shows the relation between the reciprocal of $(r - p)$ and $\log I$ for the four series of experiments made with *Pholas*. It is assumed that $p = 1.55$ hm., no measurements of the latent period having been made with these particular animals. This value of p is very likely correct, because a value of $p = 1.67$ hm. at 16.5°C. was actually obtained with 16 animals in a series of experiments on the dark adaptation of *Pholas* made within a few days of Series IV of the present experiments (Hecht, 1926–27, Table II). In making Fig. 6, the composite data of Fig. 3 were used, these in turn having been derived from Tables III and IV. The straight lines in Fig. 6 show that equation (5) describes these data, and therefore that the relationships expressed by equations (1) and (2) which enter into the derivation of equations (3) and (5) are applicable not only to the photosensory process in *Mya* and *Ciona* but in *Pholas* as well. The present experiments have thus shown that the photosensory systems of these three animals are specifically different though they are fundamentally the same.

SUMMARY.

The most effective point in the visible spectrum for the stimulation of *Pholas* is 550 mμ. On the red side, the effectiveness drops rapidly to almost zero. On the violet side, the effectiveness drops to about half, and rises again in such a way as to indicate a possible second maximum in the near ultra-violet.

On the basis of certain ideas these data are assumed to represent the properties of the absorption spectrum of the photosensitive system in *Pholas*. A comparison with *Mya* shows that the absorption spectra of the photosensitive systems in the animals are distinctly different.

Nevertheless the way in which intensity and reaction time are related in the two animals are found to be identical. The conclusion is then drawn from this and from previous work, that although the fundamental properties of the photoreceptor process show an identical organization in several different animals, the materials which compose these processes are specific.

BIBLIOGRAPHY.

Crozier, W. J., Wave length of light and photic inhibition of stereotropism in *Tenebrio* larvæ, *J. Gen. Physiol.*, 1923–24, vi, 647.

Hecht, S., The photochemical nature of the photosensory process, *J. Gen. Physiol.*, 1919–20, ii, 229; Time and intensity in photosensory stimulation, 1920–21, *a*, iii, 367; The relation between the wave-length of light and its effect on the photosensory process, 1920–21, *b*, iii, 375; The effect of exposure period and temperature on the photosensory process in *Ciona*, 1925–28, viii, 291; Zur Photochemie des Sehens, *Naturwissenschaften*, 1925, xiii, 66; The kinetics of dark adaptation, *J. Gen. Physiol.*, 1926–27, x, 781; The influence of temperature on the photosensory latent period, 1927–28, xi, 649.

Hess, C., Neue Untersuchungen über den Lichtsinn bei wirbellosen Tieren, *Arch. ges. Physiol.*, 1910, cxxxvi, 282.

Laurens, H., and Hooker, H. D., Jr., Studies on the relative physiological value of spectral lights. II. The sensibility of *Volvox* to wave-lengths of equal energy content, *J. Exp. Zool.*, 1920, xxx, 345.

Loeb, J., and Wasteneys, H., The relative efficiency of various parts of the spectrum for the heliotropic reactions of animals and plants, *J. Exp. Zool.*, 1916, xx, 217.

Mast, S. O., The relation between spectral color and stimulation in the lower organisms, *J. Exp. Zool.*, 1917, xxii, 471.