EXPERIMENTS ON THE LIGHT SENSE OF THE HAG, *MYXINE GLUTINOSA* L.

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INTRODUCTION

The general properties of the light reaction of hags have been described by Newth & Ross (1955) in another paper in this *Journal*. They show that although the time interval between the stimulus and the response is exceptionally long, the reaction is similar to those of other eyeless animals such as *Mya*, *Ciona*, *Proteus* or the ammocoete larva of *Lampetra*, and appears to conform in most respects with other photosensory systems. The experiments described in this paper were performed on some of their animals and were intended primarily to obtain information on the hag's spectral sensitivity, from which we may hope to deduce something of the nature of the underlying photochemical system. Additional information is presented on the relation between the duration and intensity of stimulation and the reaction time.

MATERIAL AND METHODS

The experiments were carried out between March and June 1952. They fall into two groups; the first with five hags was done at University College, London, the second with three some weeks later at Edinburgh. All except one of the animals had been used previously by Newth & Ross, and in the first group of tests care was taken to select those which had consistent records of behaviour in their experiments.

Table 1. *Identification of animals*

Experiment series	Animal no.	No. in Newth & Ross's experiments
Edinburgh	1	Not known
	2	Not known
	3	Not known
University College, London	4	B
	5	C
	6	H
	7	L
	8	Not used

The identification of the hags used by them and by me is given in Table 1. It is perhaps worth noting that nos. 4–7, which correspond with Newth & Ross's B, C, H and L, were all fast reacting hags. That is to say their mean reaction time was less than the mean of the whole group of twenty animals in their experiments. No. 5 (= C) consistently gave the shortest reaction time of all.

All experiments were done at room temperature. At University College this varied from 6·5 to 8·5° C.; at Edinburgh from 13·0 to 17·5° C. The latter are probably rather higher than *Myxine* normally encounters in the wild state, but no obvious signs of distress were noticed, and there seems no reason to believe that the animals' light reactions were impaired for this reason. They did not eat throughout the period of these experiments, the last of which were completed about 4 months after the arrival of the animals in this country from Sweden. Again, there is no evidence that their sensitivity to light was affected by this long fast or even that their general condition deteriorated in any way, except that towards the end they seemed to produce rather less mucus when handled.

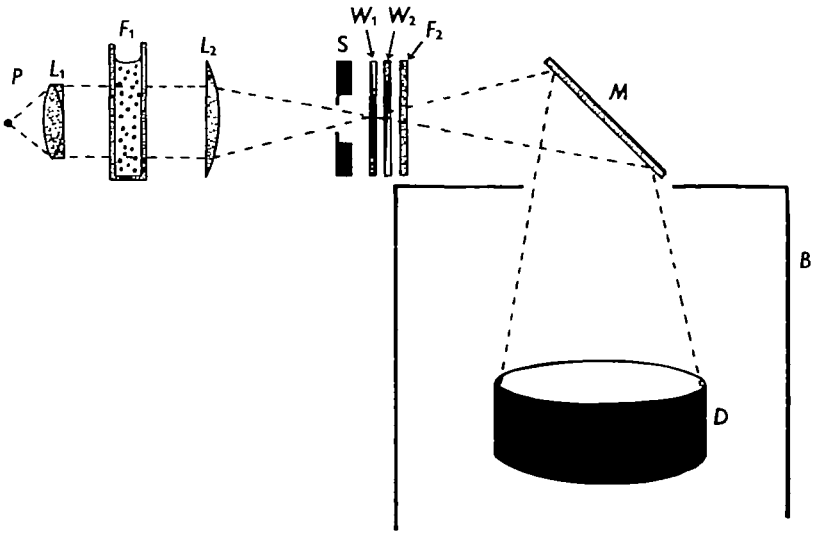

Fig. 1. To show the optical arrangement used for light stimulation of *Myxine* (not to scale). Key to lettering: *B*, dark box surrounding observation dish; *D*, glass observation dish; F_1, heat filter; F_2, colour filter; L_1 and L_2, convex lenses; *M*, mirror; *P*, light source; *S*, camera shutter and diaphragm; W_1 and W_2, neutral wedges.

The apparatus used for light stimulation was in general similar to that described by Steven (1950) for testing ammocoetes, and will not be described here in detail. The optical arrangement is represented diagrammatically in Fig. 1. Light from a source of high intensity was projected so as to illuminate from above a circular glass observation dish 30 cm in diameter. The stimulating light was switched on and off by a camera shutter and its intensity varied by means of a neutral wedge of graded density from 0 to 6, which could be moved horizontally across the path of the beam. These and colour filters when required were mounted close to the principal focus of the lens L_2. A 12 V. 24 W. tungsten filament lamp was used as light source in the experiments at University College, while at Edinburgh a higher range of illumination was obtained with a 110 V. 500 W. projector lamp at a colour temperature of about 3100° K.

The hag to be tested was placed in the observation dish in about 2 in. of clean sea water. White paper was placed under and around the dish, and the apparatus was adjusted so that the cone of light just filled its mouth. This arrangement gave very uniform illumination on the floor of the dish. At University College the experiments were carried out in a satisfactory dark room, and it was necessary only to erect a screen between the lamp and the dish to eliminate stray light. At Edinburgh the room was dimly illuminated and the observation dish was therefore placed in a tent made of cardboard mounted on a wooden frame and painted black on the inside, to which the stimulating light was admitted through an aperture in the roof. A dark cover was placed over the dish between tests so that recovery took place in total darkness.

The procedure for testing was as follows:

The light was switched on and the setting of the wedge and filters adjusted with the camera shutter still closed and the observation dish covered. The cover was then removed and the animal examined for a few seconds in dim red light from an electric hand torch fitted with a filter, Ilford no. 609, which only transmits radiation of wave-length longer than 640 mμ. If the hag was seen to be lying quite still the camera shutter was opened and a stop-watch started simultaneously. The hag was watched continuously, first by the stimulating light and afterwards by the illumination of the red torch, and the times noted of its first movement and other movements up to the commencement of swimming. Animals were usually watched up to 5 min. from the commencement of a stimulus and sometimes longer. If no movement was seen the cover was replaced on the dish and a further recovery period in total darkness allowed before the next stimulus was given.

Twenty to thirty minutes were allowed for recovery between tests at University College, but at Edinburgh periods of an hour or more were given and appeared to be followed by more consistent results. This is at variance with the opinion expressed by Newth & Ross that 20 min. is sufficient for complete dark adaptation of the hag following stimulation. No controlled experiments were done to settle this point, but the impression was formed that longer periods are required to attain maximum sensitivity.

Animals under test were kept in the same dish until a series was completed, sometimes for several days. Lumps of coagulated mucus, the only symptoms observed which might be interpreted as signs of distress, were removed and the water changed if it appeared to be at all dirty. The temperature of the water was measured after each test and at the beginning and end of each series.

The time relation between the first response and swimming

As Newth & Ross have pointed out, *Myxine* responds to light by swimming, but this is nearly always preceded by some other movement, which may be either local or general, and which may be designated the 'first response'. This may be anything from a twitch of the head or tail, or of one or two of the sensory tentacles around the mouth, to a general stirring of the whole body. Sometimes the general movements have the effect of bringing the animal from the position of rest on its side with its body in a shallow U to an upright S position, preparatory to swimming. The fact that the first response is always sharply defined makes it the best quantitative measure of the reaction time. However, the first response and the sequence of movements which precede swimming are obviously characteristics of the individual

animals, as are also the time relations between them. Just as some hags have short reaction times and others long ones, so in some the time interval between the first response and the onset of swimming is very brief while in others it is much longer. It may also be relatively constant or highly variable. There does not however appear to be any obvious relation between the reaction time measured to the first response and the interval between the first response and the commencement of swimming. In some animals with consistently short reaction times this interval is relatively long and in others the opposite is true.

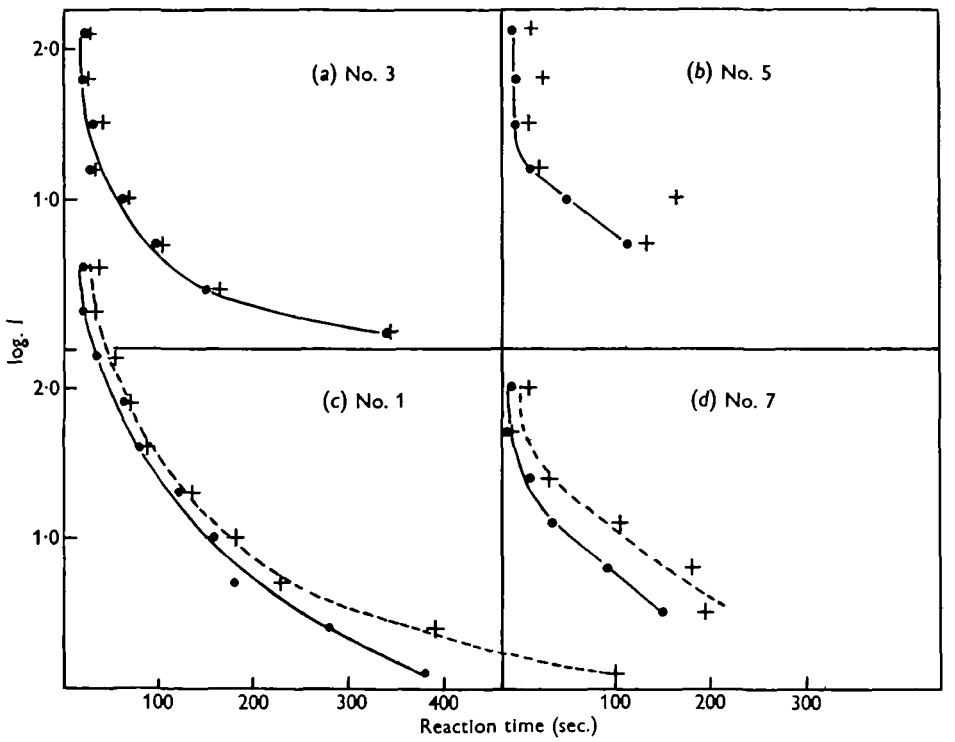

Fig. 2. The relation between the first response and the commencement of swimming of four hags. The curves show the relation between the intensity of the stimulus and the reaction time measured to the first response (dots and continuous line) and the commencement of swimming (crosses and broken line). Light intensity in arbitrary logarithmic units.

One or two examples will illustrate these points. The sequence of responses given by animal no. 3 was almost invariably a sharp twitch of the head and body followed by swimming a second or two later, without any intervening series of movements. This was true at all intensities of stimulus, as illustrated in Fig. 2*a*, which shows the relation between the intensity and the reaction time measured to the first response and to the commencement of swimming for a single series of tests between 0·4 and 50 e.f.c.* For this hag therefore the first response and commencement of swimming were almost simultaneous.

* Brightness values are expressed as equivalent foot candles, e.f.c. 3·426 e.f.c. = 1 candle/square metre.

A similar series of tests on animal no. 5, presented in Fig. 2*b*, shows a different type of relation. The time interval between the first response and swimming varies from 2 to 105 sec., and there is no obvious tendency for it to increase (as does the reaction time) as the intensity of stimulus is decreased. This suggests that photochemical processes play no part in determining this time interval, but only affect reaction times measured to the first response. Other animals, however, did show a tendency for this time interval to increase as the intensity of the stimulus was decreased. This was noticed in several experiments, and will be illustrated here by two examples. The intensity/reaction time data for animal no. 1 between 0·1 and 50 e.f.c. are presented in Fig. 2*c*. At higher intensities the interval varied in an apparently random manner between 6 and 20 sec., the average of seven observations being 15·5 sec., but at the four lowest intensities it increased progressively to the surprisingly long time of 200 sec. at 0·1 e.f.c. In this experiment the animal was illuminated continuously until the first response was seen and the effect is apparent only at levels of illumination approaching the absolute threshold of sensitivity. In the second example, shown in Fig. 2*d*, animal no. 7 was illuminated for 30 sec. at each of six intensities between 2 and 25 e.f.c. Over this range the interval between the first response and the commencement of swimming clearly tended to increase progressively as the intensity was decreased.

The findings illustrated by these last two examples were unexpected and seem to imply that at low intensities of stimulus continuing photochemical processes are in some way concerned not only with the first response but with further processes leading up to the initiation of swimming, i.e. with processes which are usually thought to be entirely secondary. This is another way of stating the point made by Newth & Ross that the light responses of animals with long reaction times, such as *Myxine*, cannot be treated in the classical manner of Hecht as though the reaction time were made up successively of an initial sensitization period followed by a latent period.

It is of course possible, though unlikely, that the intensity of stimulus is related to the commencement of swimming rather than to the first response. It seems better, however, to regard the latter as an alerting movement which can be brought about by a lesser photochemical effect than is required to initiate general locomotory activity. In support of this view it was usual at low intensities of stimulus to find a first response followed by a series of further twitches or local movements, which did not always culminate in swimming. This did not occur at higher intensities. It is difficult to avoid the conclusion that the preliminary local responses and swimming represent respectively two levels of excitation, and that the interval between them represents the time occupied by the build-up of processes to the higher level necessary to initiate swimming. It seems probable too that these processes are thermal and not concerned with the primary photochemical reaction. The position is clearly unsatisfactory as it stands. The data presented however serve to emphasize both the individuality of hags and the fact that the neurological mechanisms, involved are complex compared with those concerned in the light responses of relatively immobile animals, such as *Ciona*, *Mya* and other Invertebrates.

Analysis of reaction time

The relation between the intensity of the stimulus and the reaction time, defined as the period from the commencement of the stimulus to the first response, was studied with single hags exposed to the full radiation of the light source. Measurements of reaction time were made when:

(1) The illumination was constant and the duration of exposure varied.

(2) The duration of exposure was constant and the illumination varied.

(3) Both illumination and the duration of exposure were varied.

(1) Five series of measurements were made, three at 22·6 e.f.c. and two at 63 e.f.c. The exposure times ranged from 1 to 15 sec., the longest being slightly less than the minimum reaction time of the animals. The results are presented in Fig. 3. At 63 e.f.c. the reaction time was almost constant for exposures longer than 4 sec., but increased rapidly for exposures below this value. The reaction time was increased by about sixfold when the exposure was halved from 4 to 2 sec., and abolished when it was further reduced to 1 sec.

The three experiments at 22·6 e.f.c., Fig. 3*b*, show this inverse relation between the reaction time and the period of exposure over the greater part of the range studied. In animal no. 5, for instance, the reaction time was approximately doubled when the exposure was reduced from 7·5 to 5 sec., and doubled again when it was reduced to 2·5 sec. Similarly, the reaction time of animal no. 4 increased by about 10 sec. for each 2 sec. reduction of exposure period below 12 sec.

Approximate values for the sensitization period can be derived from these data. If a hag is illuminated until it gives a response, the reaction time observed is the minimum for that intensity of stimulus. Shorter periods of exposure may not give an increase in the reaction time until a critical value is passed, which is the sensitization period. The latter may therefore be defined as the shortest period of illumination which will give the minimum reaction time at any given intensity. Inspection of Fig. 3 shows that at 22·6 e.f.c. the sensitization period of all three animals is about 10 sec., while at 63 e.f.c. it is about 4 sec. These values can only be considered as approximations, since there is in fact no value of exposure period above which the reaction time can be said to be constant, being affected to some extent by all changes of exposure. This is no doubt due to the fact, which has been pointed out elsewhere, that sensitization and latent periods are really coextensive in time, the former being in *Myxine* a relatively large fraction of the total reaction time.

(2) Three series of measurements were made varying the intensity of stimulus and keeping the period of exposure constant. Periods of 15 and 30 sec. illumination were used, slightly less than the minimum reaction time of these hags. The results presented in Fig. 4 show that the relation between the reaction time and the logarithm of the intensity is linear over a considerable range of illumination. Only the curve for animal no. 5 shows the reaction time approaching a minimum at the higher intensities of stimulus.

(3) By varying both the intensity and duration of the stimulus responses can be obtained over a greater range of illumination than is possible by either of the fore-

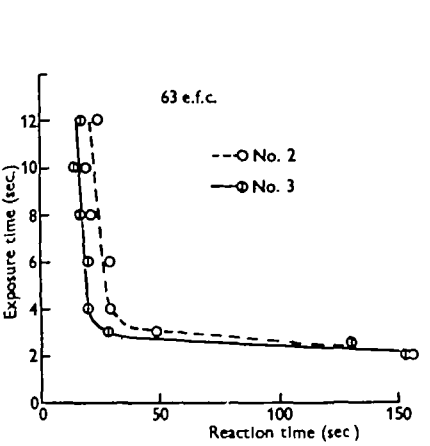

Fig. 3 *a*.

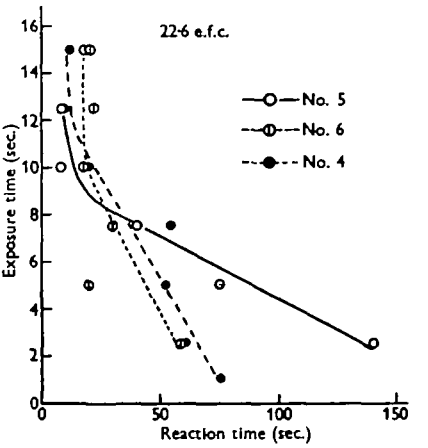

Fig. 3 *b*.

Fig. 3. The relation between stimulus and reaction time for stimuli of constant intensity and varying duration. *a*, at 63 e.f.c., 2 hags; *b*, at 22·6 e.f.c., 3 hags.

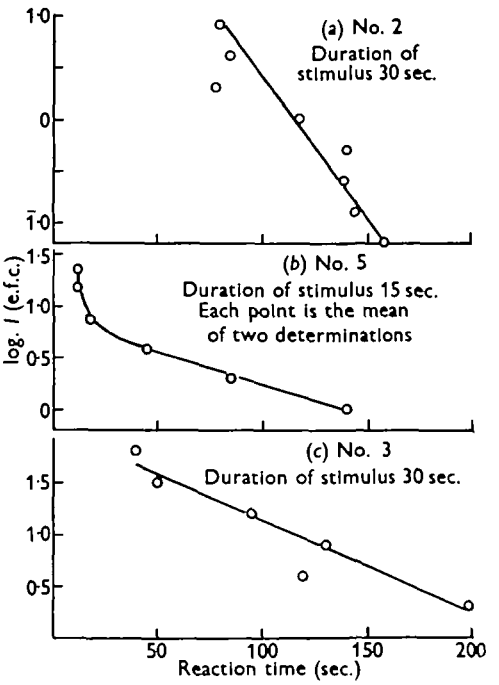

Fig. 4. The relation between stimulus and reaction time for stimuli of fixed duration and varying intensity.

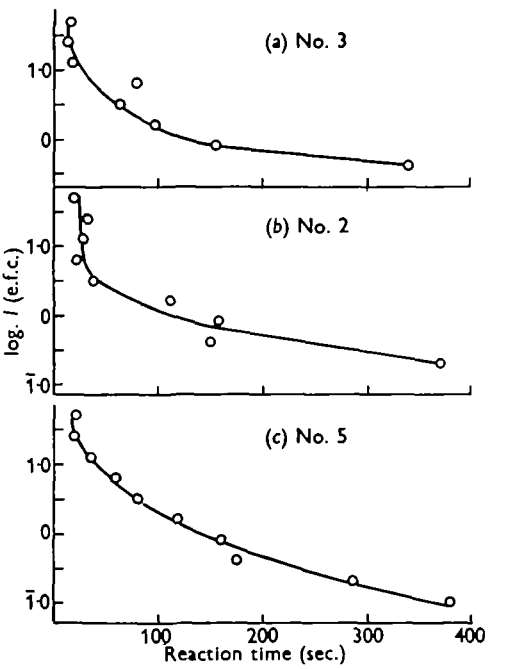

Fig. 5. The relation between stimulus and reaction time for stimuli continued until the first response.

going procedures. In this way we can approach more closely to the minimum threshold of response of each animal and thereby estimate its maximum sensitivity. The procedure was to illuminate the hag continuously until the first response was seen, so that the duration of stimulus was the same as the reaction time in each case. Five such experiments were performed. The results of two of them are presented in Fig. 2*a* and *c*, the other three in Fig. 5. The curves all show the same general characteristics. The reaction times are more or less constant at their minimum value for levels of illumination greater than about 10 e.f.c., and increase rapidly below 1 e.f.c. It is probable that hags could be made to respond to stimuli still weaker than 0·1 e.f.c., which was the lowest level attained (Fig. 5*c*), but the reaction time would be so long that the chance of spontaneous movement intervening would be greatly increased.

Spectral sensitivity

Spectral sensitivity curves were constructed from measurements of the sensitivity of hags in monochromatic light of several different wave-lengths. Spectral bands were isolated by means of the Ilford 'monochromatic' series and other combinations of filters, whose optical properties were known. The relative amount of energy transmitted by each filter in series with the light source was obtained by plotting the product of the energy of the lamp and the transmission of the filter for each 10 mμ of its transmission band. The area enclosed within each curve is proportional to the energy transmitted by that filter. A further correction was made for the quantum

Table 2. *Characteristics of the filters used to isolate spectral bands in combination with* 500 *W. lamp at* 3100° *K.*

Filter (Ilford No.)	Central wave-length (mμ)	Relative transmission
601	430	2·93
602	470	1·36
603	497	1·28
604	519	1·25
807	530	3·85
605	555	1·00
606	578	1·60
808 + 802	583	14·3
607 + 802	595	2·15
608 ± 802	650	1·58

effectiveness of the light source and the central wave-length of energy transmitted by each filter, which was in most cases a few mμ towards longer wave-lengths than indicated by the published information on these filters. The characteristics of the filters in combination with the projector lamp used in the Edinburgh experiments are listed in Table 2. The colour temperature of the lamp used at University College was not measured and the energy content of the waveband cannot therefore be calculated with the same accuracy, but the difference between the two series was certainly small and has been neglected in the calculations based on the animal's responses.

Spectral sensitivity was measured in two ways. In the first series of experiments carried out at University College the sensitivity of hags to light of different wave-lengths was calculated from measurements of their dark-adapted thresholds, that is to say by measuring the minimum intensity of light required to elicit a response within arbitrary time limits. An animal was first dark-adapted for about an hour and its threshold determined by subjecting it to a series of stimuli of increasing intensity, with a suitable period of recovery in darkness between each trial, until a response was obtained. This method suffers from the disadvantage that it is difficult to find a satisfactory criterion of a constant threshold response. In these experiments the threshold was defined as the lowest light intensity which gave a reaction time between 90 and 150 sec. in at least two out of three tests. The increment of light intensity between tests was 0·3 logarithmic unit.

Table 3. *Spectral sensitivity of three hags estimated from measurements of the threshold of response*

Wave-length (mμ)	Hag no.					
	4		5		6	
	Log. *I*	Sensitivity (%)	Log. *I*	Sensitivity (%)	Log. *I*	Sensitivity (%)
430	—	—	—	—	1·47	11
470	—	—	1·13	12	0·53	92
497	1·11	24	1·11	39	0·49	100
519	0·49	100	0·19	100	0·50	97
555	0·70	62	0·40	62	1·00	32
583	2·15	2·4	1·61	7	—	—
595	—	—	0·98	16	1·33	15
650	—	—	N.R.*	N.R.*	N.R.*	N.R.*

* N.R. = no response.

A sufficient number of observations was obtained from three animals and the results are presented in Table 3. The energy required to obtain a threshold response as defined above is expressed in arbitrary logarithmic units, and the relative stimulating capacity is the reciprocal of this value. To compare the results from different animals, however, the relative stimulating capacities are expressed in the second column as a percentage of the most effective wave-length. This is the reciprocal of $I\lambda/I_{max.} \times 100$, where $I_{max.}$ is the intensity required to obtain the response at the most effective wave-length and $I\lambda$ the intensity at any other wave-length, λ.

The method adopted in the second series of experiments was similar to that first used by Hecht (1928) with *Pholas*. Instead of measuring the stimulus required to elicit a response in a given time, reaction times for each filter were measured at several intensities of illumination. The results are listed in Table 4. Intensity/reaction time curves for different wave-lengths can be constructed from these data and those for hag no. 3 are shown in Fig. 6. The other two animals gave similar sets of curves.

The relative stimulating capacity of different parts of the spectrum can now be calculated in two ways. The most effective wave-length is that whose curve lies

Table 4. *Reaction times of three hags to stimuli of different wave-length and intensity*

(Intensity of stimulus is recorded in arbitrary logarithmic units of brightness, reaction times in seconds.)

Wave-length (mμ)	Log. *I*	R.T.	Log. *I*	R.T.	Log. *I*	R.T.	Log. *I*	R.T.	Log. *I*	R.T.
Hag no. 1										
430	0·56	292	0·87	235	1·17	160	1·47	150	—	—
470	0·24	200	0·54	178	0·84	110	1·14	125	—	—
497	0·20	220	0·50	170	0·80	120	1·10	100	—	—
519	0·19	310	0·49	232	0·80	185	1·10	125	—	—
555	0·40	230	0·70	138	1·0	105	—	—	—	—
595	1·0	249	1·34	155	—	—	—	—	—	—
Hag no. 2										
430	0·87	263	1·47	185	—	—	—	—	—	—
470	0·54	265	0·84	175	1·14	135	—	—	—	—
497	1·9	258	0·20	205	0·50	220	0·80	80	—	—
519	0·19	325	0·49	260	0·80	144	1·10	150	—	—
555	0·40	280	0·70	212	1·0	130	—	—	—	—
595	N.R.*	N.R.*	—	—	—	—	—	—	—	—
Hag no. 3										
470	0·24	310	0·84	258	1·14	180	1·45	120	—	—
497	0·20	250	0·80	172	1·1	128	—	—	—	—
519	0·19	238	0·49	178	0·80	96	1·1	78	—	—
530	0·39	283	0·68	197	0·98	176	1·29	90	1·58	48
583	1·26	210	1·56	155	1·85	112	2·15	105	—	—

* N.R. = no response.

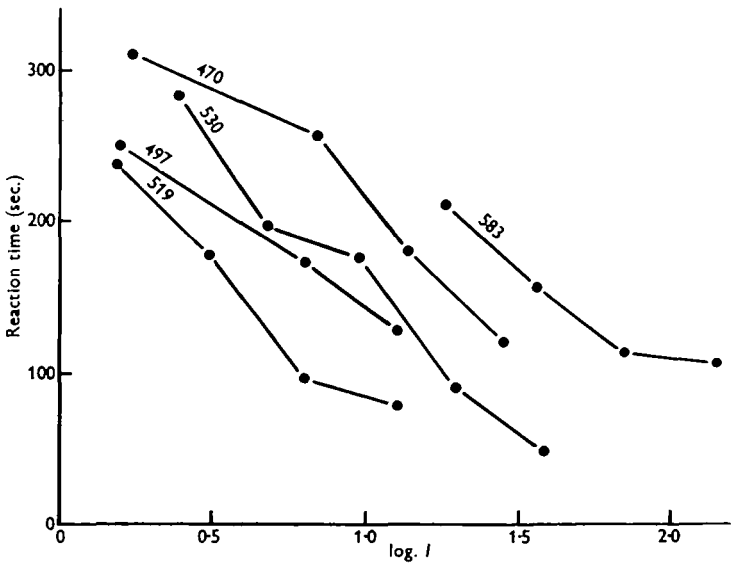

Fig. 6. Intensity/reaction time curves for animal no. 3 at different wave-lengths. The central wave-length in mμ is indicated for each filter beside the appropriate curve.

farthest to the left on the intensity axis (log. *I*), and the relative stimulating capacity of other wave-lengths is proportional to the distance they would have to be displaced along this axis in order to coincide with that for the most effective. Inspection shows, however, that although most of the curves have roughly the same slope, they are not sufficiently parallel to one another to permit them to be compared in this way. The alternative method is to estimate the relative effectiveness of different wave-lengths from the reciprocals of the values of log. *I* for several reaction times. This was done for reaction times of 150, 180, 210 and 240 sec., the results averaged, the reciprocal taken and sensitivity expressed once more as a percentage of the most effective wave-length. The result of this analysis is presented in Table 5.

Table 5. *Spectral sensitivity of three hags estimated from the intensity/reaction time data for different wave-lengths*

(The values for log. *I* are the means of the values for reaction times of 150, 180, 210 and 240 sec.; sensitivity is the reciprocal of log. *I* expressed as a percentage of the value for the most effective wave-length.)

Wave-length (mμ)	Hag no.					
	1		2		3	
	Log. *I*	Sensitivity (%)	Log. *I*	Sensitivity (%)	Log. *I*	Sensitivity (%)
430	1·08	41	0·79	21	—	—
470	0·78	58	0·80	34	0·89	38
497	0·45	100	0·27	100	0·61	70
519	0·67	68	0·61	45	0·43	100
530	—	—	—	—	0·79	55
555	0·58	77	0·68	39	—	—
583	—	—	—	—	0·72	31
595	1·51	29	N.R.*	N.R.*	—	—

* N.R. = no response.

The spectral sensitivies of the six hags vary considerably and illustrate one of the disadvantages of filters for this type of work. Since sensitivity has conventionally to be expressed as 100% at a given wave-length, a small difference in the observations may shift the maximum from one filter to another. Curves for single animals are therefore of little value, but considering the observations as a whole we may reasonably draw the following conclusions concerning *Myxine*'s spectral sensitivity:

(1) The most effective wave-length is between 500 and 520 mμ, since the most effective filter was either no. 603 or no. 604 (three hags in each case).

(2) Sensitivity decreases rapidly towards wave-lengths longer than the maximum. At 583 and 595 mμ about 10 times as much energy was required to produce the same effect as at the maximum. *Myxine* is virtually insensitive to wave-lengths longer than about 600 mμ.

(3) Sensitivity appears to decline less rapidly towards shorter wave-lengths, but observations at this end of the spectrum are too few to permit much freedom of speculation. At 470 mμ sensitivity seems to be about half what it is at the maximum.

Fig. 7, which represents the arithmetical mean of the spectral sensitivity curves of the six hags, may be considered as a summary of these conclusions. It is probably not greatly different from the true spectral sensitivity of *Myxine*.

Analysis of Vitamin A and carotenoids

Vitamin A is a component of all known photosensitive systems of higher animals, and other carotenoids are commonly associated with them. As no information seemed to exist on the occurrence and distribution of these substances in *Myxine*, two live hags weighing about 100 g. each were sent for analysis to Dr T. W. Goodwin of the University of Liverpool. He reported in a private communication (1952) that their combined livers contained 45 μg. and the rest of their bodies 110 μg. of vitamin A_1. They contained no other carotenoids and no vitamin A_2. If we

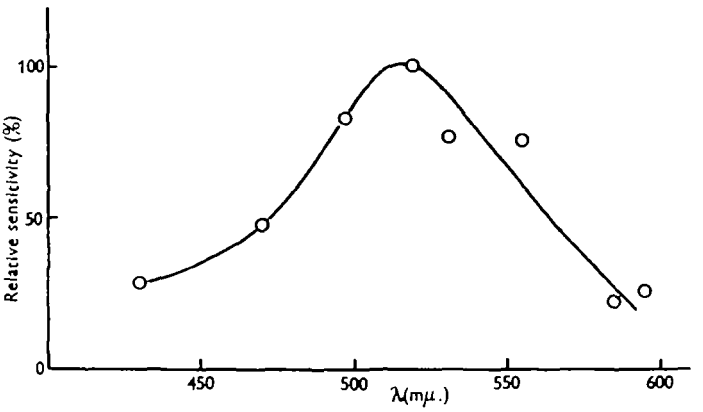

Fig. 7. Smoothed spectral sensitivity curve for six hags. Each point represents the mean value for all animals, calculated from the data presented in tables 3 and 5, and expressed as a percentage of the value for the most effective wave-length.

assume that the vitamin A was equally distributed between the two fish, each would have contained about 70–80/μg., of which about 20 μg. was in the liver. These quantities are small compared with the vitamin A content of many other fish, but it must be remembered that the hags had been kept in the laboratory for about 4 months, during which time they took no food. Although they appeared to be perfectly healthy and active up to the time they were killed, we might expect their reserves of the vitamin to have declined during this period. The normal vitamin A content of well-fed hags may therefore be much greater than these figures suggest.

DISCUSSION

The significance of the light reaction

Many of the anatomical peculiarities of hags, such as their lack of functional eyes and almost complete lack of integumentary pigment, suggest that they normally spend their lives in darkness, possibly in a marine equivalent of the cavernicolous environment. A general sensitivity to light such that illumination of almost any

part of the body surface may result in a motor response is a common feature of animals which inhabit these places, and has been studied by Hawes (1946) on the blind Urodele *Proteus*. To such animals a light sense of some kind is not, as Hawes suggested, a functionless evolutionary survival but a practical necessity and the only insurance they possess against wandering into illuminated places where they would be without protection from predators with image-forming eyes and normal vision. Newth & Ross have discussed this matter briefly, and suggest that the main role of *Myxine*'s light sense may be to keep it buried during daylight hours or to prevent it from migrating into shallow water. It seems worth while to pursue this idea a little further. If the hag's light sense plays any part in its life we might expect it to be related quantitatively to the level of illumination likely to be encountered at the depths where they are found in the sea. So far as is known hags are inhabitants of the continental shelf and appear to be confined to muddy bottoms. Gustafson (1935), who studied their habits in aquaria, found that they remain buried during the daytime but swim about freely and feed at night. If they behave in the same way in their natural habitat it follows that they must at least be able to distinguish night from day. Although they are taken sometimes from considerable depths, many known hag grounds are in quite shallow water between 10 and 50 m., where the illumination from the sea's surface during the day is by no means negligible. The illumination at any given depth is of course affected by many variables, such as the turbidity of the water, the disturbance of the sea's surface due to wave action, the altitude of the sun, latitude, amount of cloud and so on; but we need concern ourselves only with the amount of light energy which penetrates the surface and the fraction of it which is absorbed by each successive layer of water of unit thickness, a function usually expressed as the absorption or extinction coefficient per metre. The value of 120,000 lux, which is equivalent to 11,200 f.c. has been given by Sverdrup, Johnson & Fleming (1942) for the radiation penetrating the surface with a clear sky and sun at zenith. Assuming an overall absorption coefficient of 0·29 per metre for coastal water of average turbidity, the illumination at 30 m. depth will be 0·22 f.c. In clearer oceanic waters the same illumination might be encountered at 4 to 5 times this depth. Inspection of the intensity/reaction time curves (Figs. 2–5) shows that hags were activated within 3–4 min. by this intensity, which is therefore clearly within their effective range of perception.

Although these estimates are of necessity based upon arbitrary values for the amount of radiation and the turbidity of the sea, they certainly support the view that the hag's light sense is sufficiently acute to enable it to respond to the degree of illumination likely on the sea-bed in daytime at the depths in which they are commonly found. If it serves no other purpose this reaction has probably a high selective value, since it will have the effect of keeping the hag on the move until it finds itself on a suitable substrate into which it can burrow, or until it has found its way into deeper water.

Spectral sensitivity and the photochemical system

Although the data leave much to be desired, it is clear that the spectral sensitivity of the hag in its general features is similar to that of other marine animals, such as *Ciona*, *Pholas* and *Mya*, studied many years ago by Hecht. The most effective wave-length in every case is in the blue-green or green region of the spectrum: and sensitivity decreases towards both longer and shorter wave-lengths. It is axiomatic that the relative stimulating capacity of different parts of the spectrum is determined primarily by the energy absorbed by the photosensitive pigment responsible for the reaction, and the spectral sensitivity curve may therefore be regarded as an indirectly determined absorption spectrum of that pigment. Action spectra of this kind provide, in fact, the only information we possess on the photochemical systems of most animals, since our direct knowledge of the chemistry of visual pigments is confined to those of the image-forming eyes of Vertebrates, Cephalopods and some Arthropods. It is thought that in other animals the amounts of photopigment are too small or too diffusely scattered throughout the body to be detected by any of the techniques at present available. We can therefore only speculate on their photochemical systems by comparing the action spectra with those of animals possessing eyes and by analogy with those visual pigments whose chemical properties are known.

The spectral sensitivities of *Mya*, *Ciona*, *Pholas*, the ammocoete larva of *Lampetra* and the hag, differ from one another most obviously in the position of the maximum or most effective wave-length, which ranges from about 500 mμ in *Mya* to about 550 mμ in *Pholas*. This suggests that the photosensitive substances differ in detail, but are probably all of the same general type. Their relationship may well be similar to that of the different types of rhodopsin or visual purple, the best known photopigment of Vertebrate eyes. At one time rhodopsin was thought to be a single substance with well-defined physical and chemical properties, which were identical in all animals possessing it. The variations observed were thought to be due to impurities or to differences in the methods of preparation and analysis. It is now clear, however, that the position of the maximum of the absorption spectra of rhodopsin from different animals varies to some extent, and in Wald's (1953) words 'the term rhodopsin...therefore, like haemoglobin, designates a family of closely related substances'. This is due to the fact that the protein, or opsin, part of the photopigment affects the position of the absorption maximum to some extent and is a characteristic of the species. Among vertebrates this effect is rather small, only accounting for a range of about 5 mμ, but is greater in the case of rhodopsin from Cephalopods, and it seems reasonable to expect that the variation due to different proteins will be larger between animals that are more distantly related phylogenetically.

Most of the larger differences in absorption spectra are due to different prosthetic groups, and it is usual then to assign different names to the resulting pigments. The best known example of this is the case of rhodopsin and porphyropsin, which differ in the type of vitamin A in the chromophore, and whose absorption maxima lie

at about 500 and 522 mμ respectively. The absorption spectrum can also be affected by the various stereo-isomeric forms of vitamin A, or more correctly the corresponding vitamin A aldehydes, or retinenes. It seems too that photosensitive pigments, which differ chemically only in respect of their proteins but possess markedly different absorption spectra, may exist together in the eye of the same animal. Wald, Brown & Smith (1952) have recently found this to be the case in the chicken, the eye of which contains rhodopsin and another pigment, iodopsin, whose maximum lies at 562 mμ. The chromophore group of both pigments is the same, neo-retinene *b*, but the proteins are different.

It is clear, therefore, that photosensitive pigments with different spectral absorptions can be formed in a number of ways, and the range of variation of the maxima seems adequate to account for the differences between the spectral sensitivities of the animals with which we are concerned. It is indeed probable that the photoreceptor systems of all higher animals are constructed on the same pattern of a vitamin A-containing chromophore united with a specific protein.

This possibility opens up an interesting field of speculation on the evolution of photoreceptor systems. Their uniformity in all higher animals suggests that the basic chemical plan was fixed once and for all at a very remote time, certainly before the Vertebrates appeared on the scene, and that forces exist which have tended to keep them constant however much the animals may have evolved in other respects. The explanation may well lie in the spectral transmission of water, which is extraordinarily similar to the absorption spectrum of rhodopsin and to the relative sensitivity of various animals. Pure water is most transparent to wave-lengths between 400 and 600 mμ, while longer wave-lengths are progressively more strongly absorbed. Natural sea and fresh waters are more opaque, due principally to scattering of the light by small particles in suspension, and also deviate to some extent from pure water in their spectral transmission. Thus the maximum penetration of clear ocean water by light is at about 480 mμ in the blue region of the spectrum, while coastal waters are most transparent to green of about 530 mμ or even longer wave-lengths. In recent years several excellent series of measurements have been published of the transparency of sea water to light of different wave-lengths. The data are usually presented as extinction or absorption coefficients, but are more revealing for our purposes when the energy at each wave-length is expressed as a fraction of the energy at the wave-length to which the water is most transparent. The spectral distribution of energy at any depth can then be compared directly with the sensitivity curves of animals, which may be regarded as representing percentage absorption curves of a particular concentration of photosensitive pigment. Some of Utterback's (1936) figures for the average of several coastal and oceanic waters are expressed in this form in Fig. 8. They show clearly that the most effective wave-lengths in terms of their stimulating capacity are just those to which sea water is most transparent and vice versa. To emphasize this the values have been calculated for arbitrary depths of water so as to yield curves whose slope corresponds fairly closely with known spectral sensitivity curves. At greater depths the curves would be narrower and more sharply peaked, for shallower

water correspondingly broader, without however altering the position of the maxima.

The variation in spectral transmission of different types of sea water is certainly as great as the differences in spectral sensitivity of those animals which have been studied so far, and it is tempting to speculate that there may be a correlation between the ecology of an animal and its spectral sensitivity. If within the limits imposed by the general chemical properties of the system, selection can take place for a pigment of maximum effectiveness in any given environment, we might expect to find pelagic species from oceanic waters with maxima at rather shorter wave-lengths than the inhabitants of coastal seas. So far as I am aware, however, there is no experimental evidence that this is so. Bayliss, Lythgoe & Tansley (1936) attempted

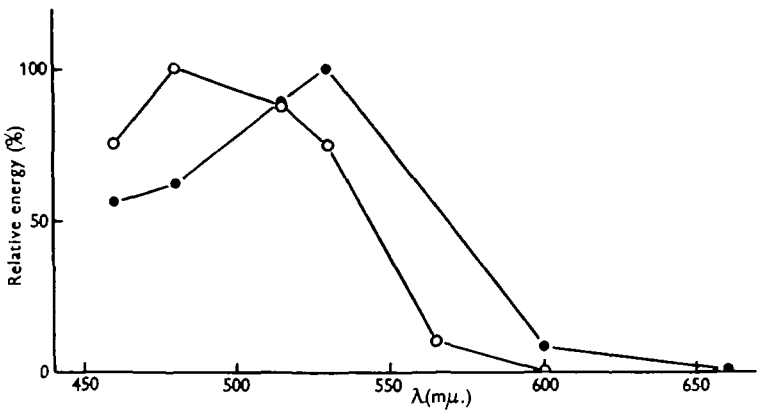

Fig. 8. The relative energy content of light of different wave-lengths penetrating oceanic and coastal waters. Open circles indicate average of ocean waters at 100 m.; filled circles average of coastal waters at 20 m. Data recalculated from Utterback (1936).

an investigation on these lines and found photosensitive pigments with maxima ranging from 505 to 545 mμ in the eyes of twelve species of fish. None of them were oceanic forms however, and new doubt has been thrown recently on the accuracy of their measurements by Kampa (1953), who found only rhodopsin in three of the species which were thought to possess pigments with the maximum at longer wave-lengths.

A further consequence of this hypothesis is that it can provide a reasonable explanation for the spectral properties of rhodopsin and the scotopic visibility curve of terrestrial Vertebrates, of which one of the most puzzling features is the virtual insensitivity to wave-lengths longer than about 600 mμ. There seems no obvious reason why the visual sense of a terrestrial animal should be limited in this way, but it is clearly adapted to the aquatic environment in which it was first evolved. On this ground alone there is good reason to consider rhodopsin and its variants as the 'primitive' visual pigment of all higher animals.

SUMMARY

1. The response of the hag to light consists of one or more local movements followed after a further interval by general locomotory activity. The first local movement has been used as a measure of the reaction time.

2. The reaction time is inversely proportional to the intensity of the stimulus at illuminations less than about 10 e.f.c. At higher levels of illumination it attains a constant minimum value. Hags respond to intensities at least as low as 0·1 e.f.c. but only after several minutes illumination.

3. Estimates of the penetration of light through sea water suggest that the hag's light sense is of functional value.

4. The spectral sensitivity maximum lies between 500 and 520 mμ. Hags are virtually insensitive to wave-lengths longer than about 600 mμ.

5. The significance of the spectral sensitivity is discussed in relation to the spectral transmission of sea water and the evolution of photosensitive systems.

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