SOME EVIDENCE FOR COLOUR-BLINDNESS IN *OCTOPUS*

BY J. B. MESSENGER, A. P. WILSON AND A. HEDGE

*Department of Zoology, University of Sheffield
and Stazione Zoologica, Naples*

INTRODUCTION

In this paper we put forward evidence from two kinds of experiments that suggests very strongly that octopuses are colour-blind.

Colour-vision in cephalopods has received intermittent attention in the last fifty years, and, using different genera and different experimental techniques, several workers have arrived at different conclusions; their results are summarized in Table 1.

With the exception of Orlov and Byzov, and of Hamasaki, all the authors are guilty of one or more of three serious errors: failure to take into account the spectral sensitivity curve of the subject, failure to control for the difference in brightness between test objects, and, in the behavioural experiments, inadequate quantification of results, which are presented without conventional statistical analysis. All this earlier evidence, therefore, is suspect.

In 1961, in a paper that seems to have been overlooked, Orlov and Byzov reported an electrophysiological investigation of the isolated cephalopod retina and found evidence that a given ERG can be produced by light of different wavelengths if compensations for brightness differences are made; and by using the method of colorimetric substitution they were able to change from one illuminating wavelength to another without affecting the ERG. They did not find a Purkinje shift. This was apparently true for a squid, *Ommastrephes sloanei-pacificus*, as well as for the large Pacific octopus, *Octopus dofleini* (Orlov & Byzov, 1961, 1962). Later Hamasaki (1968a, b) examined intact *O. briareus* and *O. vulgaris* and found that the dark-adaptation curve was monotonic, with no evidence of the discontinuity normally associated with a dual retinal system; again there was no evidence of a Purkinje shift, suggesting there is only one receptor system in the octopus retina.

Meanwhile Hubbard and her collaborators (Hubbard & St George, 1958; Brown & Brown, 1958; Kropf, Brown & Hubbard, 1959) had characterized a photopigment, rhodopsin, in several cephalopods; for *O. vulgaris* rhodopsin see Fig. 1. Then, in 1965, Hara and Hara found a second pigment in the cephalopod retina – retinochrome, whose peak absorption (490 nm in *O. vulgaris*) was close to that of rhodopsin (see also Hara & Hara, 1967; Hara, Hara & Takeuchi, 1967). It is not clear whether this pigment occurs in the receptors, however (see Discussion). Morphologically Young (1962a) had already recognized several different types of retinal cell in *Octopus*, while Hagins (1965) distinguished two types in the squid retina. Taken together these findings suggest that the cephalopod retina could possess a system for detecting wavelength.

Now octopuses are excellent subjects for learning experiments (see Wells, 1966,

Table 1. *History of research into colour-vision in cephalopods*

| Date	Author	Species	Method	Conclusion
1910	Hess	*Sepia officinalis*	Pupillary response	Colour-blind
1914	Fröhlich	*Eledone moschata* *Octopus vulgaris* *O. macropus*	ERG isolated eyes	Colour-vision
1914	Piéron	*O. vulgaris*	Learning experiment	Colour-blind
1917 a, b	Goldsmith	*O. vulgaris*	Learning experiment	Colour-vision
1921	Mikhailoff	*Eledone moschata*	Learning experiment	Colour-vision
1926	Haan	*Octopus vulgaris*	Learning experiment	Colour-blind
1929–1950 (see Kühn, 1950)	Kühn	*O. vulgaris* *Sepia officinalis*	Learning experiment	Colour-vision
1961 1962	Orlov & Byzov	*Octopus dofleinei* *Ommastrephes* *sloanei-pacificus*	ERG isolated retina	Colour-blind
1968 a, b	Hamasaki	*Octopus vulgaris* *O. briareus*	ERG intact animal	Colour-blind
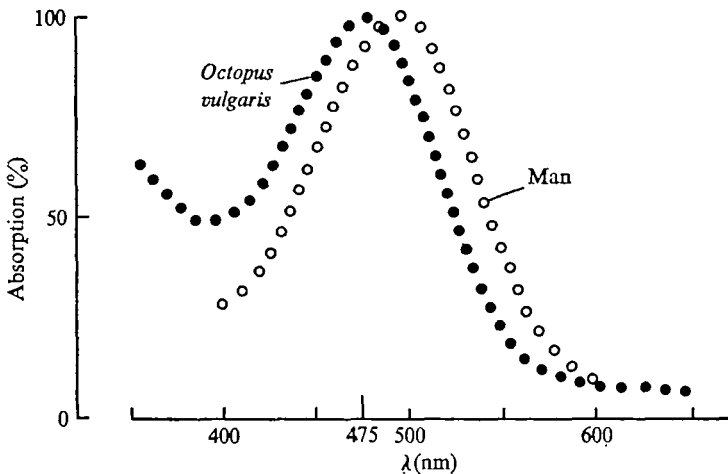
Fig. 1. Absorption spectra for *Octopus vulgaris* and human rhodopsins. The λ_{max} lies further over into the blue in octopus, as is usual in marine animals, but the shape of the curve is very similar to man's. The apparent increase in sensitivity at very short wavelengths is almost certainly not biologically significant. Redrawn to the same scale from data in Crescitelli & Dartnall and in Kropf, Brown & Hubbard.

for a brief survey of work by Boycott, Sutherland & Young) so it was decided to re-examine this problem at the level of the intact animal by testing, in a conventional experimental situation, whether discrimination-learning was possible on the basis of differences of hue. Since octopuses exhibit well-defined nystagmus and an opto-motor response in a striped background (Packard & Lucey, 1964; Packard, 1969) we also performed experiments to test whether such responses occurred with a back-ground of coloured stripes.

MATERIALS AND METHODS

Subjects were experimentally naïve *O vulgaris*, caught in the Bay of Naples.

For discrimination training, over a hundred animals of about 250–400 g were kept singly in white plastic tanks (100 × 50 × 50 cm with water to a depth of 40 cm) and fed on crabs. Experiments were carried out under fluorescent strip-lighting (40 W, 50 cycles about 1 m above the water level); this gave illumination of approximately $2 \cdot 5 \times 10^3$ lux (Table 2 below), which is not likely to be very much less than the illumination we can assume penetrates to areas that octopuses habitually hunt in around Naples (Woods, 1965; Blaxter, 1970).

Healthy octopuses that were readily attacking crabs were selected for experiment. They were *pre-trained* to the extent of showing them the positive shape for the first phase of the experiment six times and feeding them for attacking it. *Training* was by successive presentation of a vertical Perspex rectangle (10 × 2 cm) on a transparent Perspex rod, moved up and down by hand about three times per second. An attack on the positive shape was rewarded by a small piece of sardine (0·5 g): an attack on the negative was followed by a 10 V a.c. shock. Sixteen trials per session (at intervals of about 5–10 min) were presented in quasi-random order; there was one session per day. Trials lasted 30 sec, or were terminated at the time of an attack. Feedings were equalized at the end of each session. *Preference tests*, carried out on separate groups of animals, were similar but not reinforced (see below).

Red, green and blue hues were prepared with enamel paint; they were equalized for brightness by adding white or black paint until, when viewed by a human subject through red, green or blue filters respectively (Kodak Wratten No. 25, $\lambda_{max} \geqslant 700$ nm; No. 58, λ_{max} 530 nm; No 47 B, λ_{max} 430 nm) they appeared equally bright. Neutral density filters were used to compensate for the different transmission values of these filters. The three hues were thus approximately matched for physical brightness, as black and white photography with a panchromatic film confirmed. If octopuses were colour-blind the actual appearance of any one hue to the animals would depend on the spectral sensitivity curve (Fig. 1); presumably the green shape would *appear* slightly brighter than the red, but if so this did not show up in training experiments or in the preference tests (below). For each hue three arbitrary brightnesses were prepared: 'light', 'medium' and 'dark'.

For the nystagmus experiments fourteen young *O. vulgaris* (0·6 g to 16 g) were used; they were kept separately in small plastic boxes and fed on *Sphaeroma*. Small animals were chosen because of ease of handling and because their eye movements are conspicuous.

The nystagmus apparatus (Messenger, 1970) enabled the background and/or the animal to be rotated in either sense. Illumination was provided by a light source centred about 50 cm above the animal in the drum so that shadows were eliminated. For standard tests a 60 W (50 cycle) bulb was employed; a photo-flood lamp (500 W) was sometimes used for filming. Tests were also carried out in direct sunlight at noon, after tilting the apparatus to reduce shadows. The levels of illumination in all these situations are shown, in photopic units, in Table 2. The drum was 35 cm in diameter and the stripes were 5 cm wide (subtending an angle of 9° 30′); they were revolved at speeds of between 5°/s and 25°/s. Black and white stripes were used together with

Table 2. *Levels of illumination*

Conditions	Source	Illuminance*
'Dim' light (nystagmus)	Night; 40 W bulb shaded in adjacent room	< 1 lux
Learning experiments	40 W strip light	$2 \cdot 5 \times 10^2$ lux
'Standard' light (nystagmus)	60 W bulb	6×10^2 lux
	500 W flood	6×10^3 lux
'Bright' light (nystagmus)	Sun at noon†	$7 \cdot 5 \times 10^4$ lux

* Measured by meter (B. Lange, Berlin).
† In May at Naples.

a series of coloured and grey papers produced by Color Aid Co. (Messrs Longford & Hill Ltd., of Warwick Street, London W. 1). This company lists the reflectance for each paper; but, to overcome the problem of the differential spectral sensitivity of the octopus, papers likely to be suitable were first compared by a dark-adapted human subject (cf. the relative sensitivity of octopus and (scotopic) human absorption spectra, Fig. 1) and the hues matching closest for brightness were then tested on octopuses in pilot experiments. In this way we rapidly found two series of coloured papers that elicited almost no visual responses from an octopus and we could then make a full experiment (see Discussion). The combinations of papers employed, and their characteristics, are set out below in Table 4.

RESULTS

(1) *Discrimination learning*

Preference tests ($N = 28$)

Octopuses may show a marked preference for certain attributes of training shapes (Messenger & Sanders, 1972) so that it was necessary to test animals for this with the shapes being used in these experiments. Vertical rectangles were always used because, when such shapes are moved up and down, they are strongly preferred (Sutherland & Muntz, 1959) and can be expected to yield a high number of attacks. To test for possible preferences for brightness and/or hue we presented the animals with each training shape either twice (for the one-cue shapes) or four times (for the two-cue shapes) in a single session of preference training. The order of presentation followed a mirror-image design to balance any habituation effects; attacks were not reinforced but about 0·5 g of fish was given *between* trials, which lasted 30 sec or were terminated on attack.

Sixteen naïve animals were tested with the one-cue shapes; another twelve naïve animals were tested with the two-cue shapes. The latter group was given a second test 24 h later. The results are set out in Table 3. From this it is clear that none of the shapes is preferred, and analysis of variance of individual attacks gives, for the one-cue shapes, $F = 0 \cdot 67$; d.f. $= 7/120$; $P > 0 \cdot 05$; and for the two-cue shapes (first session) $F = 0 \cdot 42$; d.f. $= 3/44$; $P > 0 \cdot 05$. It is noteworthy that the level of attack by the latter group during the second preference test has fallen sharply, but evenly.

It seemed safe to conclude on the basis of these tests that preferences for individual shapes would not influence performance during training.

Table 3. *Preference tests*

Shapes in the one-cue experiments (*N* = 16)

	Dark			Light			
Black	Blue	Green	Red	Blue	Green	Red	White
19	23	22	22	23	26	26	19

The total number of unrewarded attacks is shown. Maximum possible on each shape is 32.

Shapes in the two-cue experiments (*N* = 12)

Test no.	Dark		Light	
	Blue	Green	Blue	Green
1	41	40	37	42
2	26	27	31	30

The total number of unrewarded attacks is shown. Maximum possible on each shape is 48.

Table 4. *One-cue discrimination: the experimental design*

Group	N	Stage 1. Brightness (4 sessions)		Stage 2. Hue (6 sessions)		Stage 3. Black/white (2 sessions)	
		Positive	Negative	Positive	Negative	Positive	Negative
I	7*	Dark blue	Light blue	Dark blue	Dark red	Black	White‡
II	9*	Light red	Dark red (black)†	Light red	Light green	Black	White
III	15*	Medium green	Light green	Medium green	Medium blue	Black	White

* Deaths and escapes made group sizes unequal; we aimed for 10, 10 and 16 subjects respectively.
† Sessions 1 and 2 dark red; sessions 3 and 4 black in error.
‡ One session only.

One-cue experiments (N = 31)

To begin with we ran three experiments designed to test the octopuses' capacity for discriminating between blue, green and red. First we tested blue versus red (*N* = 7) and red versus green (*N* = 9) and then, with a larger group (*N* = 15), green versus blue. Because octopuses readily make brightness discriminations, we set up an internal control for each group so that the animals had first to make a brightness discrimination and then a hue discrimination. To anticipate the results somewhat, we found that they never did discriminate between hues so that we were forced to include another brightness discrimination to ensure that the poor performance on hues was not the result of some extraneous factor. The plan of the experiment, then, was to train octopuses on a brightness, then a hue, then a black/white discrimination as is shown in Table 4. It will also be seen from the table that the positive shape for the first stage of the experiment was retained as the positive for the second stage; and that we tested for hue discrimination at all three arbitrary brightness levels. The results of such training are shown in Fig. 2. It is at once clear from this that although octopuses learn to make brightness discriminations under the conditions of this experiment they do not learn to discriminate between two hues of the same brightness.
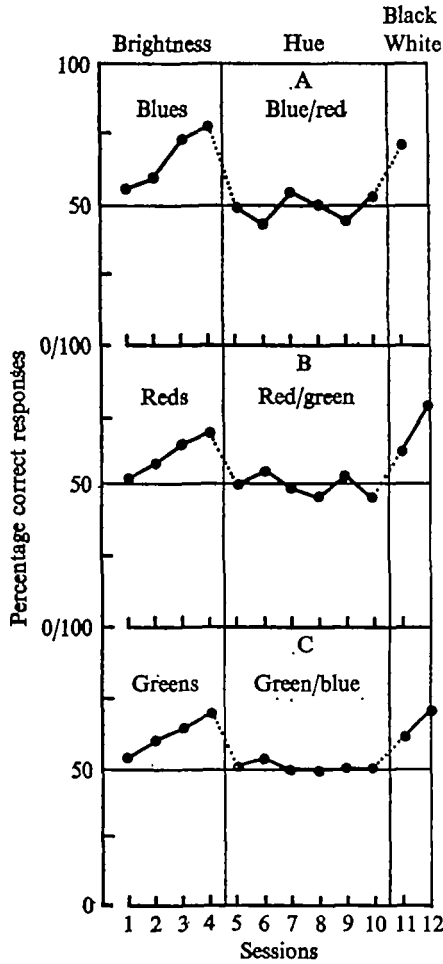
6

Fig. 2. One-cue experiments showing that octopuses that discriminated between brightnesses (left-hand and right-hand regions) did not discriminate between hues (centre). On session 4 learning performance was always significant at the 2% level (or better) (for Group A, N = 7, T = o; for B, N = 9, T = o; for C, N = 14, T = 3) as it was again on session 11 (for A and B, N = 7, T = o; for C, N = 10, T = o).

Consider the blue/red discrimination (A) prefaced by the dark blue/light blue discrimination. After only four sessions the group is making 79% correct responses on the brightness discrimination, a highly significant performance ($N = 7$; $T = 0$; $P < 0.02$; Wilcoxon matched-pairs signed-ranks test); but over the next six sessions (sessions 5–10) the performance varies about the chance level. In no one session – nor over all six sessions – does the score differ significantly from chance. Yet when the animals are given another brightness discrimination (black/white) they immediately (session 11) score 70% correct responses, again a highly significant performance ($N = 7$; $T = 0$; $P < 0.01$; Wilcoxon test). These considerations apply to the other two discriminations also (see Fig. 2).

The failure to discriminate between hues in this experiment can be shown in another way, by considering the *level of attack* on the positive and negative shape
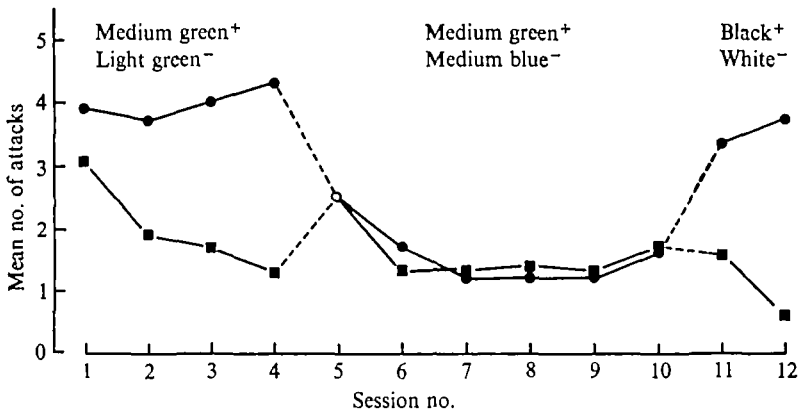
Fig. 3. Attack levels for one group during the discrimination shown in figure 2 (C). (● attacks on the positive, ■ attacks on the negative shape). Maximum on the ordinate, not shown, would be 8.

(Fig. 3). Consider the green/blue discrimination (C) prefaced by a medium green/light green discrimination. To begin with, attacks on both shapes are reasonably high, but the attack levels rapidly separate as the animal learns to make the brightness discrimination; attacks on the negative shape fall off. Yet when a hue discrimination (medium green from medium blue) is offered the attack levels become indistinguishable and remain so for six sessions. On reverting to a brightness discrimination, the attack levels on the two shapes again diverge; consider the change from session 10 to 11.

Two-cue experiments $(N = 42)$

The experiments just described show that in conditions where octopuses readily made discriminations on the basis of brightness they did not do so on the basis of hue. This does not automatically mean that hue cannot be distinguished. Suppose we were to test this more subtly, by seeing whether group performance on a brightness discrimination could be made better by adding hue as a second, relevant cue? If octopuses attend to both cues (Sutherland & Mackintosh, 1971; Messenger & Sanders, 1972) we should expect the group performance to be better than if only one cue were available for learning ('cue-additivity').

Accordingly, forty-two naïve octopuses were divided randomly into three equal groups and trained to discriminate between dark/light and blue/green, thus:

Group 1: brightness relevant, hue irrelevant (light+ versus dark−).
Group 2: hue and brightness relevant (light blue+ versus dark green−).
Group 3: hue relevant, brightness irrelevant (blue+ versus green−).

The results are set out in Fig. 4. It is at once clear that the scores of Groups 1 and 2 are identical. Indeed there is no significant difference overall ($t = 0.21$; d.f. $= 26$; $P > 0.05$) between the performance of these two groups (measured as per cent correct attack). Both learn readily and by the fourth session are discriminating at about the 80% level. On the *first* session, however, the scores of these groups do not differ significantly from each other or from those of Group 3 ($F = 0.95$; d.f. 2/39; $P > 0.05$); performance is at chance level. And Group 3 continues to perform ran-
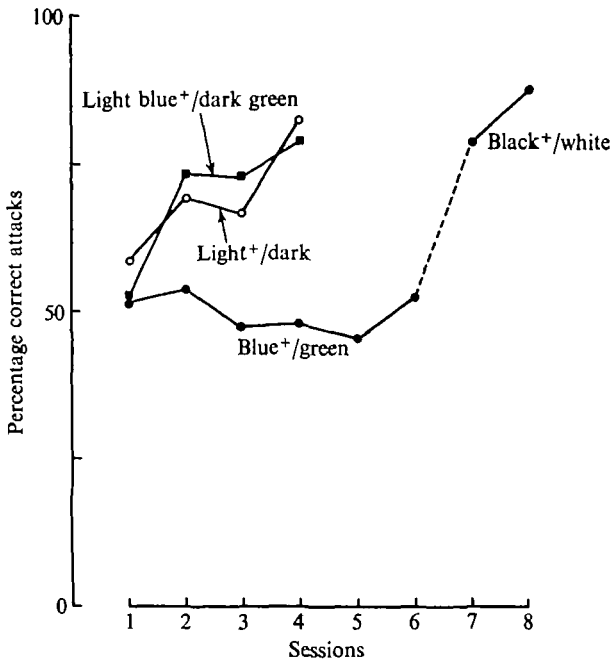
Fig. 4. Two-cue learning (1) with brightness only relevant (O); (2) with both cues relevant (■); and (3) with hue only relevant (●). Groups 1 and 2 scores are identical.

domly for *six* sessions, by which time the level of attack is so low that a black and white discrimination has to be substituted for the hue discrimination. At once (session 7) the animals show learning at the 80 % level and this score improves to the 87 % level in the next session (Fig. 4).

The implications are clear: in this experiment none of the animals is attending to hue. Group 3 comprises another fourteen octopuses that no more discriminated blue from green than did the fifteen animals in the earlier, one-cue experiment. Since Group 2 scores are not significantly greater than Group 1 scores, we need assume that Group 2 animals are discriminating only in terms of brightness. And if the performances of individual animals are looked at there is no evidence that any animal is making a hue discrimination. Finally, we transfer-tested six animals after they had learned the discrimination light blue positive/dark green negative. These animals made significantly more attacks on the white transfer shape than on the black ($t = 5·29$; d.f. $= 10$; $P < 0·001$) but attacked medium blue and medium green shapes equally ($t = 1·53$; d.f. $= 10$; $P > 0·05$). Attacks on transfer shapes were not rewarded.

(2) *Nystagmus and the optomotor response*

The fact that the octopuses in our training experiments did not discriminate between hues does not necessarily mean they are colour blind. Apart from the shortcomings of our relatively crude experimental procedure it could be that the octopus eye can detect differences in wavelength but that this information is not available for learning. To examine whether wavelength is detected peripherally we set out to see whether

Table 5. *Characteristics of papers employed in the nystagmus experiments*

Stripes	Color Aid	Wavelength[*]	Brightness[†]
Orange/blue	YO hue	*ca.* 575 nm	—
	BV 1	435 nm	—
Striped grey	Grey 3A	—	31%
	Grey 6	—	13%
	Grey 3	—	26%
	Grey 6A	—	14%
Plain grey	Grey 7A	—	11%
Mixed hues:			
Blue	BV 1	435 nm	—
Cyan	BG hue	500 nm	—
Green	G hue	519 nm	—
Red	R 2	*ca.* 625 nm	—
on a grey ground	Grey 5	—	15%
Reds	R hue } *ca.* 625 nm		{ 13%
	R 4		{ 44%
Cyans	BG hue } 500 nm		{ 10%
	BG 3		{ 31%

* Peak transmission by reflectance spectrophotometry (Unicam SP 735); peaks were not well defined for YO, or for R.
† Reflectance according to Color Aid specification.

octopuses showed nystagmus or optomotor responses, with stripes of widely differing brightness and with stripes of the same subjective brightness but of different wavelength. This gives the animal opportunity for making simultaneous comparison of hues, incidentally (see Discussion).

Definitions. Both *Octopus* and *Sepia* make visually induced, compensatory eye movements (Packard & Lucey, 1964; Packard, 1968; Collewijn, 1970; Messenger, 1970). In the classic situation, where an octopus is surrounded by a revolving black-and-white-striped cylinder, these compensatory movements show up (1) as nystagmus, with its slow ('drift') component in the same sense as the rotation of the drum and its fast ('flick') component in the opposite sense; (2) as movements of the head, in the same sense as the drum rotates; and (3) as movement of the whole animal often about its own axis, again in the same sense. In this account we define an *eye movement* as the 'flick' of nystagmus, and an *optomotor response* as movement of the head or the whole animal.

The combinations of Color Aid papers employed, and their characteristics are set out in Table 5.

Experiment 1 (N = 10)

In the first experiment we exposed octopuses in the nystagmus apparatus to a series of seven backgrounds: black/white; mixed hues; striped grey; plain grey; striped grey; orange/blue; black/white. With each background the procedure was the same: both the animal and the background were rotated, then the background alone, and then the animal alone, first clockwise, then anticlockwise (see Table 6).

The tests generally followed one another without a break and lasted 15 sec. During this period the number of eye movements was recorded as well as the number of seconds during which optomotor responses occurred.

Table 6. *Individual performance in a nystagmus experiment*

	Background												
	Black and white		Mixed hues		Grey stripes		Plain grey		Grey stripes		Orange/ blue		Black and white	
Test	Flicks	OMR	Flicks	OMR	Flicks	OMR	Flicks	OMR	Flicks	OMR	Flicks	OMR	Flicks	OMR
Both CW	1	—	—	—	2	—	—	—	—	—	—	—	—	3
Screen CW	6	10	—	—	2	11	—	—	—	15	1	3	3	15
Animal CW	—	14	—	—	3	12	—	—	2	14	1	—	5	9
Both ACW	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Screen ACW	6	12	—	—	4	13	—	—	2	15	—	—	4	10
Animal ACW	2	12	—	—	1	14	—	—	—	13	—	—	—	13
Total	15	48	0	0	12	50	0	0	4	57	2	3	12	50

Animal II/2 (12. v. 72). CW = clockwise; ACW = anticlockwise; 'Flicks' = no. nystagmic movements/15 sec; 'OMR' = no. seconds showing optomotor responses/15 sec.
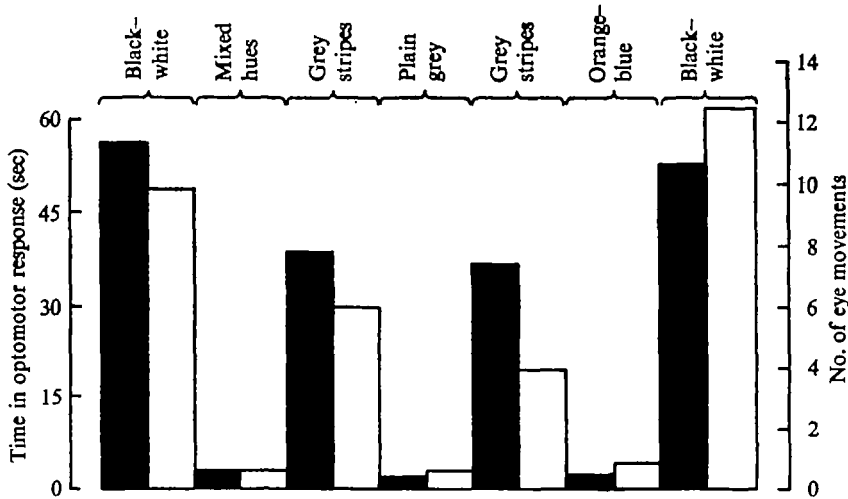

Fig. 5. Histogram showing the amount of time in optomotor response (black; left-hand ordinate) and the number of eye movements (white; right-hand ordinate) with the different backgrounds indicated. Mean of ten individuals.

A typical individual response is shown in Table 6 and the mean performance of ten animals is plotted as a histogram in Fig. 5. This diagram speaks for itself: under the conditions of the experiment the eye movements and optomotor responses that occur with stripes of a different grey or of black and white do not appear with coloured backgrounds, *which are treated by octopuses as a plain grey background*. There are, of course, some eye movements and the occasional turn of the head, but it is always difficult to eliminate all such movements by a 'plain' background (Collewijn, 1970; Messenger, 1970); and as there was no significant difference ($F = 0.19$; d.f. $= 2/27$; $P > 0.05$) between the number of eye movements made to the mixed, orange/blue or plain grey background, this may be regarded as background noise. There were, however, highly significant differences between performance with all these backgrounds and with grey stripes ($F = 8.70$; d.f. $= 4/45$; $P < 0.001$) or black-and-white stripes. (The latter elicited significantly more attacks than the striped greys incidentally, $F = 3.87$; d.f. $= 3/36$; $P < 0.05$.)
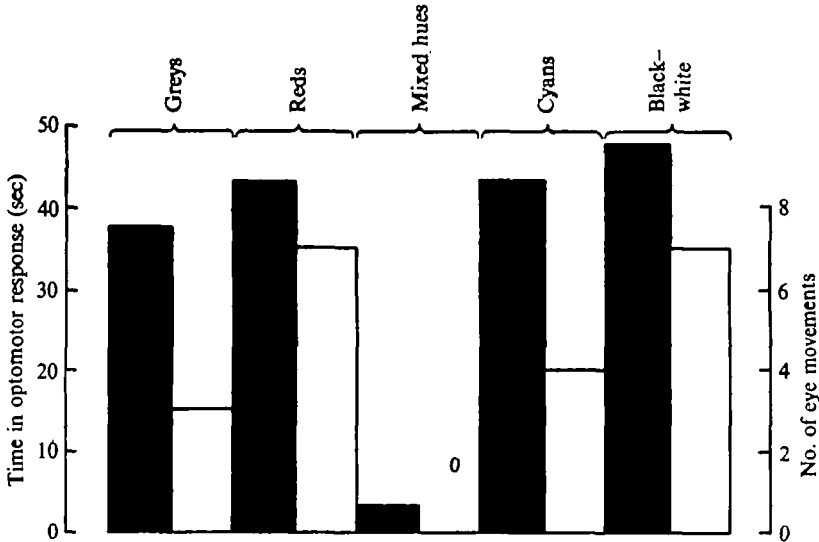
Fig. 6. The mean performance of three animals in a similar test with light and dark reds and cyans. Conventions as in Fig. 5.

Experiment 2 (*N* = 3)

To show that octopuses give nystagmic responses to different intensities within a relatively restricted waveband three animals were tested under exactly the same circumstances, and with the same procedure, in a sequence of backgrounds that included light and dark reds and light and dark cyans:

grey stripes; reds; mixed hues; cyans; black/white.

The results were unequivocal and are shown in Fig. 6. Octopuses respond equally well to backgrounds that differ in brightness (the differences being about 100 % for black/white, 31 % for reds, 21 % for cyans and 18 % for greys) but again they do not respond when there are only differences in hue.

Yet it is possible to elicit visual responses from the orange/blue or mixed-hue backgrounds, by interposing suitable monochromat filters between the light source and the drum. With neutral density filters no such effect appears, but with a Wratten filter 47B or 25 which similarly lower the intensity of illumination, nystagmus and optomotor responses are made. The former is a blue filter that 'bleaches' the blue (BV) stripes; the latter is a red filter that 'bleaches' the red (R) or orange (YO) stripes. Through lack of suitable filters we were unable to test this for the cyan and green stripes, but the point we wish to emphasize is that with the illumination used there was sufficient energy to elicit a visual response provided that we selectively altered the *relative* brightness of the stripes.

Experiment 3 (*N* = 3)

The experiments so far described were carried out under electric light giving illumination slightly above that likely to prevail at the surface of the sea at dawn and dusk (Blaxter, 1970). At noon, however, the illumination level even under water may be much higher, especially in shallow (sub)tropical seas. To test whether there is a colour-detecting system in *Octopus* operating at higher photopic levels we tested three

Table 7. *Nystagmus in bright light*

Animal no.	Black and white		Mixed hues		Plain grey		Orange/blue		Grey stripes	
	Flicks	OMR	Flicks	OMR	Flicks	OMR	Flicks	OMR	Flicks	OMR
II/1	6	56	1	2	0	0	0	2	5	32
II/2	0	26	0	4	0	15*	0	2	2	38
II/3	4	54	0	1	0	12	0	4	0	34
Total	10	136	1	7	0	27	0	8	7	104
Mean	3·3	45·3	0·3	2·3	0	9	0	2·7	2·3	33·3

$N = 3$; 29. v. 72 noonday sun; * would not settle; abbreviations as Table 6.

octopuses with an abbreviated version of Expt 1 under noon-day sun after 30 min light adaptation. In these conditions animals were extremely restless and it was deemed advisable to curtail the experiment especially as results from the first three animals all agreed and told the same story (Table 7). Responses were made to stripes differing in brightness, but when the stripes differed only in hue the octopuses remained immobile and gave no eye movements. It is interesting that two animals actually made more responses to 'plain' grey than to the hues.

Experiment 4 $(N = 2)$

Finally, two octopuses were dark-adapted for 30 min and tested at night when there was just enough light in the background to enable the observer to see the animal. This was to preclude the unlikely possibility that octopuses have a unique visual system that shows scotopic colour vision. There was no evidence for this: while black and white stripes elicited weak nystagmus, orange/blue and mixed stripes did not.

DISCUSSION

We have been considering evidence from two kinds of experiment. The first asked the question: is information about the wavelength of light available as a basis for discrimination learning? The second asked: is information about wavelength used peripherally, to regulate a visual reflex? The answer to both questions seems to be no, but it is impossible to prove a negative and there are in any case a number of caveats that must qualify the conclusions of these experiments.

It is obvious, for example, that in the one-cue learning experiments the octopuses get very limited experience of the test shapes. Octopuses must attack to learn, yet training with these hues was only for six sessions, representing a *maximum* of $(6 \times 16) = 96$ trials. But in fact the octopuses attacked much less than this as the discrimination became difficult and indiscriminate attacks resulted in shock. Fig. 3 shows that these octopuses were making only about *three* attacks per session, i.e. about 20 trials in all. Yet in their recent experiments Daw & Pearlman (1970) needed 1500 trials to show colour vision behaviourally in the cat, where there has also been a history of con‑ flicting opinions. Of course the very reason for curtailing the training was the low attack level, so to meet this criticism we decided to run another group of animals without shocking, so that the attack level would be kept up. Unfortunately this was combined with another new feature, also designed to make the discrimination easier

for the octopus: simultaneous presentation of the shapes (Sutherland & Muntz, 1959). This combination proved unsatisfactory, however, as strong side preferences developed without any shocking, so that the large group that started training effectively dwindled to two animals only. Nevertheless, there was no evidence after eleven sessions (176 trials) that these animals could discriminate red shapes from yellow. This, incidentally, is a discrimination that also proved impossible in a pilot experiment ($N = 8$) with successive training.

Admittedly then, a long-term experiment is still required before we can be certain that the octopuses cannot be trained to discriminate between hues. Yet what characterizes octopuses, and makes them most useful subjects for learning experiments (cf. Messenger & Sanders, 1972), is that when they do learn they learn extraordinarily rapidly. This is shown here in both experiments. After only *four* sessions all octopuses in the one-cue experiment are making brightness discriminations at a level significantly above chance; similarly, in the two-cue experiment Groups 1 and 2 are scoring at the 80% level by this stage. In the one-cue experiment we can note again that when octopuses revert to a black/white discrimination (after failing to make a hue discrimination) they score significantly above chance *after only one session* (16 trials) (Fig. 2).

This last fact emphasizes that there is no reason to suppose that the animals in the experiments were sick and that whatever the defects of the experimental situation it enables learning to develop (and to be expressed) very rapidly indeed where the discriminands are discriminable. In short, octopuses do not learn about wavelength differences under conditions where they rapidly learn about brightness differences.

This is also true if we consider the two-cue experiments (Fig. 4). Adding hue as a relevant, second cue made no difference to the group performance (Group 1 scores and Group 2 scores were indistinguishable) and where hue alone was relevant (Group 3) there was no learning. Under similar circumstances Muntz & Cronley-Dillon (1966) were able to demonstrate relatively rapid learning in the goldfish with its well-developed colour vision. We also have evidence from another group ($N = 7$) that when octopuses trained on the discrimination dark blue positive/light green negative are given the new discrimination dark *green* positive/light *blue* negative their scores remain the same or improve, which would be expected if they were attending only to brightness. Again we are forced to the conclusion that if colour is perceived by an octopus it is relatively unimportant. Incidentally Sutherland (see Sutherland & Mackintosh, 1971) might object to our two-cue experiment just because of this. According to him, cue-additivity only appears in a group when the cues are of approximately equal strengths to the animal, a point we had overlooked in designing our experiment.

In summary, although our learning experiments may not have been subtle enough, especially as wavelength seems to be unimportant, they show that information about wavelength is probably *not* made available to higher centres in the brain for learning, despite the claims of earlier workers like Goldsmith (1917 *a*, *b*), Mikhailoff (1921) and Kühn (1950).

Yet it could be that there is a peripheral system for wavelength detection; the retina may respond to wavelength though it does not make this information available for learning. Wells (1961) has shown that a situation like this occurs with proprio-

ceptive information detected in the arms in *Octopus*; and Neuhaus (1963) claims this
is true of olfactory information in birds. Accordingly, we investigated the optomotor
responses of octopuses. The establishment of colour vision on the basis of nystagmus
is questionable of course, for eye movements may be induced by brightness differences,
but if there is *no* nystagmus with differently coloured stripes we have strong evidence
for colour-*blindness*.* This is assuming that information about the relative movement
of hue is fed into the optomotor control system; it *need* not be, nor is it apparently in
some flies that are known to have colour-vision (Kaiser, 1968). There was, in any case,
an element of circular argument in our experimental procedure here for in practice
we could only match two hues for brightness by seeing whether the octopus failed to
respond to them in the nystagmus drum. Empirically we found the best Color-Aid
papers to test by dark-adapting and viewing them ourselves, but inevitably the
differences between the human and octopus eye caused some discrepancies; it was
interesting that we had great difficulty matching colours for the octopus in the green–
yellow range, which is what might be expected since the rhodopsin extinction curve
is steepest in this part of the spectrum (Fig. 1). With a complete range of papers it
would doubtless be possible to find a match in this region.

With the two series of Color-Aid papers selected, however, our results with
octopuses were remarkably consistent (Fig. 5). Again and again coloured stripes were
treated like plain grey. Qualitatively, a most dramatic experiment was to have con-
centric paper backgrounds and remove the inside one while the drum was rotating.
If we removed a black/white and exposed a coloured ground optomotor responses
stopped. If we removed a coloured to reveal a black/white ground they started.

It is interesting that the performance with grey stripes was lower than with black
and white stripes. Four shades of grey were used (because of shortage of suitable
papers); the maximum brightness difference between these shades was 18%, the
minimum 2%. Now physiologically Orlov & Byzov (1961) showed that the *Octopus*
eye cannot reliably discriminate between a brightness difference of less than 8%.
This relatively crude sensitivity to brightness agrees with the difficulty encountered
in training octopuses to discriminate between greys (Messenger & Sanders, 1971)
though it is difficult to reconcile with the excellent tone-matching of the background
by the chromatophore system (see below).

The backgrounds that elicited no response from octopuses were also tested with
two teleosts (*Mugil* sp. and *Serranus cabrilla*) in an experimental sequence like that
in Expt 1. Both genera gave frequent eye movements and some optomotor responses
to the coloured stripes though few to the plain grey background.

Finally, the design of the nystagmus experiments was such that responses to
intensity were called for – and made – late in the sequence so that we can fairly
safely exclude sensory or motor fatigue as factors contributing to the absence of eye,
or body, movements with the coloured backgrounds. It seems that, since nystagmic
eye movements do not occur when stripes of the same brightness but different hue
move relative to the retina, the *Octopus* retina is probably incapable of detecting the
wavelength of light.

* A similar argument can be used against the objection that we employed paints, not light, in the
training experiments. These are indeed more difficult to match but, whatever small differences there
may have been between shapes apart from hue, the fact remains that octopuses *did not discriminate*
between them.

This finding, then, is in agreement with electrophysiological evidence from the isolated retina of *O. dofleini* (Orlov & Byzov, 1961, 1962) and from intact *O. briareus* and *O. vulgaris* (Hamasaki, 1968 *a*, *b*). The significance of the different types of retinal cell (Young, 1962 *a*; Hagins, 1965) remains a puzzle as does the function of Hara and Hara's (1965) second pigment, though perhaps, as the authors themselves claim, it may play an accessory role in the visual process 'acting as a direct supply of active retinal for the rhabdomes' (Hara, Hara & Takeuchi, 1967). In any case retinochrome seems to be located 'just behind the rhabdomes' (*loc. cit.*), certainly not in the rod outer segment so that it is almost certainly not a true visual pigment. It seems that as a working hypothesis we can regard octopuses, though not necessarily all other cephalopods of course, as functionally colour-blind. We must briefly consider the implications of this.

The demonstration that an animal is colour-blind may seem an uninteresting finding, even if it were absolutely conclusive and even if it resolved a long controversy. Cephalopods are remarkable animals, however, not least for their ability to change colour with breathtaking rapidity. In the littoral octopods and benthic sepioids (e.g. *Sepia, Sepiola*) some of these colour changes are for concealment, by cryptic coloration (combined with disruptive coloration together with distortion of the skin itself); and their superlative ability to match the background must be difficult to imagine for someone who has never watched them doing so. Some idea of it can be gauged from the colour plates in Packard & Sanders (1969). How could a colour-blind animal match its background? There seem to be at least three methods used in the skin of *O. vulgaris*:

(1) *The transmission of reflected light.* This applies only to very young animals, whose skin, particularly in the web and along the arms, is very thin and transparent. In simple experiments with coloured stones it is easy to demonstrate that the predominant wavelength reflected off the background will be transmitted through the skin, especially if all the chromatophores are contracted.

(2) *The reflexion of incident light.* The body skin of cephalopods contains *leucophores, iridiocytes* and *chromatophores* (Packard & Sanders, 1971). The leucophores contain a creamy-white substance that acts as a good reflector of incident light, and it is simple to show that white or monochromatic incident light is reflected very well from the octopus body, presumably from these structures. This is especially obvious after the chromatophores have been made to contract experimentally by sectioning the mantle connective unilaterally; that side of the body becomes very pale and takes up the background coloration by reflexion much better than the intact, darker side does. Thus an octopus has only to contract its chromatophores to reveal a reflecting system that could automatically match the background. The iridiocytes in the skin also reflect light and so probably contribute to this effect. They also give 'structural' colours by interference. How important this is in the mantle skin is not known, though elsewhere these structures may form very important reflecting layers – e.g. in the iris, or, in *Sepia*, on the belly (Denton & Land, 1970).

(3) *The reflexion of incident light after absorption.* In *Octopus* there are two or three kinds of chromatophores, which are elastic sacs containing pigments in the yellow–orange–red (black) range of the spectrum. Thus, when they are expanded and incident white light falls upon them, they strongly absorb the shorter wavelengths. These sacs

can be expanded differentially so that, by a kind of *pointillist* effect, black, orange, red and yellow dots can be produced. These will be combined by the eye of an observer to produce a variety of colours especially in the red–yellow region of the spectrum to match the brown, rocky background. Furthermore, this system of dots of different intensities permits excellent *tone-matching*, which contributes hugely to the success of the cryptic 'coloration'. What must be emphasized (cf. Packard & Sanders, 1971) is that the 'observers' for whom these chromatophore pigments were designed in evolution were probably the teleost fish, animals with colour vision that prey upon cephalopods and that evolved contemporaneously with them. Indirect evidence for this is the fact that known *intraspecific* communication in cephalopods is mediated by a message framed in terms of brightness differences; in *Sepia* the 'stars-and-stripes', and in *O. horridus* the stripes, of the mating display (Young, 1962b). (See also Packard, 1972).

We are quite ignorant of the processes that control chromatophore activity. Perhaps octopuses select an appropriate pre-programmed display from a large repertoire, on the basis of information about the intensity of the background gained by a colour-blind eye; so far we have not tested this experimentally. Yet it is certain that octopuses can match a background by a combination of devices: transmission, passive reflexion, and reflexion with differential absorption. The first two processes operate automatically if the chromatophores are 'switched-off' and give good matching for wavelength. The third may act principally by producing a good intensity match, which could be achieved with information from a colour-blind eye.

SUMMARY

1. Groups of *O. vulgaris* were trained successively to make brightness and hue discriminations with painted vertical rectangles. These were of approximately the same physical intensity and were equally preferred.

2. Using one-cue discrimination training we found that (*a*) Seven octopuses that discriminated between dark blue and light blue did not discriminate between dark blue and dark red shapes. (*b*) Nine octopuses that discriminated between light red and black did not discriminate between light red and light green shapes. (*c*) Fifteen octopuses that discriminated between medium green and light green did not discriminate between medium green and medium blue shapes.

All these octopuses later discriminated between black and white shapes.

3. Another group of octopuses was trained with two cues, one or both of which were relevant. The cues used were brightness (dark or light) and hue (blue or green). (*a*) Group 1 ($N = 14$) discriminated between dark and light shapes where hue was irrelevant. (*b*) Group 2 ($N = 14$) discriminated between dark blue and light green shapes. (*c*) Group 3 ($N = 14$) failed to discriminate between blue and green shapes where brightness was irrelevant.

Overall the scores of Groups 1 and 2 did not differ significantly.

4. Thus under experimental conditions where octopuses rapidly learned to make brightness discriminations they did not learn discriminations based on hue alone; even if colour is perceived, it seems a relatively unimportant cue for this animal.

5. Another group of octopuses ($N = 10$) was tested, in a special apparatus, for nystagmus and optomotor responses.

6. Such responses were always given when stripes of different brightness moved relative to the retina, but when stripes of the same subjective brightness but different hue moved across the eyes they rarely occurred. Such occurrences were no more frequent than the responses given to a plain grey background.

7. Similar results were obtained under conditions of illumination ranging from less than 1 lux to $7 \cdot 5 \times 10^4$ lux.

8. The absence of visual response to moving coloured stripes suggests that the failure of octopuses to learn a hue discrimination may be the result of an inherent incapacity at the retinal level. Together with other findings, which are discussed, this strongly suggests that *O. vulgaris* is colour-blind.

REFERENCES

BLAXTER, J. H. S. (1970). Light. In *Marine Ecology* (O. Kinne, ed.), 1, 213–85. London: Wiley.

BROWN, P. K. & BROWN, P. S. (1958). Visual pigments of the octopus and cuttlefish. *Nature, Lond.* 182, 1288–90.

COLLEWIJN, H. (1970). Oculomotor reactions in the cuttlefish, *Sepia officinalis. J. exp. Biol.* 52, 369–84.

CRESCITELLI, F. & DARTNALL, H. J. A. (1953). Human visual purple. *Nature, Lond.* 172, 195–7.

DAW, N. W. & PEARLMAN, A. L. (1970). Cat color vision: evidence for more than one cone process. *J. Physiol.* 211, 125–37.

DENTON, E. J. & LAND, M. F. (1971). Mechanism of reflexion in silvery layers of fish and cephalopods. *Proc. Roy. Soc. Lond.* B 178, 43–61.

FRÖHLICH, F. W. (1914). Beiträge zur allgemeinen Physiologie der Sinnesorgane. *Z. Sinnesphysiol.* 48, 28–164; Weitere Beiträge. *Z. Sinnesphysiol.* 48, 354–438.

GOLDSMITH, M. (1917a). Quelques réactions sensorielles chez le poulpe. *C. r. hebd. Séanc. Acad. Sci., Paris* 164, 448–50.

GOLDSMITH, M. (1917b). Quelques réactions du poulpe. Contribution à la psychologie des invertébrés. *Bull. Inst. gén. psychol.* 17, 25–44.

HAAN, J. A. BIERENS DE (1926). Versuche über den Farbensinn und das psychische Leben von *Octopus vulgaris. Z. vergl. Physiol.* 4, 766–96.

HAGINS, W. A. (1965). Electrical signs of information flow in photoreceptors. *Cold Spring Harb. Symp. Quant. Biol.* 30, 403–18.

HAMASAKI, D. I. (1968a). The electroretinogram of the intact anaesthetized octopus. *Vision Res.* 8, 247–58.

HAMASAKI, D. I. (1968b). The ERG-determined spectral sensitivity of the octopus. *Vision Res.* 8, 1013–21.

HARA, T. & HARA, R. (1965). New photosensitive pigment found in the retina of the squid *Ommastrephes. Nature, Lond.* 206, 1331–4.

HARA, T. & HARA, R. (1967). Rhodopsin and retinochrome in the squid retina. *Nature, Lond.* 214, 573–5.

HARA, T., HARA, R. & TAKEUCHI, J. (1967). Rhodopsin and retinochrome in the octopus retina. *Nature, Lond.* 214, 572–3.

HESS, C. (1910). Neue Untersuchungen über den Lichtsinn bei wirbellosen Tieren *Arch ges. Physiol.* 136, 282–367.

HUBBARD, R. & ST GEORGE, R. C. C. (1958). The rhodopsin system of the squid. *J. gen. Physiol.* 41, 501–28.

KAISER, W. (1968). Zur Frage des Unterscheidungsvermögens für Spektralfarben: Eine Untersuchung der Optomotorik der königlichen Glanzfliege *Phormia regina* Meig. *Z. vergl. Physiol.* 61, 71–102.

KROPF, A., BROWN, P. K. & HUBBARD, R. (1959). Lumi- and meta-rhodopsins of squid and octopus. *Nature, Lond.* 183, 446–50.

KÜHN, A. (1950). Über Farbwechsel und Farbensinn von Cephalopoden. *Z. vergl. Physiol.* 32, 572–98. (Includes earlier references to same author.)

MESSENGER, J. B. (1970). Optomotor responses and nystagmus in intact, blinded and statocystless cuttlefish (*Sepia officinalis* L.). *J. exp. Biol.* 53, 789–96.

MESSENGER, J. B. & SANDERS, G. D. (1971). The inability of *Octopus vulgaris* to discriminate monocularly between oblique rectangles. *Intern. J. Neuroscience* 1, 171–3.

MESSENGER, J. B. & SANDERS, G. D. (1972). Visual preference and two-cue discrimination learning in *Octopus. Anim. Behav.* 20, 580–5.

MIKHAILOFF, S. (1921). Expériences réflexologiques. Expériences nouvelles sur *Eledone moschata. Bull. Inst. Océan.* 398, 1–11.

MUNTZ, W. R. A. & CRONLEY-DILLON, J. R. (1966). Colour discrimination in goldfish. *Anim. Behav.* 14, 351–5.

NEUHAUS, W. (1963). On the olfactory sense of birds. In *Olfaction and Taste* (ed. Y. Zotterman). London: Pergamon.

ORLOV, O. YU. & BYZOV, A. L. (1961). Colorimetric research on the vision of molluscs (Cephalopoda). *Dokl. Akad. Nauk SSSR* 139, 723–5. (In Russian.)

ORLOV, O. YU. & BYZOV, A. L. (1962). Vision in cephalopod molluscs. *Priroda, Mosk.* 3, 115–18. (In Russian.)

PACKARD, A. (1969). Visual acuity and eye growth in *Octopus vulgaris* (Lamarck). *Monitore Zool. Ital.* 3, 19–32.

PACKARD, A. (1972). Cephalopods and fish: the limits of convergence. *Biol. Rev.* 47, 241–307.

PACKARD, A. & LUCEY, E. (1964). *Optomotor responses in cephalopods.* 16 mm colour film with sound track. Institute of Animal Genetics, Edinburgh.

PACKARD, A. & SANDERS, G. D. (1969). What the octopus shows to the world. *Endeavour* 28, 92–9.

PACKARD, A. & SANDERS, G. D. (1971). Body patterns of *Octopus vulgaris* and maturation of the response to disturbance. *Anim. Behav.* 19, 780–90.

PIÉRON, H. (1914). Contribution à la psychologie du poulpe; la mémoire sensorielle. *Année psychol.* 20, 182–5.

SUTHERLAND, N. S. & MACKINTOSH, N. J. (1971). *Mechanisms of Animal Discrimination Learning.* London: Academic Press.

SUTHERLAND, N. S. & MUNTZ, W. R. A. (1959). Simultaneous discrimination of training and preferred directions of motion in visual discrimination of shape in *Octopus vulgaris* Lamarck. *Pubbl. Staz. zool. Napoli* 31, 109–26.

WELLS, M. J. (1961). Weight discrimination by *Octopus. J. exp. Biol.* 38, 127–33.

WELLS, M. J. (1966). Learning in the octopus. *Symp. Soc. exp. Biol.* 20, 477–507.

WOODS, J. (1965). Octopus-watching off Capri. *Animals* 7, 324–7.

YOUNG, J. Z. (1962a). The retina of cephalopods and its degeneration after optic nerve section. *Phil. Trans. R. Soc. Ser.* B 245, 1–18.

YOUNG, J. Z. (1962b). Courtship and mating by a coral reef octopus (*O. horridus*). *Proc. Zool. Soc. Lond.* 138, 157–62.