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THE FUNCTION OF PHOTOMECHANICAL MOVEMENTS IN THE RETINA OF THE RAINBOW TROUT (*SALMO GAIRDNERI*)

BY R. H. DOUGLAS*

Department of Biology, University of Stirling, Stirling FK9 4LA, Scotland

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SUMMARY

1. The function of photomechanical movements in the retina of rainbow trout (*Salmo gairdneri*) was investigated by determining both the effect of light on the level of extractable visual pigment, and the electroretinographic b-wave sensitivity, during various stages of photomechanical light and dark adaptation.

2. Dark-adapted fish, light-adapted fish, and dark-adapted fish exposed to ten minutes direct sunlight had on average visual pigment concentrations of 100, 82 and 36% respectively.

3. The intensity of illumination required to bleach a specified amount of visual pigment in the light-adapted retina was found to be 1·29 log units higher than that needed to bleach the same amount of visual pigment in a dark-adapted eye.

4. The level of extractable visual pigment was observed to be relatively constant over natural twilight periods.

5. A close temporal correlation was observed between the time course of electroretinographic adaptation, measured by the b-wave sensitivity, and photomechanical changes.

6. All these observations tend to support the hypothesis that photomechanical movements serve, at least in part, to protect the rod visual pigment from overstimulation in the light-adapted retina.

INTRODUCTION

The retinal epithelial pigment, cones and rods of many lower vertebrates, including teleosts, undergo positional changes depending on the ambient lighting conditions. Ever since the discovery of these photomechanical, or retinomotor, movements attempts have been made to determine their adaptive significance. The great abundance of such hypotheses around the turn of the century led Arey (1915) to state that 'Many such explanations reveal the resourcefulness of the human mind rather than the ingenuity of nature.' Most of these early explanations, which have been summarised by Arey (1915), Detwiler (1943) and Ali (1971, 1975), however, can now

* Present address: Universität Ulm, Abt. Klinische Morphologie, 79 Ulm (Donau), Nebau Oberer Eselsberg, West Germany.

be discarded, and only the two major contending hypotheses will be further discussed here.

Herzog (1905) and Exner & Januschke (1906) saw retinomotor movements as a means of adapting the eye to photopic and scotopic vision. During daylight the cones are contracted and are thus the first receptors to intercept the light, while the rods are buried in the expanded pigment epithelium, and so protected from overstimulation by the high levels of illumination. Conversely, at night the cones, which do not function at low levels of illumination, expand and accompanied by retraction of the epithelial pigment leave the contracted rods free to receive the low intensity illumination.

The obvious advantage of such a system is that the receptors involved in vision at any one time are the ones primarily stimulated by the incident light. An additional advantage is that the rod visual pigment is protected from bleaching by the high levels of illumination during the day.

An alternative function for these retinal migrations was proposed by Garten (1907). He suggested that the ellipsoids (and oil droplets) of cone outer segments in teleosts, and the oil droplets in the cones of amphibians, reptiles and birds, would cause a lot of light to be refracted out of the cell. The pigment epithelium, interdigitating with the cones when light adapted, would serve to stop this stray light from stimulating neighbouring visual cells. This will serve to increase acuity, and the underlying rods will also be protected, minimising the bleaching of rhodopsin. Conversely, the retraction of the pigment epithelium and expansion of the cones during dark adaptation, coupled with the contraction of the rods, ensures maximal use of low level illumination. As the rods are no longer isolated from one another, any light escaping will stimulate adjacent receptors, thus serving to further increase sensitivity.

Despite these theoretical speculations, the precise function of the pigment epithelium in retinomotor movements is still uncertain, and all workers since Arey (1915) have noted that the diversity of retinal responses is so large that a single theory, that can account for all the observed species differences, is unlikely to exist (Arey, 1915; Detwiler, 1943; Ali, 1975).

At least a partial solution to this problem comes from the recognition that the two major contending hypotheses are not really mutually exclusive. In both theories the rods are protected from overstimulation when light adapted, and on dark adaptation the rods are positioned so as to make maximal use of the impinging illumination. The cone isolation when light adapted proposed by Garten (1907) is not excluded by anything said by either Herzog (1905) or Exner & Januschke (1906). The only real conflicting statement between the two theories concerns the position at twilight. Garten (1907) proposes that both the rods and cones are active at these intermediate levels of illumination, while Herzog (1905) and Exner & Januschke (1906) adhere to a theory of either rod or cone vision.

Up until the present time retinomotor function in vertebrates is an area where there have been very few critical experiments. Evidence for one theory or another has come mainly from theoretical speculation and incidental observations. The following paper outlines experiments carried out in the rainbow trout to investigate

one of the possible functions of the epithelial pigment, namely that it serves to protect the rod visual pigment from being bleached when the retina is light adapted.

Since the visual pigment in retinal rods can easily be extracted, it is possible to observe the effect of illumination on the visual pigment levels in various adaptive states. In this way it can be determined (1) if rods that are covered with pigment epithelium are less susceptible to bleaching than ones that are exposed, (2) how efficient the epithelial pigment is in its shielding role, and (3) if the visual pigment is actually protected in fish experiencing natural conditions of illumination.

The function of photomechanical changes was further investigated by monitoring electroretinographic sensitivity changes during both light and dark adaptation and relating these to the position of the retinal elements. A close temporal correlation between photomechanical adaptation and sensitivity changes would indicate the involvement of retinomotor movements in determining retinal sensitivity.

METHODS

All fish (*Salmo gairdneri*), which ranged in size from about 10 cm in Expts 1–3 to 18 cm in Expt 4, were obtained from Howietoun fisheries, Bannockburn.

The positions of the cones and pigment epithelium are expressed as cone and pigment indices, respectively. Rods were not examined. The procedure for fixation and measurement are described in the preceding paper (Douglas, 1982).

Visual pigment extraction and analysis

It is usual when extracting visual pigment to separate the retina from the rest of the dark-adapted eye, and use it alone for extraction. This was avoided in the present study as pigment was extracted from light- as well as dark-adapted animals. When dark adapted, rods are easily separable from the epithelial pigment as this is aggregated at the back of the eye. When light adapted, however, the pigment completely surrounds the rods, and any attempt made to separate the two would result in the loss of a substantial proportion of the rods. Pigment was therefore extracted using digitonin from complete, macerated, hemisected eyes.

The resulting extracts were analysed using a Cecil CE 505 double beam UV spectrophotometer and absorption spectra constructed for the pigment before and after a 10 min tungsten light bleach. The amount of pigment in an extract is represented by the maximum change in density between the bleached and unbleached pigment.

Variations in the amounts of pigment due to different sizes of eyes were corrected for by calculating the surface area of each eye (assuming the retina is a hemisphere) through measuring the diameter of each eye before extraction. All eyes sampled at the same time were pooled and the resulting maximum pigment density divided by the sum of the surface areas. This figure is taken as representing the relative concentration of pigment in that sample.

Electroretinograms

Following anaesthesia in 0·45% urethane, the fish was placed in a Perspex holder and kept moist by damp tissue paper. A grounding electrode was placed around the

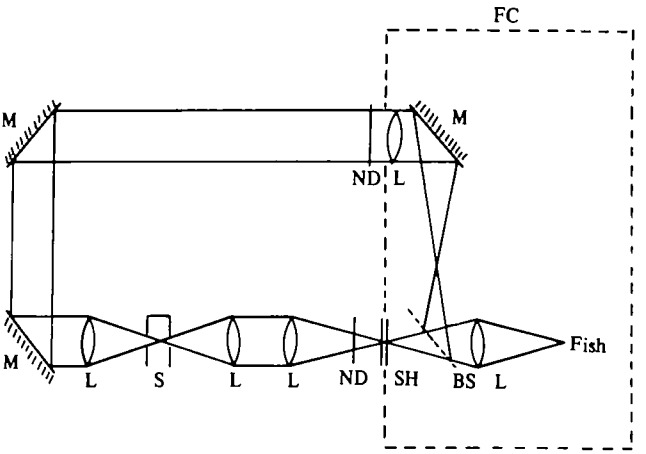

Fig. 1. Optical system used for stimulating the eye when recording the electroretinogram. M, Mirror; L, lens; S, light source; ND, neutral density filter; SH, shutter; BS, beam splitter; FC, Faraday cage.

body, and a urethane solution kept circulating over the gills. The holder was then placed in position in the optical system (Fig. 1). Fish treated in this way survived for up to 3 h without serious deterioration of the response.

As temperature affects both the photomechanical movements and the electroretinographic response to flickering and flashing stimuli, it was recorded throughout the experiments.

The active and reference electrodes were placed on the cornea of the stimulated and unstimulated eye respectively. In this way potentials, such as heart beat, were picked up by both electrodes and cancelled out, and only the electroretinogram (ERG) was recorded on a storage oscilloscope. Both electrodes were cotton wicks, soaked in teleost Ringer's, surrounded by chloride coated silver wire and encased in a glass tube to facilitate positioning. The fish, electrodes, and immediately surrounding optics were enclosed in a Faraday cage.

The stimulus and adapting background were identical rectangular fields superimposed on one another at their focal points, supplied by the Maxwellian view system shown in Fig. 1, from the same 24 V 150 W tungsten iodide projector bulb (colour temperature 3120 °K). The intensity of the stimulus was controlled by Wratten neutral density filters. The stimulus was delivered by opening a shutter.

EXPERIMENTAL DESIGN

(1) *Extractable visual pigment levels in light-adapted, dark-adapted and bleached eyes*

Three groups of three fish were exposed to different conditions of illumination:

Group (1) – $3\frac{1}{2}$ h in direct sunlight (approximately 5×10^4 lx).
Group (2) – $3\frac{1}{2}$ h in darkness over the same period as group (1).
Group (3) – as group (2) followed by 10 min exposure to sunlight.

After each treatment, one eye from each fish was fixed in Bouin's fixative for histological examination while the visual pigment was extracted from the other. For extraction of the pigment the three eyes of each group were treated as a single unit as described above ('Visual pigment extraction and analysis'). This experiment was carried out twice at the same time of day.

(2) *Degree of rod protection afforded by the epithelial pigment*

Preliminary experiments showed that the total amount of pigment extracted from the two eyes of a given individual differed on average by 13%, in both the light and dark adapted state. It was therefore possible to measure the effect of various intensities of bleaching light on the amount of visual pigment in individual eyes, and express this as a percentage of the total amount of visual pigment in the unbleached eye of the same individual.

Following either 2 h light or dark adaptation the fish were killed, and their eyes hemisected after removal from the orbit. This and all subsequent manipulations were carried out using a dim red torch. One eye was then placed directly into McIlvanie's buffer, while the other was first exposed to a bleaching spot of light, which completely filled the retina, for 45 s. The visual pigment from both eyes was then extracted. By varying the intensity of the spot the amount of visual pigment in both light- and dark-adapted eyes exposed to different degrees of bleaching was determined. This, expressed as a percentage of the total visual pigment content, gave the relationship between the intensity of the bleaching light and the amount of pigment bleached.

(3) *Extractable visual pigment levels during twilight*

Fish were collected from the fish farm 4 h before the first animals were sampled at dusk, and placed in tanks, supplied with running water, outdoors, away from any artificial lighting. At intervals during the subsequent fall and rise of natural illumination fish were killed, and one eye immediately immersed in Bouin's fixative. The other was placed in a Polypropylene bottle, wrapped in tin foil, and submerged in liquid nitrogen. The extractable visual pigment was removed from the frozen eyes at a later date, while the former were used in the histological determination of the position of the retinal elements. At all times during the experiments the only artificial lighting came from a dim red torch.

During the two dusk periods investigated three fish were sampled at approximately 15 min intervals (see Figs 4, 5 for details), while during the single dawn followed on three occasions only one fish was sampled and at the others only two.

The levels of illumination at the times of sampling were obtained from U.S. Naval Natural Illumination charts (1952).

(4) *Relationship between electroretinographic sensitivity changes and photomechanical movements during light and dark adaptation*

Following either light (650 lx at the water surface) or dark adaptation for 3 h, fish were transferred to the experimental set-up and left undisturbed in the apparatus for 10 min. The light was then either turned on or off (time 0). For light adaptation

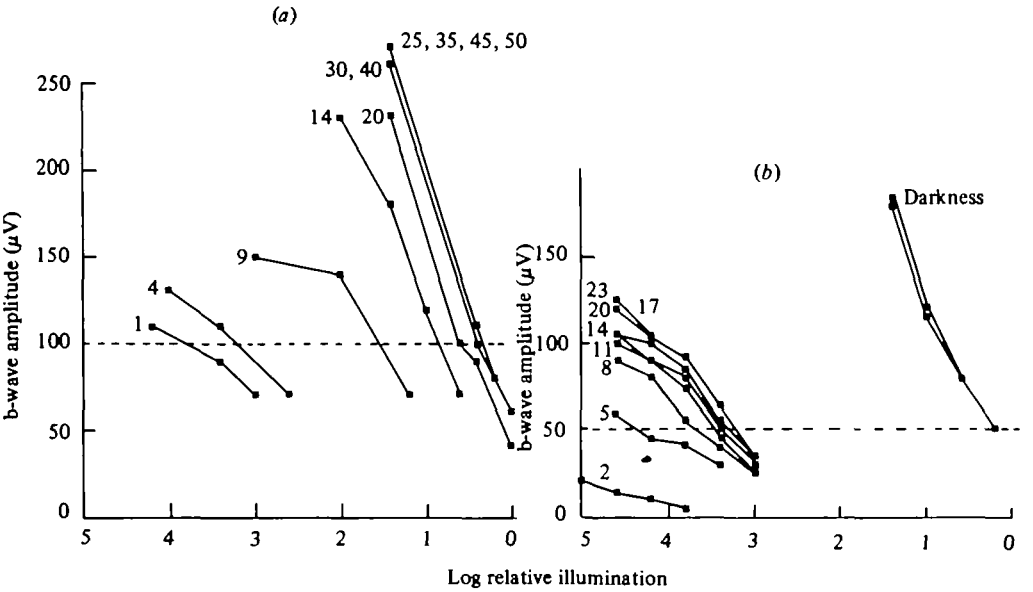

Fig. 2. Relationship between b-wave amplitude and stimulus intensity at various times during dark (*a*) and light (*b*) adaptation. The numbers on the lines indicate the length of time (minutes) that the fish have been adapting.

the eye was exposed to a background illumination of 12 lx. Light intensities were measured using a U.D.T. 40x optometer.

At intervals after the beginning of adaptation the eye was stimulated by a series of 0·2 s stimuli of differing intensities. Each set of presentations took approximately one minute to complete. In this way a series of response (b-wave amplitude) vs intensity curves at different times during adaptation was obtained for each fish (e.g. Fig. 2*a*, *b*). From such a group of curves the intensity of stimulation necessary to elicit a specified amplitude of b-wave was determined (100 μV during dark adaptation, and 50 μV during light adaptation).

A preliminary study showed that urethane had no effect on the movement of the retinal elements, confirming the early observation of Arey (1916). It was therefore possible to follow the retinomotor movements of fish exposed to exactly the same conditions as those experienced by fish during the electroretinographic determination of sensitivity changes during adaptation. Both groups of fish were treated in an identical fashion, even to the extent of putting electrodes on the eyes of fish later used for histological sectioning. A different number of fish were killed at various stages during adaptation and all index values at any one time averaged (light adaptation; 3 fish killed at time 0, 5 and 20; 4 at 10 and 25, 6 at 15 and 2 at 30, dark adaptation; 3 at time 0, 10 and 20, 2 at 25 and 30, and 1 at 40, 50 and 60).

RESULTS

(1) The amounts of extractable visual pigment in the three groups of fish are shown in Table 1. The fish that had been in the dark for the whole time, and therefore not exposed to any bleaching light, contained most pigment (100%), although

Table 1. *Visual pigment levels in light-adapted (group 1), dark-adapted (group 2) and bleached (group 3) eyes in Expt 1*

(The concentration of visual pigment is given as optical density change $mm^{-1} \times 10^3$. Each figure is the total amount of pigment from three eyes.)

Treatment of fish	Concentration of visual pigment, Expt 1	Concentration of visual pigment, Expt 2	Average concentration of visual pigment
Light adapted	0·600	0·610	0·605
Dark adapted	0·624	0·852	0·738
Dark adapted & 10 min bleach	0·261	0·265	0·263

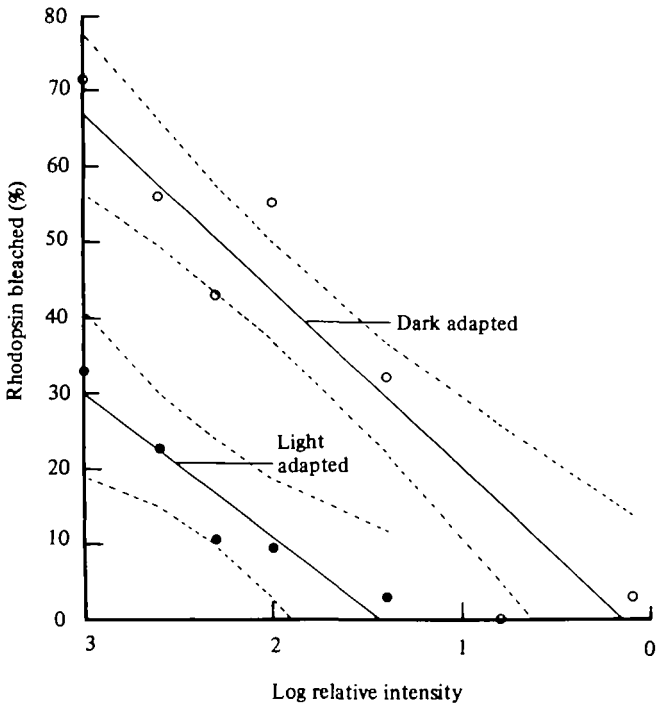

Fig. 3. Percent of rhodopsin bleached in light- and dark-adapted hemisected eyes by a 45 s bleach of varying intensity. The filled circles (●) represent the average values for light-adapted eyes and open circles (○) those of dark-adapted eyes. The dashed curves (---) are the 95 % confidence limits of the regression lines. Dark adapted $y = -23{\cdot}62x + 67{\cdot}2$, light adapted $y = -19{\cdot}23x + 30{\cdot}02$.

fish that had been in the light for 3½ h, and whose rods were therefore covered with epithelial pigment, had on average only slightly less (82%). Whereas, dark-adapted fish whose exposed rods were subjected to 10 min of bleaching had considerably less, with only 36% as much visual pigment relative to the dark-adapted controls.

The histological results confirm that fish kept in the light and dark only, are respectively totally light and dark adapted in terms of their photomechanical changes. Group (3) eyes, which were only in the light for 10 min, were also light adapted. This is to be expected, however, as in the laboratory, under weaker light adaptation

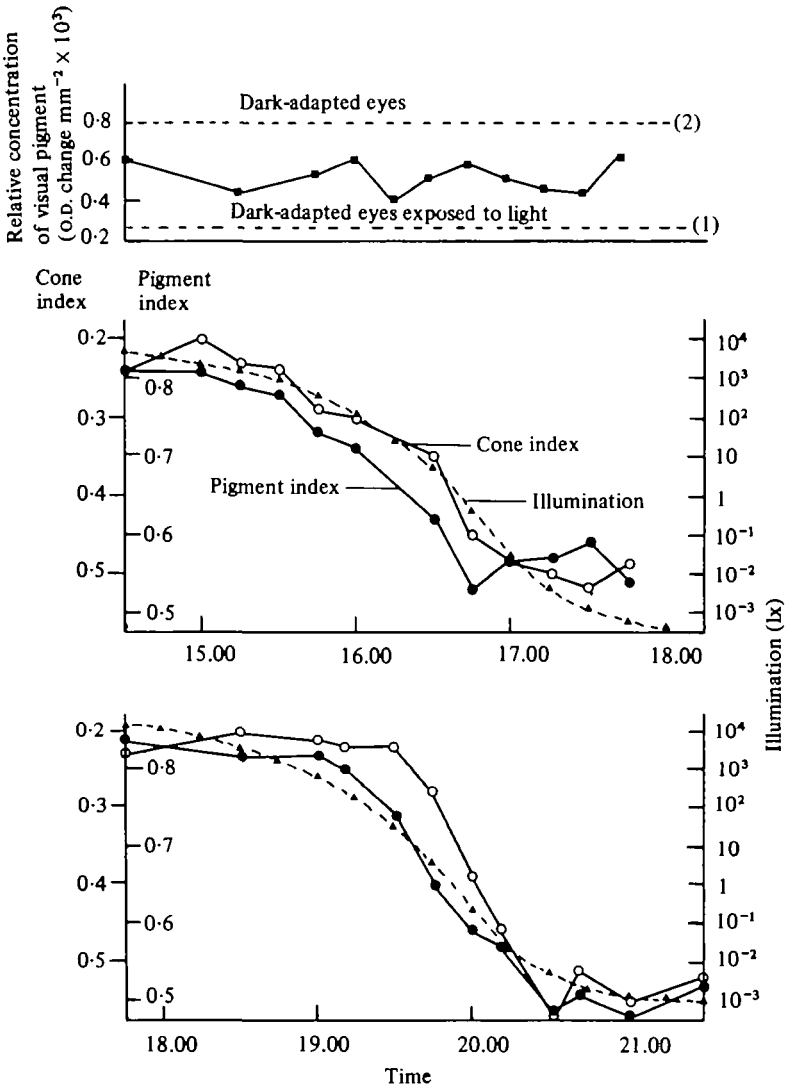

Fig. 4. Average concentration of extractable visual pigment at equal intensities during two dusk periods. The concentration of visual pigment represents the average concentration in three eyes at various intensities during two dusk periods. The position of the retinal epithelial pigment and cones on the two occasions are plotted below these. The retinal pigment index is shown by the filled circles (●), the cone index by the open circles (○), and the intensity of the light by the broken line and filled triangles (▲---▲). (1) and (2) are the levels of visual pigment found in dark-adapted eyes followed by a 10 min bleach and fully dark-adapted eyes respectively, as described in the text.

(650 lx), the retinal elements take only 15 min to light adapt (Douglas, 1980). Group (3) were, however, fully dark adapted when first exposed to the light as they came from the same tank and were removed the same time as fish in group (2).

(2) The efficiency of the photomechanical movements in shielding the rods can be determined from Fig. 3, which shows the percentage of visual pigment bleached

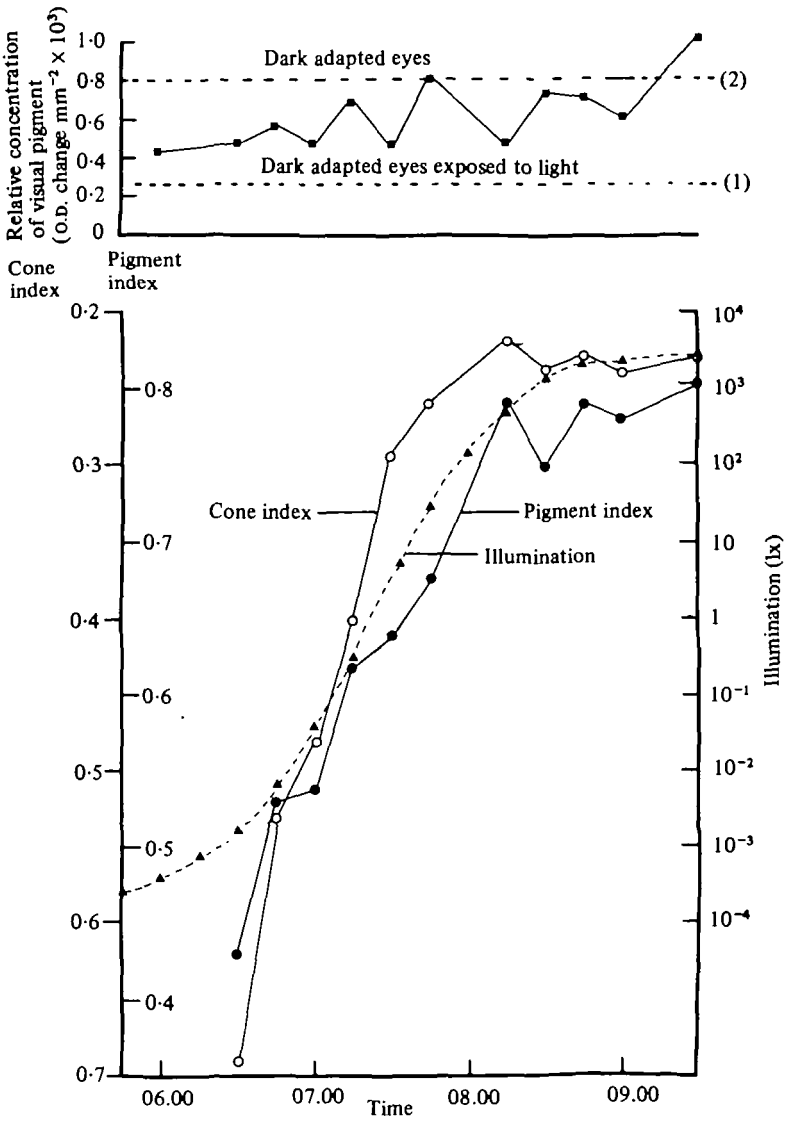

Fig. 5. Relative concentration of visual pigment at various intensities during dawn. See Fig. 4 for details of the symbols.

by different intensities of light in both light- (●) and dark-adapted (○) eyes. A straight line has been fitted to the points by regression.

Figure 3 shows that a given light intensity bleaches more pigment in dark-adapted eyes than in light-adapted ones. A measure of the degree of protection given by photomechanical movements is indicated by the amount the light-adapted line must be transposed along the abscissa in order to differ minimally from the dark-adapted curve. As there was no significant difference between the slopes of the light- and dark-adapted lines (see Fig. 3 caption for the regression equations) this is simply a matter of determining the difference between the intercepts on the abscissa. This gives a 'protection factor' of 19·5 (1·29 log units).

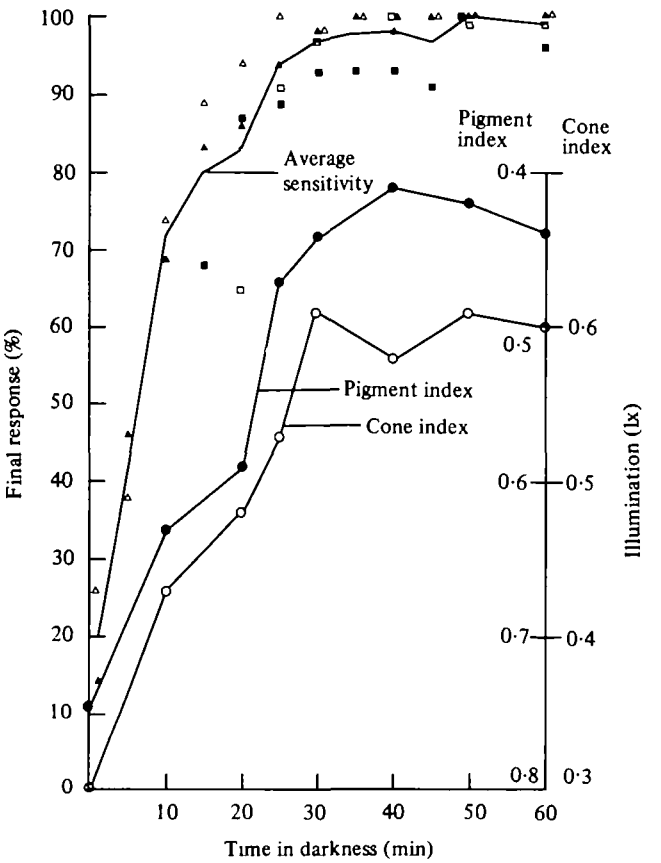

Fig. 6. Sensitivity changes of four fish, as measured by the ERG b-wave, with associated retinomotor changes, during dark adaptation. The results of four individuals have been averaged as described in the text. Each of the four symbols (▲, △, ■, □) represents an individual fish. The retinal pigment index is given by the filled circles (●) and the cone index by the open circles (○).

(3) This experiment examined the protective ability of the epithelial pigment during natural twilight periods. Fig. 4 shows the average amounts of visual pigment obtained at equal light intensities during the two dusk periods investigated. The extraction results are plotted above histological results at the times corresponding to these intensities. Not all values of the amount of visual pigment extracted are included in this figure, as the same intensities were not sampled in both experiments.

Results for a single dawn are shown in Fig. 5. The large variation in the visual pigment content is probably due in part to the relatively small number of fish sampled.

In both Figs 4 and 5, (1) represents the amount of visual pigment in dark-adapted eyes following 10 min exposure to full daylight, and (2) is the amount of pigment in fully dark-adapted eyes (groups 3 and 2 as described in Expt 1). During neither dawn nor dusk did any group contain as little visual pigment as bleached dark-adapted eyes (1).

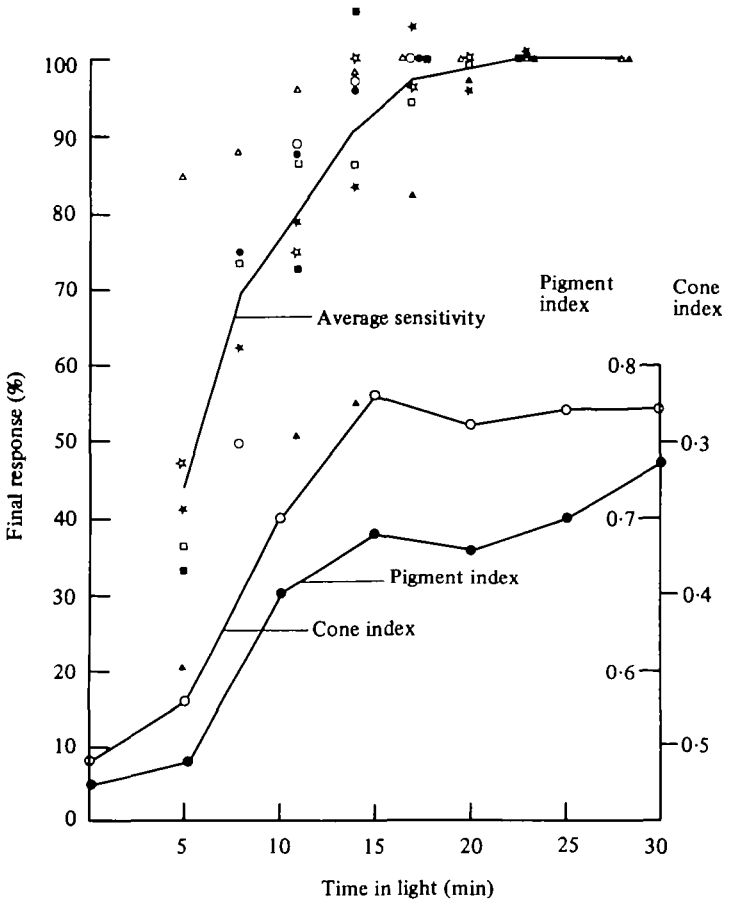

Fig. 7. Sensitivity changes of eight fish, as measured by the ERG b-wave, with associated retinomotor changes, during light adaptation. Each of the eight symbols (▲, △, ■, □, ★, ☆, ●, ○) represents an individual fish. Other symbols described in Fig. 6.

(4) In order to examine the relationship between retinal sensitivity changes during dark and light adaptation and photomechanical movements, the ERG b-wave sensitivity and the position of the retinal elements was determined during various stages of adaptation.

Immediately following the change in conditions of illumination, sensitivity dropped to a very low level so that often in the early stages of adaptation no b-wave that reached threshold could be recorded. In order to give an indication of the average time course of adaptation, the final response of each fish was arbitrarily assigned as 100%, and all other thresholds for that fish plotted relative to this. The line joining the average of these responses represents the average adaptation time course.

Figs 6 and 7 show the position of the retinal elements after varying periods in the dark and light respectively, along with the associated changes in sensitivity. In both cases photomechanical and electroretinographic adaptation were complete at about the same time (25–30 min for dark adaptation and 15 min for light adaptation).

DISCUSSION

It is generally believed that the role of the retinal epithelial pigment in photomechanical movements is either, to increase acuity by isolating neighbouring receptors, or to protect the rods from overstimulation when light adapted, but neither function has previously been effectively demonstrated in teleosts. The aim of this study was to show that at least one of these mechanisms, namely the protection of rods, is operational in the rainbow trout.

If the covering of the rods by the pigment epithelium serves to protect the visual pigment, light- (1) as well as dark-adapted (2) eyes should contain a large amount of visual pigment, while dark-adapted fish whose exposed rods are subjected to full daylight illumination (3) should have very little. These expectations were fully confirmed in the first experiment (Table 1).

This finding is backed up by the observation that the level of visual pigment throughout natural twilight periods does not vary in the manner expected if the rods were not shielded in some way. The amount of visual pigment present in bleached eyes (group (1) in Expt 1) is the amount that would be present in the eyes of those fish exposed to daylight if the rods were not protected. As this low level of pigment was not reached during any stage of either dawn or dusk (Figs 4, 5), the pigment must be shielded from the incident light. Furthermore, the level of visual pigment did not increase during dusk or decrease during dawn, as would be expected if the high levels of illumination experienced during the day bleached the pigment. The level of visual pigment during dusk was relatively constant, while the increase in pigment levels during dawn is in fact in the opposite direction to that expected if the pigment was being bleached. Such protection ensures that the fish go into the dusk period with eyes that are fully functional for low light intensity vision, which will save both the time and metabolic energy needed for pigment regeneration.

At no time during dusk was the level of visual pigment as high as that in fully dark-adapted eyes in the laboratory ((2) in Fig. 4). This may be due to differences in the techniques of extraction, as in the field eyes were frozen before extraction while in the laboratory the pigment was extracted immediately following the death of the fish. It might be argued that at no time during dusk did the eyes have a full complement of visual pigment, and that the pigment was only fully regenerated after several hours in the dark, at which time no fish were sampled. That this is unlikely to be the case is indicated by the level of pigment at the beginning of dawn (Fig. 5), before the intensity of illumination has started to rise. These eyes have been in the dark overnight and should thus contain the maximum amount of visual pigment.

Experiment 2 determined how efficient the pigment epithelium is in protecting the rod visual pigment. Photomechanical movements were found to reduce the light intensity affecting the rods by a factor of 19·5. Such a protective function of the epithelial pigment has only been quantitatively demonstrated, in terms of visual pigment content, once before in vertebrates. Bäck, Donner & Reuter (1965) determined the degree of protection in the common frog, *Rana temporaria*, and found that when the pigment epithelium is in the light-adapted position it reduces the effective light intensity at the rods by a factor of 3, which is very much lower than that observed here for rainbow trout.

The experimental procedures used on the frog and trout differ in certain respects and these differences may be part of the cause for this discrepancy. In the first place, Bäck *et al.* (1965) used excised eyes during the adaptation period that preceded bleaching. Intact fish were used in the present study to avoid any possible degeneration or abnormal reaction of the retina that may be associated with isolation. Secondly, in the previous study only the small flat portion of the centre of the eye was used, so as to avoid bleaching in peripheral parts of the retina where light would strike at an angle. This was felt to be an unnecessary precaution since the Stiles–Crawford effect is largely restricted to the cones (e.g. Flamant & Stiles, 1948). The use of such a small area gave rise to several problems in the study on frogs, owing to the small amount of pigment it yielded. The authors had to use eyes that had been dark adapted for 15–30 min as their light-adapted eyes, presumably because they were unable to get enough pigment from truly light-adapted eyes. Using larger pieces of eye allowed extraction from fully light-adapted eyes in the present study. Bäck *et al.* (1965) also blame the large variation in pigment content of light-adapted eyes (25%) on the small amounts of visual pigment. By using larger pieces of retina such problems were minimized. In the frog study, pigment was only extracted from one dark-adapted animal at each intensity of bleach. A greater number was not necessary as the results were confirmed using microdensitometry. In the present study at least three, and on two occasions six, fish had their pigment extracted at each intensity. Similarly, light-adapted eyes from the trout were sampled at five intensities of bleaching light, while in the former study light-adapted eyes were only sampled at one intensity. Finally, Bäck *et al.* (1965) used only a twofold range of bleaching intensity (0·3 log units) compared to the range of nearly three log units used in this study. It is felt that a greater range and an increased number of light-adapted bleaching intensities allows a more accurate determination of the degree of protection.

Although it is possible that differences in procedure between the two studies may be responsible for part of the observed disparity, it is unlikely to account for all of it. The majority of the observed difference in protective efficiency between *Salmo gairdneri* and *Rana temporaria* is probably due to species difference. The photomechanical movements of the trout are thus much more efficient at shielding the rods than those of the frog.

The fitting of a straight line to the data in Fig. 1 is in no way justified on the basis of the underlying photochemistry, but complicating factors such as self screening make it unclear what the exact photochemical basis would be. A straight line was thus only fitted in order to estimate the protective ability of the pigment epithelium and should not be confused with the undetermined photochemical relationship between the amount of pigment bleached and the intensity of the bleaching light.

If, as has been indicated above, the rods are shielded from light by the epithelial pigment one would expect that initially, when a fish is transferred from light into darkness, sensitivity would be low as all the rods are masked by the pigment epithelium. As the epithelial pigment and cones retract and the rods advance during adaptation an increasing number of rods will be exposed, thus increasing sensitivity, until maximal sensitivity is attained when the retinomotor changes are complete. Conversely, if a fish was suddenly exposed to light, having previously been in the dark, the cones would be in an inappropriate position at the back of the eye, masked

by the rods. Maximal sensitivity would again only be reached when photomechanical changes are complete and light can reach the cones unimpeded by the layer of rods. If retinomotor movements are involved in mediating sensitivity, as described above, one would expect the observed close temporal relation between the time taken to complete photomechanical and sensitivity changes during light and dark adaptation (Figs 6, 7). If, on the other hand, the pigment epithelium served solely to isolate cones, such a close relationship would not necessarily be expected.

A similar function to the one indicated by the experiments outlined above has never before been demonstrated in teleosts using controlled experiments, but some previous observations tend to support a protective role for the epithelial pigment. Blaxter & Jones (1967), Blaxter (1968), Blaxter & Staines (1970), Ali (1959) and Ali & Wagner (1975), for instance, have shown that almost universally throughout the teleosts, larval stages have pure cone retinas, and that retinomotor movements only develop along with the appearance of rods. This may indicate that in pure cone retinas photomechanical movements would serve no purpose.

Although a direct link between photomechanical movements and the ERG has never before been demonstrated in vertebrates, Gramoni & Ali (1970) did note two similarities between the fish ERG and retinomotor movements. Both responses depend on the intensity of preadapting illumination, and light adaptation is more rapid than dark adaptation.

Ali & Kobayashi (1968*a*) compared the ERGs of albino and pigment *Salvelinus fontinalis*, and found the components of the albino's ERG to have higher amplitudes and shorter latencies compared to pigmented fish. These differences indicate a higher sensitivity of the albino trout, presumably at least partially caused by the lack of rod shielding by the absent pigment epithelium. Similarly, at low levels of illumination the ERG flicker fusion frequency of the albino was higher than that of the normal trout, and the maximum fusion frequency was obtained at a much lower intensity in the albino compared to the normal trout (Ali & Kobayashi, 1968*b*). These differences can also be explained in terms of the pigment shielding the receptors in the normal fish, thus reducing the effective amount of light and consequently the response. A relationship has also been shown to exist between retinomotor activity and the ERG during hypoxia in the rainbow trout by Hoffert & Ubels (1979).

Dodt & Jessen (1961) found that in the frog the threshold changed by a factor of 32 during light adaptation. Injection of dark-adapted frogs with adrenalin, which causes the pigment epithelium to move to a light-adapted position, reduced the sensitivity change to a factor of two to four over subsequent light adaptation. The authors thus concluded that pigment migration changes sensitivity by a factor of 8–16.

All the evidence presented above, both from this paper and from previous authors, gives strong support to the idea that the epithelial pigment serves to protect the rods from overstimulation when light adapted. It should however be remembered that this in no way excludes the possibility that it could also serve to isolate cones from one another, thus improving acuity.

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