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GRADED POTENTIALS OF BREAM RETINA

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The large-amplitude graded potentials which can be obtained following illumination of fish retina were first reported by Svaetichin (1953). Although there is still evidence (Oikawa, Ogawa & Motokawa, 1959) to support the original hypothesis that they represent the activity of single cones, most subsequent studies have concluded that their source is located in more vitreal regions of the retina (Tomita, 1957; MacNichol & Svaetichin, 1958; Tomita, Murakami, Sato & Hashimoto, 1959). The amplitudes of these responses range from 10 to 30 mV and they are superimposed upon a negative d.c. potential of 10–50 mV. Two distinct types have been reported, a predominantly negative potential whose amplitude varies approximately linearly with the logarithm of the intensity of the stimulating light (Svaetichin, 1953) and a less common response which changes polarity with wave-length (Svaetichin & MacNichol, 1958; Motokawa, Oikawa & Tasaki, 1957).

This investigation is concerned with bream retina, which was originally reported to have the negative intraretinal potentials only (Svaetichin, 1953), but which will also be shown to exhibit responses that reverse polarity when the intensity of illumination is changed. Factors have been studied which would give an indication of the anatomical structure involved in the production of these graded intraretinal potentials, including the effects of light intensity, stimulus area, and electric current. Histological stains were employed in order to locate the position of the recording pipette and to examine retinal structures at this site. Spike activity in the immediate vicinity of the micro-electrode recording graded intraretinal potentials is described. Evidence is given to indicate that both the threshold and the effective stimulus area of the graded intraretinal potential is greater than what would be expected of the response of a single photoreceptor. Histological evidence strongly supports the hypothesis (MacNichol & Svaetichin, 1958) that the source of these potentials resides near the horizontal cell layer of fish retina. The electrical resistance of the structure across which these potentials are generated is relatively low and may be responsible for the ineffectiveness of current in altering the response.

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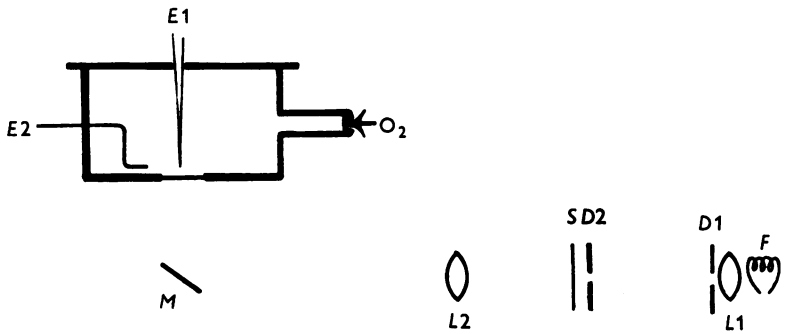
METHODS

Preparation. The results presented in this paper have been obtained from the excised retinas of bream (*Abramis brama*) but in the few studies *in vivo* of the e.r.g. anaesthetized animals were also used. Similar responses were obtained from the retinas of roach, trout, carp and tench but are not included in these results. The posterior hemisphere of the excised eye was cut in half and a strip of retina measuring about 10×6 mm at its widest margins was lifted away from the pigment epithelium, a manoeuvre facilitated by prior dark adaptation. The flat segment was mounted with its vitreal surface down on a thin cover-slip in the bottom of a Perspex chamber (Text-fig. 1), into which moistened oxygen was continuously pumped at a temperature of 18–22° C. The recording or stimulating micro-electrode was introduced through a circular hole in the movable upper section of the chamber while the secondary lead entered through a wax-sealed opening on its lateral side. The chamber was



Text-fig. 1. Apparatus: $D1$, variable stops imaged on retina by $L2$; $E1$, micro-electrode; $E2$, reference electrode; F, tungsten filament imaged at S by $L1$; $L1$, condensing lens; $L2$, focusing lens; M, front-face mirror; S shutter.

held in a mechanically driven micro-manipulator to facilitate electrode positioning and focusing of the stimulating light spot. The light entered through the bottom of the chamber and the vitreal surface of the retina, as in the intact eye. The entire preparation was viewed with a dissecting microscope at a magnification of $\times 40$.

Optics. The light source was a 6 V 36 W car head-lamp with a coiled tungsten filament. It was operated at 6 V d.c. and monitored by a voltmeter in parallel with the bulb. The filament was focused by lens $L1$ on a flag shutter S where the beam could be readily interrupted (Text-fig. 1) by a hand operated electric relay. The lens $L1$ was focused on the preparation by lens $L2$, which was so positioned as to intercept the entire beam entering the diaphragm $D2$. Diaphragms of different diameters or neutral filters were interposed immediately in front of lens $L1$ to control either the area or intensity of illumination. The maximum illumination of the preparation measured by a Salford Electrical Instruments exposure photometer was approximately 21,000 lm/m^2.

Electronics. The most frequently used electrodes were 3 M-KCl single and double barrelled micropipettes with resistances of 5–20 MΩ. Pipettes filled with potassium ferricyanide and saline (0·9 % w/v) solutions were also employed. Electrochemically sharpened tungsten micro-electrodes, insulated up to 5–10 μ from the tip, were used routinely to record high-frequency impulse activity. The secondary junctions were chlorided silver wires which led to the customary cathode-follower input stage with grid current of 10^{-11} A. The chlorided silver wires were checked before experimentation for photo-electric effects. Direct coupling was used at all times for the study of low-frequency activity. The amplified signals were photographed from the tube face of a Cossor oscilloscope. Electric current was passed

through one barrel of a Y-shaped double-barrelled pipette, by means of a variable 122 V dry-cell battery in series with a 1000 MΩ Victoreen resistor, which was used to minimize current changes due to changes of the electrode's resistance. Most electrodes tolerate the passage of as much as 0·1 μA without marked changes in their resistances, but higher values proved more difficult, especially with electrodes having resistances greater than 5 MΩ. The most elusive property proved to be the coupling resistance, which varied not only from electrode to electrode, but also with the electrode's position in the tissue. The coupling resistances of most electrodes, as measured either in saline or on the surface of the retina, ranged from 50 to 200 kΩ but this value invariably increased markedly with subsequent insertion into the retina. Nevertheless, coupling resistances were never recorded high enough to reduce current passed through the electrodes by more than 50 % and usually a much smaller reduction occurred.

Histology. In order to locate the position of the recording pipette a staining technique identical to that recently reported by Tomita (1959) was used. Micropipettes were filled with a saturated solution of potassium ferricyanide and selected for resistances of 1–2 MΩ to provide large current-carrying capacity and at the same time to retain the ability to record the typical intraretinal graded potentials. The technique consisted in stopping the insertion of the micro-electrode immediately after recording the first graded potential, disconnecting the cathode-follower heater supply to prevent current leak, passing 1–2 μA for 1 min, quickly withdrawing the electrode and almost simultaneously dropping ferrous sulphate solution over the recording site. With the aid of the dissecting microscope, a small blue spot could be seen forming when current durations longer than 30 sec were used. To facilitate later sectioning the electrode tip was thereupon broken and the blunt end relowered to the surface of the retina in order to form a large marking spot. The size and density of the ferroferricyanide precipitate was dependent upon the time elapsing between the electrophoresis of the ferricyanide ion and the application of the ferrous salt. Only very faint spots were obtained when this duration exceeded 2 min. If the ferrous ions were applied before the electrode was withdrawn from the retina, however, the precipitate was formed principally at the electrode's tip and would be dislodged proximally upon withdrawal. The segments were fixed in Susa's reagent, imbedded in paraffin and sectioned at a thickness of 20 μ. These sections were stained with haematoxylin and eosin. Other sections of normal retina were studied with Azo carmine, iron haematoxylin and Van Gieson stains.

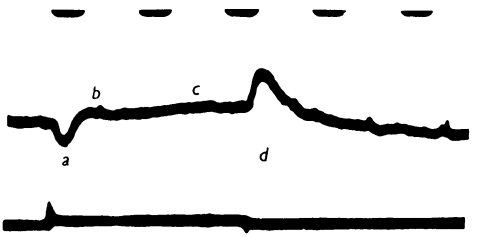
RESULTS

Low-frequency response of bream retina

If the conventional e.r.g. is recorded from the intact bream eye, the characteristic *a*-, *b*-, and *d*-waves are readily obtainable, as is shown in Text-fig. 2. The *b*-wave is largest after dark adaptation, as Day (1915) also reported to be the case in pike. A slowly developing positive *c*-wave can also be seen in Text-fig. 2. A few minutes after excision, however, the *b*-wave rapidly declines and the residual e.r.g. becomes diphasic, with a maintained negative wave at 'on' followed by a transient positive 'off' response. Such a change, presumably the result of deterioration, prompted the earliest retinal physiologists to study the amphibian retina, where similar changes are not seen for many hours after dissection (Day, 1915). If the excised fish eye is quickly opened and the extracted retina placed in an oxygenated moist chamber, the following pattern is observed. When the area of stimulation is less than approximately 3 mm in diameter a

micro-electrode on the photoreceptor surface will record a maintained positive wave during illumination and usually a transient 'off' response of opposite polarity. If either the area is increased, or the electrode is moved to an unilluminated region or if the retina itself is damaged, as, for example, after the electrode's insertion and withdrawal, the surface response will invariably become negative and can then reach amplitudes as large as 2 mV.

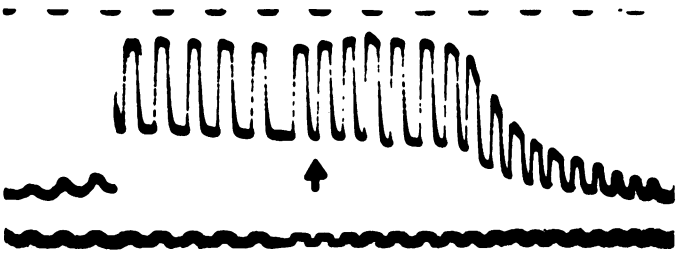
Accompanying the insertion of a micro-electrode into the retina from either the photoreceptor or vitreal surface there is an initial increase in its resistance of as much as 50 MΩ. If at this point the electrode is lowered further or if the table is gently tapped, the resistance is usually suddenly diminished and large graded photic potentials of 5–35 mV appear, super-



Text-fig. 2. E.r.g. *in vivo* after dark adaptation. Light stimulus begins at upward deflexion of lowest trace and ends at downward deflexion. Cornea positivity is upward on trace. Recording d.c. 'Off' response is 350 μV. Respiratory artifacts are visible. In all figures, time marker 1 sec.

imposed upon a negative d.c. potential of 5–50 mV. Only a negligible portion of this d.c. change is attributable to grid current. The over-all pattern of these events is ubiquitous across fish retina. With any given response there is a limited duration for which it can be maintained and within a few minutes there is always a reduction in the size of both the graded and d.c. potentials. The larger responses are more susceptible to this deterioration and often diminish markedly in a few minutes. Responses of 5–10 mV are readily obtainable with electrodes having tip diameters as large as 20 μ, whereas the larger responses require fine micro-electrodes. Text-figure 3 shows a typical 25 mV negative response to an intermittent light appearing superimposed on a negative 12 mV d.c. potential upon lowering the electrode. At the arrow the electrode is gradually withdrawn and the response increases transiently. With further withdrawal the response diminishes together with the concomitant d.c. potential. The surface-negative response is now larger than the one elicitable before insertion. If the electrode is reinserted into the same area similar responses are reobtainable, but seldom are they as large. If the electrode is moved to a region only 200 μ away, however, larger responses are again obtainable.

If it is lowered deeper after a characteristic response is recorded, the response will gradually diminish together with its d.c. potential. Two or three such responses may be obtainable in a single traverse, as noted by Oikawa *et al.* (1959). Upon withdrawal, the same sequence of events may be reversed but the responses are invariably smaller. Such a pattern is also found when the micro-electrode is inserted from the vitreal side, but the responses are never as large as they are in the approach through the photoreceptors. The resistant hyalo-vitreal membrane of fish eye, upon which the retinal vessels course (Rochon-DuVigneaud, 1943), may be responsible for this difference. As a consequence, most results are based upon recordings obtained from the approach through the photoreceptors, and the vitreal approach was only used in later attempts to facilitate locating the site of the micro-electrode tip by iontophoresis.

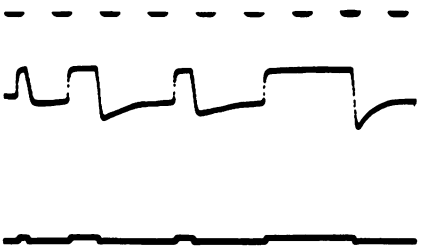


Text-fig. 3. Graded potential. Negativity upwards. Lowest trace, stimulus artifact; light 'on' when trace up. Maximum photic response 25 mV.

There are all gradations in the size of these potentials, from those that appear identical to the low-amplitude surface response to others as large as 35 mV. There are also differences in configuration, although they can be classified into two types representing extremes between which all intermediary responses are found. At one extreme is a predominantly negative response, which is the most frequent and the largest obtainable from bream retina (Text-fig. 3). At the other extreme is a response which at supramaximal levels of stimulation is also negative but at cessation of illumination exhibits a marked positive after-potential or 'off' response. This positive after-potential is very sensitive to the duration of illumination, being both larger and more accelerated with longer periods. A plot of response amplitude of this 'off' response against duration of illumination reveals an almost linear relationship up to periods of 5 sec, where a plateau is reached. Such a response is shown in Text-fig. 4, where the largest and most rapidly developing after-potentials are seen to follow the longest period of illumination. Svaetichin (1953) has also reported an acceleration in the rise and decay times of these intraretinal potentials, which occurred with light adaptation. The alternating stimuli of short and long duration

serve to demonstrate that this effect is not cumulative but immediate and transient. Such a dependency of 'off' responses on the duration of the preceding period of illumination has also been observed by Adrian & Matthews (1927) and Granit & Riddell (1934) in the amphibian e.r.g.

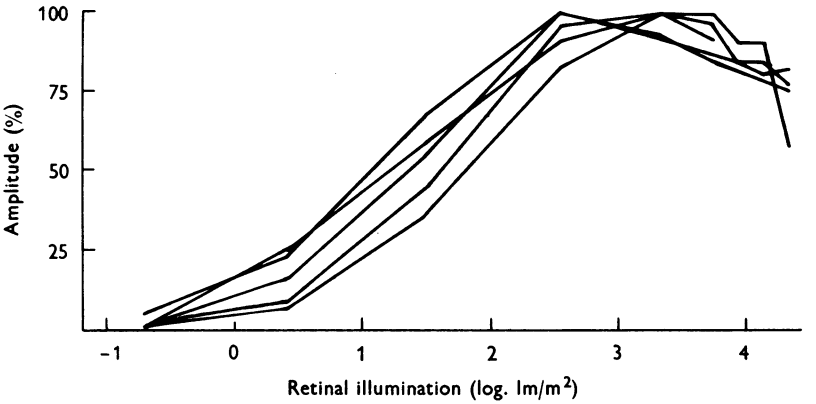
The effect of intensity on response amplitude. The amplitude of the intraretinal potentials of bream retina have been reported to vary linearly with the logarithm of the light intensity up to a saturation point at high intensities (Svaetichin, 1953). Absolute measurements on the level of retinal illumination, within which this linear relationship holds, are lacking. Although Mitarai & Yagasaki (1955) have reported increases in the d.c. potential, the effect of light adaptation has been found to be negligible (Svaetichin, 1953). It was considered important to determine the lowest



Text-fig. 4. Graded potential with 'off' response. Voltage calibration as Text-fig. 6. Negativity upwards. Lowest trace, stimulus artifact; light 'on' when trace up.

level of retinal illumination at which these responses are recordable, the level within which they vary linearly with the log. intensity and the point at which saturation occurs. During this study negative after potentials which were dependent upon the intensity of illumination were noted. These latter effects are not considered adaptational in that they curtail the maximal elicitable response rather than the sensitivity. The entire retinal segment was illuminated in order to obviate the marked dependency of these potentials on stimulus area (Tomita, Tosaka, Watanabe & Sato, 1958; Oikawa *et al.* 1959). The stimuli were flashes of 1–5 sec duration. Because of the diversity in response amplitude and configuration, as well as the inevitable deterioration that occurs with a prolonged study of an individual response, the following criteria were adopted in selecting data. Only predominantly negative potentials of greater than 15 mV with maximal stimulation were studied and responses which were not maintained almost constant to a submaximal stimulus throughout the test period were discarded. The latter criterion was the most difficult to attain because the test period lasted longer than 3 min, during which time there was frequently a diminution in the amplitude of the larger potentials.

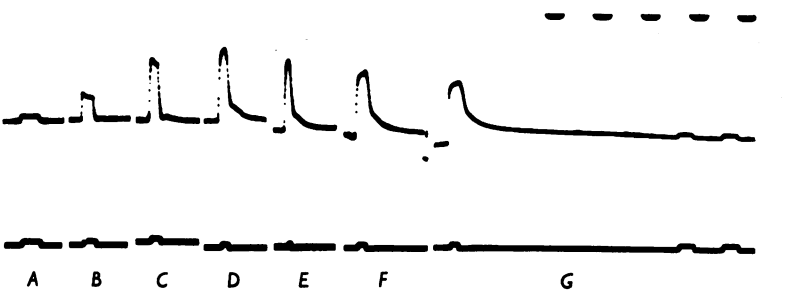
Text-figure 5 shows the result of this study where response amplitude is plotted as a percentage of the maximum response in order to minimize scatter. The responses are detectable only with difficulty below a retinal illumination of 0·1 lm/m², but vary almost linearly with the logarithm of the light intensity from 1 lm/m² up to retinal illuminations of 1000 lm/m², where saturation occurs. The decrease in response amplitude with high intensities represents the effect of both deterioration and the superposition of responses on a lingering after-potential. Text-figure 6 demonstrates a set of typically negative responses to increasing intensities of illumination. After-potentials characterized by an elevation of the negative d.c. potential occur minutes after intense 1–2 sec flashes. If threshold stimuli are given



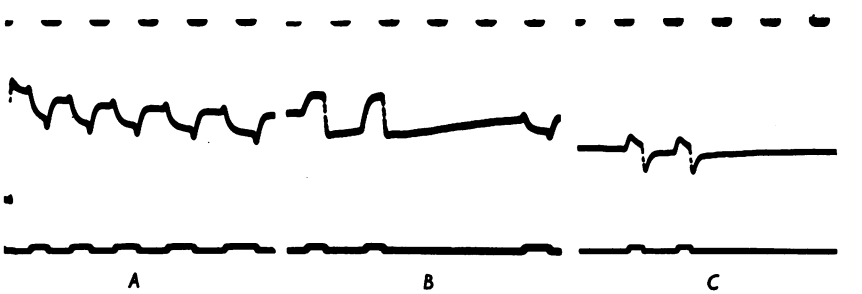
Text-fig. 5. Graph of amplitude of graded negative potentials plotted against retinal illumination. Semi-log. scale.

during a negative after-potential, the amplitudes of the threshold responses are unchanged, indicating that there is no change in sensitivity. In addition to the negative after-potential, another change can be seen to occur with increasing intensities of stimulation. At 35 lm/m² there is an initial transient at the beginning of stimulation. Such a transient is also apparent at 350 lm/m², but is lost at higher levels of illumination. This is shown in its most exaggerated form in the case of responses with large positive after-potentials demonstrated by Text-fig. 7. Stimulation with 3 lm/m² (*A*) produces a maintained positive potential but with an initial negative transient at the beginning of illumination, as in the case of the response of Text-fig. 6. With 350 lm/m² (*C*) the response becomes negative during illumination but also has an initial transient. At cessation of stimulation there is a marked positive 'off' response. With 1900 lm/m² (*B*) the initial negative transient disappears and the response is almost a square negative wave during illumination and a rapidly developing

positive after-potential at 'off'. If a second stimulus is delivered during the course of the positive after-potential the amplitude of the negative wave is correspondingly greater. It can be seen that despite the thousand-fold increase in stimulus intensity the amplitude of the positive after-potential is fixed, although its duration varies considerably. There appears to be a saturation point that is rapidly reached at both the positive and negative levels of these intraretinal responses, so that any additional



Text-fig. 6. Graded response to *A* 0·2, *B* 3, *C* 35, *D* 350, *E* 1900, *F* 5300, *G* 21,000 and 0·2 lm/m² terminally. +10 mV calibration at *F*. Negativity upwards. Lowest trace, stimulus artifact; light 'on' when trace up.

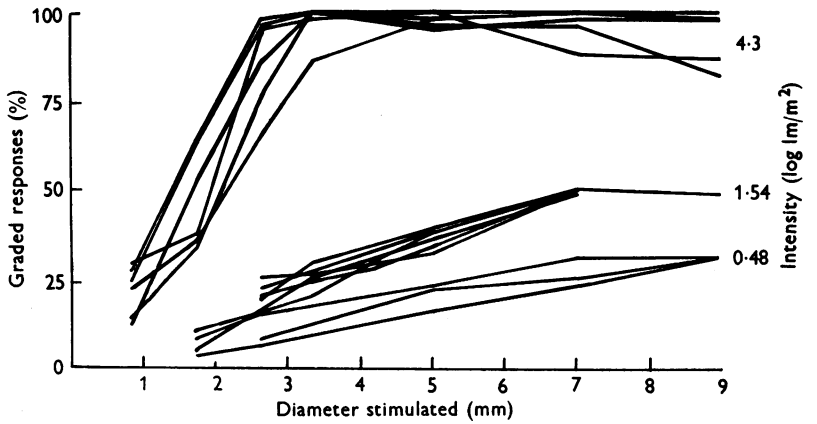


Text-fig. 7. Graded response. *A* 3, *B* 1900 and terminal 3 lm/m², *C* 350 lm/m². d.c. potential at beginning of *A* trace, reduced at *C*. Voltage calibration as Text-fig. 6. Negativity upward. Lowest trace, stimulus artifact; light 'on' when trace up.

increase in stimulus intensity serves only either to prolong the duration or to accelerate the rise or fall times of the response. The initial negative transient and the prominent 'off' response may indicate that the positive component of this response develops and decays more slowly than the negative component, in a manner similar to the mechanism proposed for the positive component of the colour response of *Mugil* retina (MacNichol & Svaetichin, 1958). The delay in the development of this component may also explain the noticeable dependency of the positive after-potential on the duration of illumination.

The effect of area on response amplitude. Two previous studies (Tomita

et al. 1958; Oikawa *et al.* 1959) have reported the marked dependency of these intraretinal potentials on the area of stimulation. The earlier workers, employing an illumination of about 200 lm/m^2, noted that they became very small when the diameter of the light spot was 0·2 mm, and increased in amplitude progressively to diameters of 3·2 mm. The subsequent workers, although not stipulating light intensity, found two different patterns with increasing areas of stimulation. The most common was similar to that which Tomita *et al.* (1958) described, in which the response amplitude increased up to diameters of 1·5–2·0 mm. A second, less common pattern, obtainable only with electrodes having tips finer than 0·2μ in

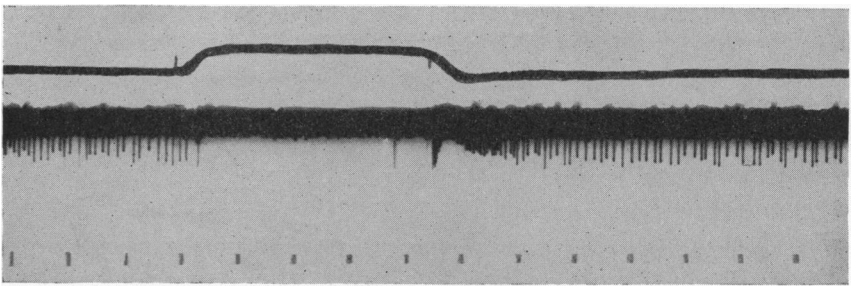


Text-fig. 8. Graph of amplitude of graded responses plotted against diameter of retina stimulated with supramaximal, submaximal and threshold illumination.

diameter, showed no increase in response amplitude with diameters greater than 0·16 mm, although changes in the after-potentials were observed. As a result of this and additional histological evidence Oikawa *et al.* (1959) attributed the latter responses to cones.

In this study the effect of area was also investigated but with more attention being given to the intensity of stimulation. The criteria for selecting responses were identical to those of the previous section. Stimulating spots of gradually increasing diameters were focused on the retina around the micro-electrode, and the effective area for a response was defined as the area beyond which illumination produced no increase in response amplitude. Text-figure 8 shows the results with amplitude again being plotted as percentage of the maximum response. Despite supramaximal stimulation, a retinal area of greater than 2 mm in diameter has to be illuminated in order to obtain a maximal response. This value must be a lower limit of the effective area of the structure generating the intraretinal response. Complete elimination of non-focal stray light can only

increase this value and focal stray light can be considered inconsequential. On the other hand, with stimuli only slightly above threshold the effective area is found to include the entire retinal segment. In this case focal stray light may be a contributing factor in increasing the limit, whereas non-focal stray light can be disregarded. Nevertheless, the area influencing the generator of the graded intraretinal potentials must be considered relatively large, having a lower limit diameter of 2 mm and an upper one of at least 8–10 mm. The small receptive fields described by Oikawa *et al.* (1959) were not encountered. The fact that these investigators did find changes in the after-potentials with larger areas may indicate that they



Text-fig. 9. Graded response and adjacent spike activity. Graded response 7 mV and spike amplitude 50 μV. Negativity is upward for upper trace, downward for lower. Upward artifact on upper trace indicates light 'on' and downward light 'off'.

were operating in the plateau of the intensity–amplitude curve, above retinal illuminations of 1000 lm/m^2.

There is a marked similarity between this result and that obtained by Wirth & Zetterström (1954) in their study of the effect of area on the size of the cat's e.r.g. In order to obtain an e.r.g. having the characteristic features of the high-intensity response it was necessary to stimulate the retina with diameters of 3–5 mm, and with stimuli less than 2 mm in diameter no e.r.g. could be recorded.

High-frequency unit activity

'Spikes' are easily recorded with insulated tungsten micro-electrodes from either the vitreal or photoreceptor surfaces of bream retina. The responses in general resemble those of ganglion cells of other vertebrate retinae (Hartline, 1938; Granit & Svaetichin, 1939). One noteworthy characteristic encountered in the study of discharges at the vitreal surface is their frequent sensitivity to the pressure of the recording electrode. Gradual and continued pressure can produce discharge rates of 200–300/sec in a reversible manner similar to what Alanis & Matthews (1952) described

of central neurones. The activity recorded at the photoreceptor surface is of more interest, however, in that it would appear to represent propagated responses of layers more external than the ganglion cells. Although spikes are obtained at the photoreceptor surface, they are always increased in amplitude by inserting the electrode into a retinal region within or just beyond which the large intraretinal potentials are recorded. With stimuli greater than 2000 lm/m^2 the 'spikes' at this location are invariably 'off' units, which always maintained a spontaneous rhythmic discharge in the dark. Simultaneous recordings of both the graded intraretinal potentials and the neighbouring spike activity are shown in Text-fig. 9. At this level of retinal illumination the graded potentials are all negative during the period of illumination, as described previously, and respond at 'off' with varying after-potentials. Spike activity always decreases during illumination and increases at 'off' in direct relationship to the positive after-potentials of the graded response. Single units can be maintained relatively constant as long as 2 hr after dissection. With prolonged study, however, a change occurs in the discharge, initially noticed as an increased frequency of firing in the dark. Illumination is only able to curtail the discharge momentarily, after which there is inevitable escape. Eventually the unit disappears in a high-frequency discharge in the dark and can only be made to fire during illumination. This rapid increase in frequency with subsequent cessation of activity is similar to depolarization or cathodal block described of other nerve cells (Eccles, 1957), but although temporarily offset by illumination, is irreversible in the case of these cells. The amplitudes of the 'spikes' are inversely related to the frequency of firing, being smallest during the brisk 'off' response as seen in Text-fig. 9, and largest when firing slowly.

Location of the micropipette and histology of bream retina

In order to determine the intraretinal region from which the graded potentials are best obtainable, the Turnbull's blue-staining technique described above was used (Tomita *et al.* 1959). The earliest attempts revealed that the external nuclear layer of the retina has to be penetrated before the large graded responses are obtainable (Pl. IA). Subsequent stains produced by electrodes introduced from the more difficult vitreal approach were localized in the horizontal cell layer, without any evidence of disruption or dye in the region of the outer or inner limbs of the photoreceptors or in the external nuclear layer, confirming the observations that these layers are not the source of the large graded potentials (Pl. IB). Reduction in both current intensity and duration of iontophoresis produced the smallest stain obtainable in this study (Pl. IC) which is less than 30 μ at its widest diameter and is located almost entirely at the horizontal

cell layer of the retina. Six successive attempts with this technique were localized in this region of the retina.

The prominence of this cell layer in fishes was observed by early retinal histologists (Schiefferdecker, 1886). Although working predominantly with information obtained from macerated material Schiefferdecker compiled the comparative anatomy of the vertebrate horizontal cell layer. In fishes individual cells are flat and stellate in appearance with processes as long as 500 μ. The cells are reported to form three distinct and almost continuous layers beneath the external plexiform layer. Mammals are second only to fishes in the prominence of this retinal layer, which is least developed in amphibians. The existence of intercellular anastomoses, observed by most early histologists, has been subsequently refuted by Polyak (1941), but only recently resupported by Chalissery (1955).

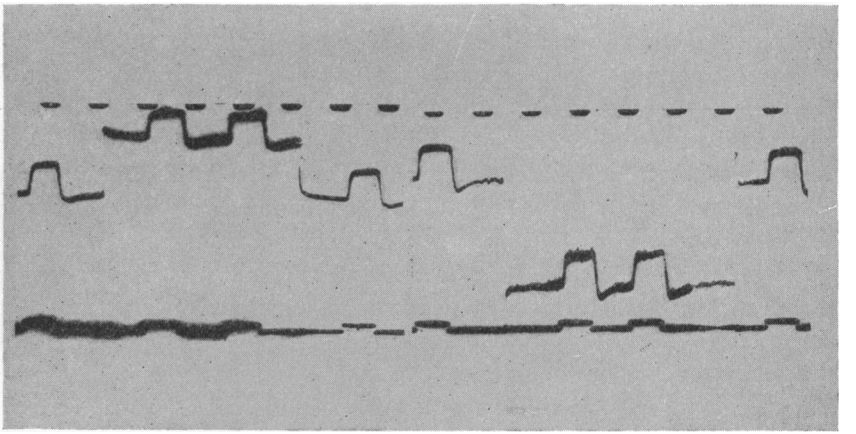
The present histological studies show that the horizontal cell layer of bream retina forms an almost continous sheet of closely packed cells extending immediately beneath the external plexiform layer. The diameter of the somata of the largest cells is 40 μ if a 20% shrinkage factor is assumed. Plate II B shows a tangential section through the somata of these cells, revealing the layer as the micro-electrode 'sees' it. Between their hexagonal somata are oval extracellular spaces through which the fibres of both Müller and bipolar cells have been reported to course, the former to form the external plexiform layer. The large processes of the horizontal cells extend from their bipolar surface to run both horizontally and vertically interdigitating in a dense network at the outer surface of the bipolar cell layer. Plate II A reveals such a single process extending 150 μ from its soma, which is visible in the same section. It can also be seen that in any one vertical section the somata of the horizontal cells overlap, forming what may have been interpreted as more than one layer by investigators using macerated preparations. With these staining techniques it was never possible to discern intercellular anastomoses between adjacent horizontal cells.

The effect of electric current

If these graded intraretinal potentials represent the transmembrane potentials of single cells it would be expected from analogy with receptor and post-synaptic potentials (Eccles, 1957) of more extensively studied nerve cells that alterations in the d.c. potential would produce alterations in response amplitude. MacNichol & Svaetichin (1958) have already reported that 10^{-8} A passed through the recording barrel of a micropipette failed to effect the response. If the response is intracellular, then either the membrane potential was not changed sufficiently by the current because of the cell's low total resistance, or the generator of these potentials

is an active ionic movement independent of membrane potential, somewhat analogous to what Lundberg (1956) reported of salivary-gland cells. If the recorded response is extracellular the explanation is simplified, because the current would then produce little change across the active membrane.

These possibilities can be investigated by the use of double-barrelled electrodes, which allow membrane potential to be measured simultaneously with the passage of current. The most difficult problem associated with this measurement proves to be the coupling resistance between the barrels of the electrode. The coupling resistance is lowest at the surface of the retina (25–200 kΩ) and largest during insertion (up to 100 MΩ). The most



Text-fig. 10. Graded potentials while $1{\cdot}2 \times 10^{-7}$ A being passed through one barrel of double-barrelled pipette. Two different responses: left, hyperpolarization and decrease in negative response; right, depolarization and increase in negative response. Voltage calibration as Text-fig. 6. Negativity upwards. Lowest trace stimulus artifact; light 'on' when trace up.

typical sequence is to record an enormous increase in coupling resistance just before recording the d.c. potential and the large graded responses. At this point the coupling resistance suddenly drops to approximate to the surface value. To obtain the total resistance of the hypothetical cell, the coupling resistance as measured at the surface of the retina is subtracted from the resistance measured during the recording of the large graded response. The resistances so calculated range from 10 to 200 kΩ and are obtained with currents as great as 10^{-7} A. Because of these relatively low values, current of 10^{-7} A is unable to alter the resting potential by more than 10–20 mV. In two cases, however, the response was altered with maximum current intensity. Text-figure 10 shows a

reduction of 1·5 mV in the amplitude of a negative response produced by inward current of 10^{-7} A. In a second case outward current increased the amplitude of a similar response by 0·5 mV. This alteration of response amplitude is in the direction that would be expected for intracellular responses resulting from permeability changes of a cell membrane. It may be equally explicable, however, by a decrease in resistance due to illumination of a structure across which the current is passing. A decrease in the radial resistance of frog retina has been proposed to explain changes in the amplitude of the *b*- and *d*-waves of the e.r.g. with polarizing currents (Brindley, 1956) and a decrease in the resistance of goldfish retina during illumination has recently been found (Tasaki, 1960).

DISCUSSION

In favour of the view that these intraretinal potentials are intracellular are the resting negative potential, the large amplitude of the graded potential, the tendency for both to deteriorate concomitantly and relatively rapidly when recorded with even fine pipettes, and the individuality of response pattern at different regions of a completely illuminated retina. Against it are Tomita's (1957) simultaneous recording of graded responses from both barrels of a concentric micro-electrode, the tips of which were separated by 50 μ, the fact that they are obtainable with relatively large electrodes (20 μ diameter), that receptive fields include at least half of the retina, that intracellular spikes are absent and that the response amplitude tolerates extremely large currents.

Measurements with double-barrelled pipettes indicate that the resistance between the recording electrodes is 10–200 kΩ greater than the surface measurement when the recording pipette is in that region of the retina where the large graded potentials are found. This increase in resistance can be attributed to either the micro-electrode's mechanical alteration of the retinal tissue, or its encroachment upon a high resistance structure, or its entrance into an electrically isolated intraretinal compartment. The latter hypothesis is supported by the following facts. The change in resistance occurs concomitantly with the appearance of a steady negative potential of 5–50 mV, which is independent of the position of the external reference electrode; that is, the d.c. potential exists when the retinal segment is completely submerged in a conducting solution. This negative potential, the large graded responses, and the increased interelectrode resistance occur suddenly and after only a slight movement of the recording pipette, observations which seem more explicable by a compartment hypothesis. There is a gradual and greater increase of interelectrode resistance just before the appearance of the above phenomenon, which seems more attributable to either mechanical changes or the pipette's

approaching a high-resistance structure. This change occurs at the region of the photoreceptor nuclei as indicated by the marking techniques and may be due to a structure similar to the R membrane reported to be present in frog, rat and rabbit retina (Brindley, 1958).

Iontophoretic stains locate this hypothetical compartment at the external plexiform or horizontal cell layers. Histology reveals that the horizontal cells of fish retina are relatively large and also form a compact tangential structure shunted only by cylindrical extracellular spaces which form 10–20 % of any cross-sectional area (Pl. II). These spaces are in addition filled with both nerve and Müller fibres that would act to increase the radial resistance of this layer. That the graded responses are most readily found in fish retina where the horizontal cell layer is most prominent, and are almost absent in amphibian retina where it is least developed (Shiefferdecker, 1886), suggests that this layer plays a part in the genesis of the large graded potentials. Similar responses have also been detected, although with less facility, in mammalian retinae (Grüsser, 1957; Brown & Wiesel, 1958), which are second to those of fish in the prominence of the horizontal cell layer (Schiefferdecker, 1886).

The compartment may be the intracellular space of a horizontal cell, although its specific membrane resistance would have to be extremely low if its surface area were even as large as a motoneurone (Eccles, 1957) to have a total resistance as low as 10 kΩ. If the syncytial hypothesis is true, then the lower resistance would be expected, although the demonstrations of these anastomoses extant in the literature only show processes having the dimensions of fine dendrites (Chalissery, 1955). Another hypothesis is offered which would envision the external nuclear layer or the external limiting membrane and the compact horizontal cell layer of fish retina forming one or a number of relatively large isolated compartments of the external plexiform layer and this would explain most of the results of this investigation more satisfactorily.

There are many similarities between the graded responses and the lower amplitude e.r.g. Both require large areas of illumination, both have relatively high thresholds, both have 'off' responses which are very dependent upon the duration of illumination and both have fast initial negative components. In addition, changes in amplitude of both these responses require rather large currents and the direction of these changes can be explained by a decrease in resistance during illumination of the structure across which the current is passing. In general, there is only a small d.c. component in the vertebrate e.r.g. during illumination as demonstrated *in vivo* by the fish e.r.g. of Text-fig. 2. The surface response of isolated fish retina, however, frequently shows a pronounced d.c. change during illumination, similar to that of the graded intraretinal response.

The vertebrate e.r.g. appears to be generated across a high-resistance tangential structure in the retina, which has been identified as the external limiting membrane and across which current is most likely carried by the photoreceptors (Brindley, 1958). If the potentials resulting from this current are generated across the high-resistance boundary on the photoreceptor side of the hypothetical compartment, this current could also be the source of the graded intraretinal potentials, and a micro-electrode located within the compartment would be expected to record these potentials at a greater amplitude than it would at the retinal surface.

In addition to the e.r.g., focal intraretinal potentials have occasionally been reported to occur in amphibian retina (Tomita & Torihama, 1956). These potentials differ from the e.r.g. in their greater susceptibility to deterioration and their complex changes to stimulation (Brindley, 1958). Such complexities are reminiscent of the polarity reversals found in the graded intraretinal responses of fish retina. These potentials may be a limited version of the phenomenon proposed to be occurring in fish retina, since they are less frequently detected, deteriorate quickly, and are of a much smaller amplitude. The fact that they are not as well maintained during illumination as the graded intraretinal potentials of fish may reflect their relationship to the amphibian e.r.g., which is as dissimilar from the surface response of excised fish retina.

The presence of extracellular spikes both at the photoreceptor surface and within the region from which large graded responses are recorded suggests that their source is more external than the ganglion-cell layer. There is a close relationship between the graded potentials and neighbouring spikes, with positive graded potentials being associated with increased frequency and decreased amplitude of the spikes and the reverse relationship for the negative potentials. Such a correlation between spikes and graded potentials would not be expected if these activities originated in separate cells. It is also in agreement with the conventional intracellular changes at receptor and dendritic terminals with increased positivity being associated with depolarization and increased frequency of propagated action potentials, and the reverse with negativity. This association may indicate that the graded fish potentials represent the activity of bipolar processes, isolated as they are within the external plexiform layer by the horizontal cells, and the spikes the propagated responses of the bipolar somata occurring outside this hypothetical compartment and hence of smaller amplitude.

The fact that two processes of opposite polarity appear to be acting in varying degrees in the graded potentials of bream retina indicate that the negative response does not merely sum luminosity. At low intensities the positive potential is most conspicuous, but may remain observable in some

responses even at the highest intensities, as an after-potential or 'off' response. At illuminations greater than 350 lm/m^2 the responses are always negative during illumination and are associated with curtailment of neighbouring spike activity. The fact that some responses change polarity with intensity make them seem analogous to the wave-length reversing potentials of other fish retina, although colour-discriminating responses have been reported to be absent from bream retina (Svaetichin, 1953). This polarity reversal is not due to partial activation of a tangential structure, since it occurs with illumination of the entire retina. The author has observed polarity reversals with colour while studying bream retina, but they were never properly controlled for changes in intensity. The fact that intensity does play a part in producing polarity reversal demands that a careful distinction be made between intensity and wave-length in any spectral scanning study. Although two processes of opposite polarity and different spectral sensitivities are summing in the same response in the case of *Mugil* retina (MacNichol & Svaetichin, 1958), since multiphasic patterns were not obtained in a spectral scan it would be interesting to know whether these two processes also differ in their threshold intensity. The complexity of such a system would prevent a straightforward comparison of spectral sensitivity curves of intraretinal potentials with those of photochemical pigments or with those obtained at a different intensity level and would perhaps explain some of the variation in such findings at the present time.

The role of these responses in fish vision is undoubtedly of major importance. They are either responses of the terminals of photoreceptors in the external plexiform layer or adjacent second-order neurones consisting of either horizontal cells or the dendrites of bipolars. That two processes of different threshold and temporal relationships occur suggests that there are either at least two types of photoreceptor responses, or two types of transmitter substances released at this stage in the visual pathway which sum in different degrees to produce the graded intraretinal potential.

SUMMARY

1. Evidence is given to support the hypothesis that the graded intraretinal potentials of bream retina originate near the horizontal cell layer of the retina.

2. Two components of opposite polarity and different temporal sequence sum to varying degrees in the graded intraretinal response. With total retinal illumination greater than 350 lm/m^2 the graded potentials are negative, but at lower intensities slower developing maintained positive potentials are found which are manifest only as 'off' responses at higher intensities.

3. The graded negative intraretinal response is recorded with difficulty below a retinal illumination of 0·1 lm/m²; from this point it varies almost linearily with the logarithm of illumination to 1000 lm/m². Greater illumination produces increases in the duration of the after-potentials but no increase in amplitude.

4. The minimum area of retina that must be illuminated to produce maximum response with supramaximal stimuli is 2 mm in diameter and summation occurs over areas as large as 8 mm in diameter.

5. The resistance of the structure across which these potentials are generated ranges from 10 to 200 kΩ and supports the hypothesis that these potentials arise in extracellular intraretinal spaces.

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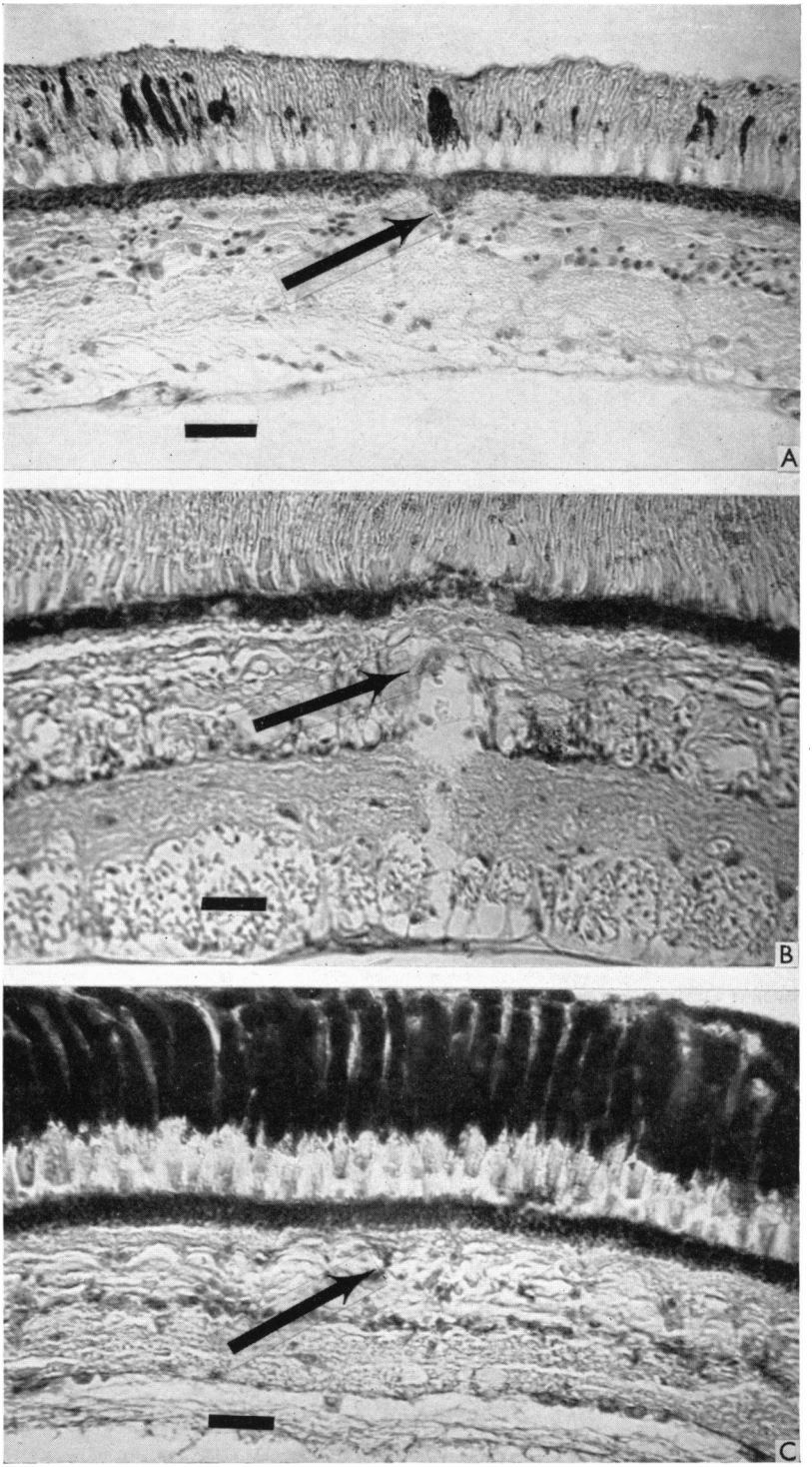
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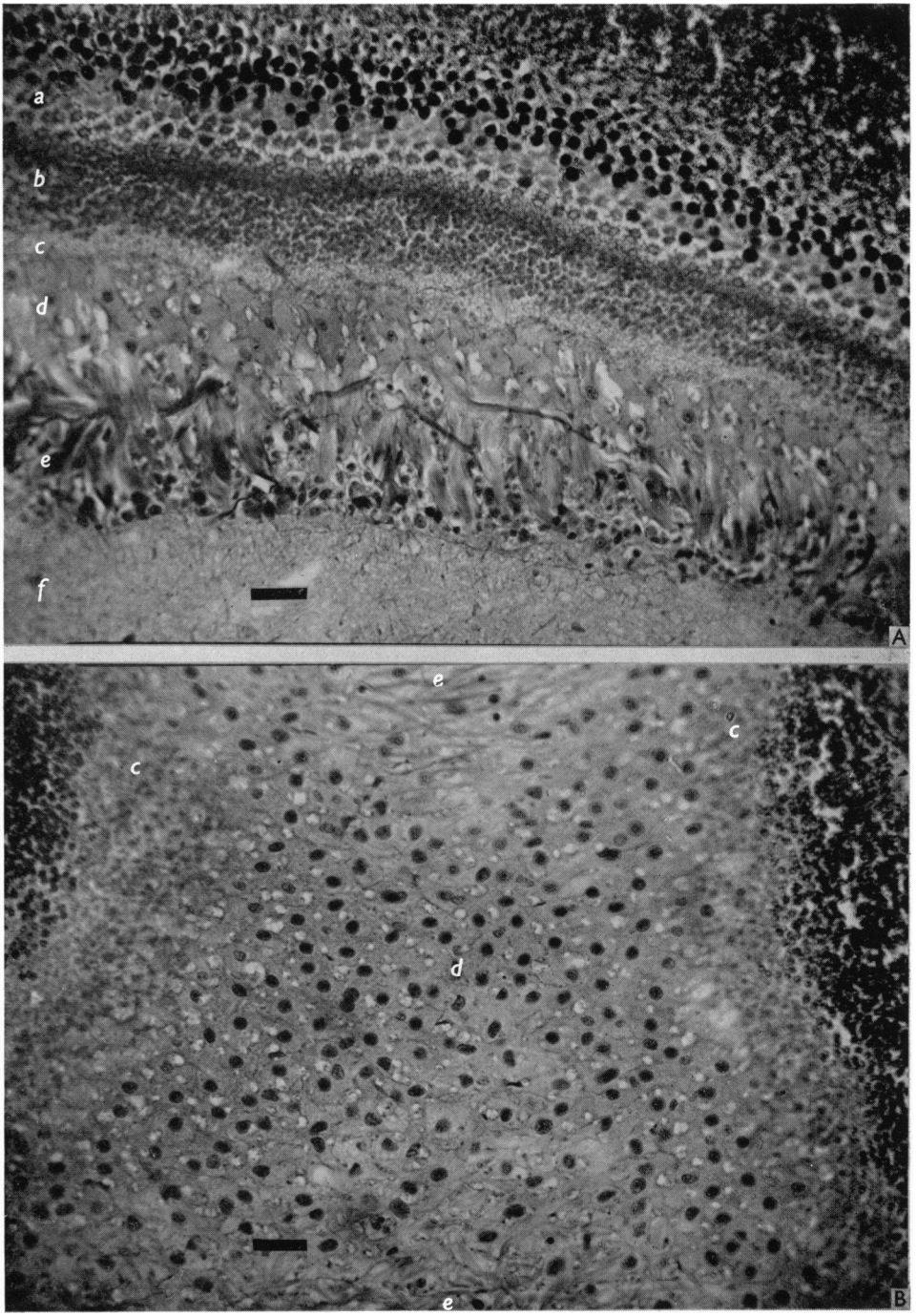
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EXPLANATION OF PLATES

PLATE 1

Location of distinctly blue ferroferricyanide precipitates intraretinally. A and C via photoreceptors; B via vitreal approach. Lines represent 40 μ.

PLATE 2

A, radial section of bream retina; B, tangential section. *a*, layer of rods and cones *b*, layer of photoreceptor nuclei, *c*, external plexiform layer, *d*, horizontal cell layer, *e*, bipolar cell layer, *f*, internal plexiform layer. Lines represent 40 μ.