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THE SOURCES OF SLOW ELECTRICAL ACTIVITY IN THE FROG'S RETINA

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In three earlier papers (Brindley, 1956*a*, *b*, *c*) in which slow electrical responses of the excised eye of the frog to illumination were investigated, the following tentative conclusions were drawn:

(i) The layer of high electrical resistance and capacity (R membrane), which a micro-electrode penetrates when it is about 230μ from the inner boundary of the retina, is the external limiting membrane.

(ii) A complex sequence of responses to diffuse illumination of the whole retina, recorded by a micro-electrode advanced in steps through the retina, similar to that described by Tomita (1950), is a normal phenomenon. A simple sequence of responses (Ottoson & Svaetichin, 1953; Brindley, 1956*c*, Fig. 6) is found in retinae which have lost some, but not all, of their normal functions.

(iii) The electroretinogram (excluding its c-wave, for which no evidence is available) is entirely or almost entirely generated by the rods and cones.

The present paper presents new evidence which supports these three conclusions, and also provides some indication of the sources of the electrical activity which is present in those retinae which give the complex (Tomita, 1950) pattern of responses and absent in those which give the simple (Ottoson–Svaetichin, 1953) pattern.

METHODS

These were mainly those described in Brindley (1956*a*). Opened excised eyes of *Rana temporaria* were used wherever the contrary is not stated. The following features are new:

Preparation. In a few experiments pithed frogs were used, the cornea, iris and lens of one eye being removed, the body of the frog attached to the horizontal micromanipulator with the opened eye facing upwards, and the micro-electrode lowered into the retina in the usual way. The 'indifferent' electrode was placed in the mouth; with this precaution, only a small fraction of the electrocardiogram appeared on the record.

Amplifier. The directly-coupled amplifier shown in Fig. 1 was used. The 'cascode' arrangement of the first two valves is familiar in communication engineering, where its principal advantage is in

minimizing shot noise (Valley & Wallman, 1948). It does not seem to have been used in electrophysiology, where, besides the low noise, it has the advantages of great simplicity and very satisfactory control of grid current. With the present circuit the grid current taken by V_1 could, by altering the potential of the grid of V_2, be varied from $+3 \times 10^{-9}$ A through zero to -2×10^{-11} A without substantial change in gain.

In all records the beginning of the stimulus is marked by a downward and its end by an upward artifact. Its duration is always 0·63 sec.

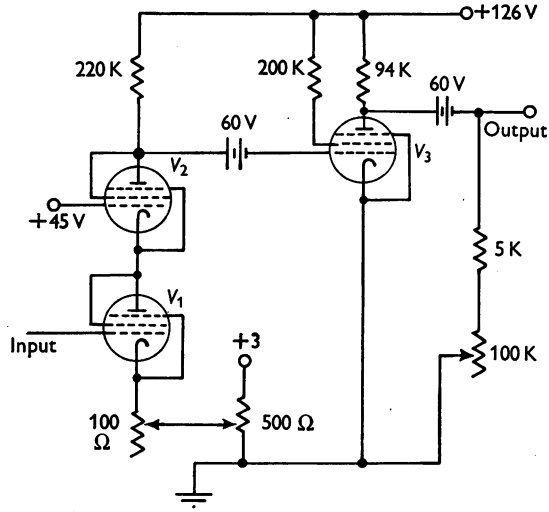

Fig. 1. Circuit-diagram of preamplifier. V_1 and V_2 are Mullard EF 37A. V_3 is Brimar 6 BR 7. The coupling batteries are pairs of 'Ever-Ready' B123 hearing aid batteries. The variable cathode load of the input stage serves as a fine balance-control.

RESULTS

The nature of the R membrane

Presence of an R membrane in mammalian eyes

Fig. 2 shows the result of passing square pulses of current between the vitreous and sclera of an opened excised rat's eye, and recording the resulting potential pulses between a large electrode in contact with the sclera and a micro-electrode inserted into the retina from its inner surface, as described earlier for the frog (Brindley, 1956*a*, 1957*a*). The records are very similar to those obtained with frogs' eyes (see Brindley, 1957*a*, Fig. 2), and indicate the presence of a structure of high electrical resistance and capacity ('R membrane') which the electrode penetrates between 250 and 300μ from the inner surface of the retina as it is advanced. Closely similar records were obtained from seven of twelve other eyes tested, the R membrane being sometimes penetrated on the same step in withdrawal as in advancement, sometimes 50μ nearer inner surface. The lack of success in five experiments, in which the potential pulses were very nearly square when the micro-electrode was in the vitreous, and did not change suddenly at any point within the retina, may well

be attributable to mechanical injury to the retina during dissection. This is more difficult to avoid in the rat than in the frog, because the rat's sclera has very little rigidity, and the eye is apt to collapse when opened.

For the experiment of Fig. 2, the change in height of pulse between the inner surface of the retina and the point immediately before the R membrane was reached corresponds to a resistance of 89 Ω cm^2, and that on crossing the R membrane to 99 Ω cm^2. The mean values of these quantities for all the eight successful experiments were 72 and 112 Ω cm^2. These are very similar to the corresponding quantities for the frog (97 and 100 Ω cm^2).

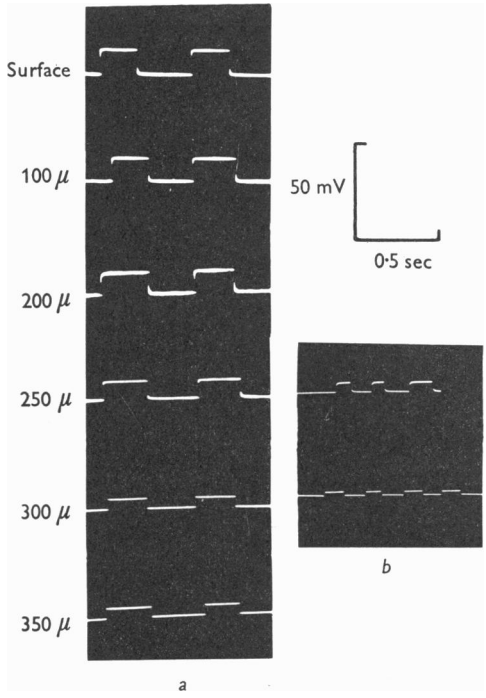

Fig. 2. (*a*) Potential pulses recorded between a micro-electrode, inserted in steps of 50 μ into a rat's retina from its inner surface, and a large electrode in contact with the sclera, when square pulses of current of 16·8 μA were passed between the sclera and vitreous. (*b*) Potential pulses recorded between electrodes in the vitreous and in contact with the sclera of a rabbit's eye when square pulses of current of 61 μA were passed between the sclera and vitreous. Above, retina intact; below, after removing the retina.

Fig. 2 also shows the potential pulses recorded by electrodes in contact with the vitreous and sclera of a rabbit's eye on passing square pulses of current between a second similar pair of electrodes. As with the frog and rat, the pulses recorded with the retina intact are rounded, as if there is a capacity as well as a resistance between the electrodes. When the retina is removed, this rounding is

lost, and the size of pulse diminished by an amount corresponding to a resistance of 154 Ω cm².

Absence of an R membrane from isolated retinae

The retinae were removed from freshly excised eyes of six fully dark-adapted frogs. Each retina was mounted with the receptors uppermost on a dome of porous earthenware soaked with Ringer's solution, similar in size and shape to the vitreous body of an average frog. A ring, 1·5 mm in diameter, of fine silver wire was gently lowered on to the surface of the retina, upon which it rested, touching along its whole circumference. The upper surface of the retina remained exposed to the air. In each retina inspection of the surface with a microscope revealed a continuous pattern of purple and green rods within the ring and for at least 1 mm in all directions outside it, except for a few small patches of adherent pigment epithelium; and all points within this region tested with a micro-electrode gave responses to local illumination roughly similar to those shown in Figs. 1 and 2 of Brindley (1956*c*). In all retinae, when square pulses of current were passed between the ring and the earthenware dome and the resulting potential pulses recorded between the dome and a micro-electrode whose tip touched the retina at the centre of the ring, the potential pulses were found to be square. On advancing the micro-electrode through the retina they decreased only slightly in size, and at no point changed in shape. One retina was fixed and embedded in paraffin at the end of the experiment. Examination of stained sections confirmed that rods and cones were present over the whole of the central part of the retina, from which the electrical records were taken, though they were bent in several regions and obviously broken in a few, and many of the rods appeared to have lost their extreme outer ends. The external limiting membrane and the layers in front of it showed no signs of damage anywhere.

Normality of Tomita's complex sequence of responses to illumination

Persistence of the complex sequence of responses in a preparation with intact blood supply

In excised eyes the complex sequence of responses to uniform illumination of the whole retina is usually obtained for the first half-hour or so after excision. Thereafter the complexities diminish and then disappear, leaving an eye in which a typical normal electroretinogram can be recorded from all points in front of the R membrane, and little or no response to illumination from points behind it. With further lapse of time, the electroretinogram itself diminishes in size and finally disappears. Thorough removal of the vitreous humour, which should assist the exchange of oxygen and carbon dioxide between the retina and the atmosphere, usually delays the disappearance of the complexities, and also the final disappearance of the electroretinogram. These observations

suggest that the complexities are a normal phenomenon, and their disappearance a sign of deterioration, perhaps due to lack of oxygen or poisoning with carbon dioxide. It might, however, be objected that they could result from a disturbance of the retina caused by removing and opening the eye, a disturbance from which it recovers in about half an hour. This objection is made much less plausible by the fact that in pithed frogs, in which the retina continues to be supplied with blood, the complexities may last very much longer. The pithed frog is not a wholly satisfactory preparation, for in some, perhaps those with a relatively high blood pressure, approaching that of an intact frog, the cut edge of the choroid bleeds profusely and covers the anterior surface of the retina with clot, and in others, thought to be those with an unusually low blood pressure, the retina lasts no longer than that of an excised eye. However, from one pithed frog typical complex responses were recorded without substantial change during a period of 7·5 hr, and from three others for periods exceeding 2·5 hr. No excised eye ever gave complex responses for more than 70 min.

Evidence that the complexities are not caused by the presence of the electrode

The region from which a micro-electrode is recording might be atypical because the electrode casts a shadow on it, because potassium chloride solution leaks from the tip, because the grid current taken by the input valve of the amplifier flows through it, or because it is mechanically deformed by the insertion of the electrode. Against these possibilities we have the following arguments:

(i) If a grating having 25 lines/cm is used as stimulus instead of a uniform field of light, it makes almost no difference to the response whether a line or a space falls on the electrode (see Fig. 3).

(ii) The complex series of responses can be recorded with an electrode filled with Ringer's solution (Tomita, 1950) or with silver wire (Tomita & Funaishi, 1952).

(iii) Variations of grid current from -24 pA through zero to $+530$ pA have no effect on the responses (see Fig. 3).

(iv) Almost the same sequence of responses is obtained on withdrawing the electrode as on advancing it, although the mechanical deformations must be very different.

Origin of the electroretinogram

The possibility of obtaining the simple (Ottoson–Svaetichin) pattern of responses at all points of a retina

It was earlier observed (Brindley, 1956*c*) that when the complex sequence of responses has disappeared from one region of a retina it is usually found to be absent also from other regions, even though the electroretinogram may be com-

pletely normal. Tomita & Torihama (1956) seem to have observed the same phenomenon, for they write: 'The focal potentials are very susceptible to ageing at higher temperatures. At 17° C and above they usually disappear in 10 minutes or less, while the e.r.g. does not change much,' and later: 'Once the focal potentials have disappeared at one site, punctures of other regions of the same retina usually fail to detect them, or if detected, they are very feeble.'

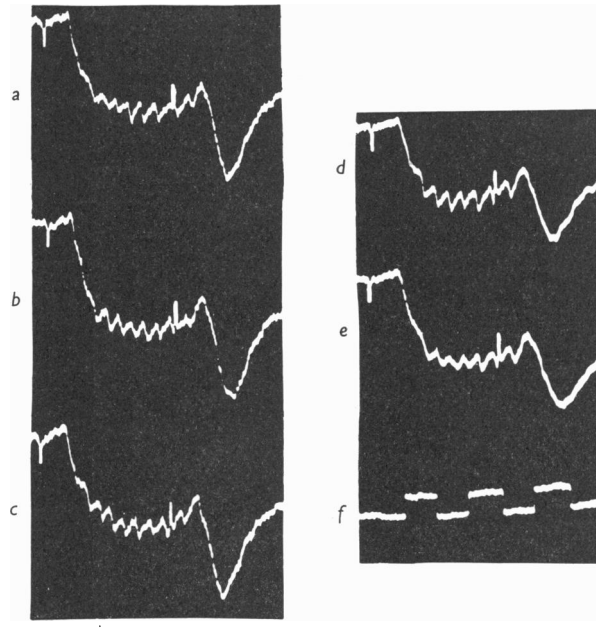

Fig. 3. Left: responses to uniform illumination at 43 lm/m², recorded with a micro-electrode inserted from the inner surface of the retina of an excised frog's eye to a depth of 160μ, the amplifier drawing a grid-current of (*a*) +530pA, (*b*) +1pA, (*c*) −24pA. Right: responses recorded from the same point on the retina, the stimulus being a grating of equal lines and spaces having 25 lines/cm: (*d*) centre of a dark line falling on the electrode; (*e*) centre of a space falling on electrode. (*f*) 154μV.

The following experiment was done to verify that it is possible to obtain the simple (Ottoson–Svaetichin) pattern of responses at all points of a retina which gives an e.r.g. of normal shape and nearly normal size. From each of six opened excised eyes the anterior parts of the sclera, choroid and retina were cut away with scissors, so that the posterior pole which remained formed only about a one-sixth segment of a sphere, and hence, when placed in the apparatus, was wholly accessible to the micro-electrode, which could enter the retina at any point without great obliquity. The preparation was kept in the apparatus at about 18° C for 25 min. Then a number of points scattered all over the retina were tested, responses to illumination being recorded at the surface of the

retina and at depths of 100, 200 and 300μ. In the two best of these six experiments 25 and 21 points were tested, and all gave the simple pattern of responses, though the e.r.g. was of normal shape, and its amplitude was about 400μV at the beginning of the experiment and about 200μV at the end.

The effect of intensity of stimulus on the complex (Tomita) pattern of responses

Fig. 4 shows the responses to spatially uniform stimuli at three intensities, recorded at the inner surface of a retina and at five depths within it, all stimuli being superimposed on a steady background ten times less bright. The effect of increasing intensity on the electroretinogram (surface record) is simply to increase its amplitude and shorten its latency and time course. Opposite effects, or effects different in kind from these, were never found in any range of intensities. The responses recorded from within the retina, on the contrary, may increase in amplitude and shorten in time course with increasing intensity in one range of intensities, but decrease and lengthen in another, as is seen at 200μ in Fig. 4; and they may show features, such as the diphasic off-effect at 100μ in Fig. 4, which are conspicuous at some intensities and wholly absent at others.

Additivity in the electroretinogram

It was shown earlier (Brindley, 1956*b*) that for fields of 0·5 mm^2 area and over, if the effects of stray light are eliminated by superimposing the test flash on a steady background, the e.r.g. obtained on illuminating any region of an opened excised frog's eye is the sum of the responses obtained on illuminating the parts of that region separately. This sufficed to allow the large departures from additivity at comparable field sizes, found by Adrian & Matthews (1927) in the *absence* of steady background, to be attributed to stray light, and hence discarded as evidence concerning the origin of the e.r.g. However, the range of field sizes concerned was one in which even the local responses recorded intraretinally were almost additive, so that the finding of additivity in the e.r.g. provided no substantial evidence that these local responses do not contribute to it.

Improvements in the electrodes and amplifier, and the device of using multiple fields, have now made it possible to test for additivity of the e.r.g. with fields small enough for the local responses recorded intraretinally to show very large departures from additivity (see Fig. 9 of Brindley, 1956*c*; the degree of non-additivity seen there is typical of that found in many similar experiments). For the e.r.g., no departures from additivity were found. In particular, a single circular field of area 2·08 mm^2 was found consistently to give the same response as a field of practically the same total area (2·02 mm^2) made up of 24 small circles, each about 0·3 mm from its neighbours. Fields made up of three circles each of area 0·19 or 0·96 mm^2 gave responses

indistinguishable from the sums of the responses to their components applied separately. The response to three circles each of area 0·96 mm² was indistinguishable from a 0·96/0·19 times multiple of the response to three circles each of area 0·19 mm². Typical records, and photographs of the fields with which they were obtained, are shown in Figs. 1*a* and 1*b* of Brindley (1957*b*).

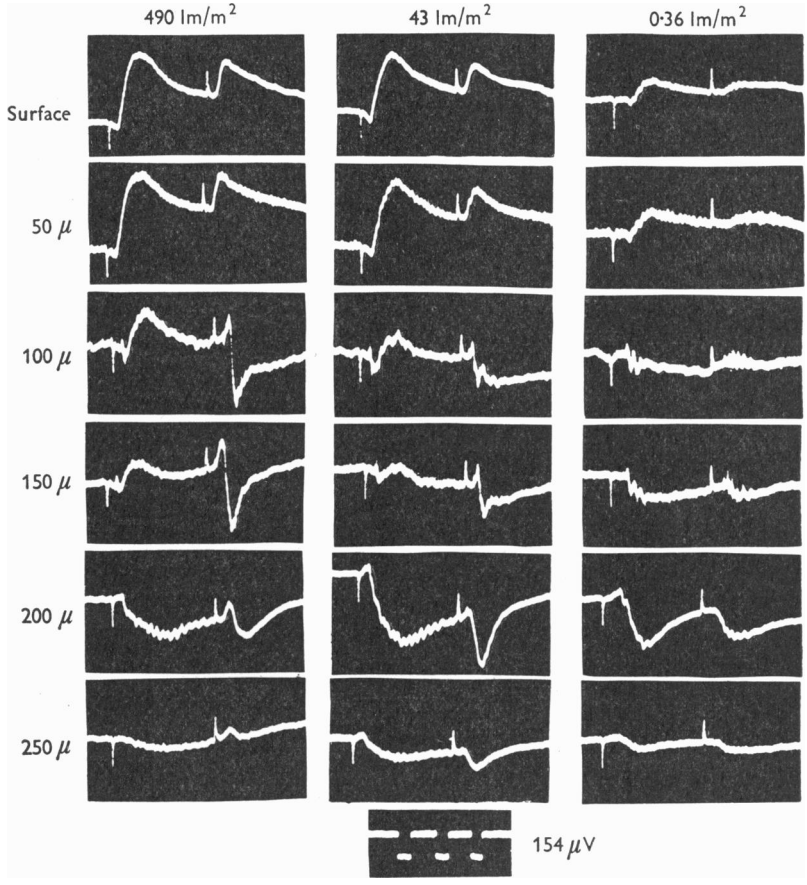

Fig. 4. Effect of varying the intensity of the stimulus on the e.r.g. and on the responses to uniform illumination of the retina, recorded with a micro-electrode inserted into it from the inner surface.

Proportionality of response to stimulus at very low intensities

If the e.r.g. is generated by structures, such as the rods and cones, which may be expected to respond independently of each other, the electroretinographic response to a short flash should be proportional to its intensity for flashes weak enough to provide an average of substantially less than one quantum absorbed per receptor. It has been found impossible to test this

inference in the frog. The weakest short (8 msec) flash which gave a clearly measurable response (of about $10\mu V$) was 8×10^{-5} lumens × seconds/m^2 at wave-length 520 mμ, or about 4×10^{-5} quanta/mm^2 incident. Inferring from the data of Denton & Wyllie (1955) that there are about 3×10^4 rods/mm^2 in *Rana temporaria* and that about 65% of incident light at wave-length 520 mμ is absorbed in rhodopsin, this corresponds to 8·6 quanta absorbed per rod.

This result, though disappointing in itself, does suggest that, given a preparation a little more stable and sensitive than the excised frog's eye and an efficient means of improving the signal-to-noise ratio by averaging many similar responses, the e.r.g. might be detected for flashes providing an average of less than one quantum absorbed per receptor, and the inference of proportionality be tested.

DISCUSSION

The nature of the R membrane

The presence of an R membrane in mammalian eyes shows only that it is not a structure peculiar to the frog or to amphibia. It is consistent with the hypothesis that it is the external limiting membrane, without giving that hypothesis any strong support.

The absence of an R membrane in isolated retinae, the whole relevant region of which had an intact pattern of rods as far as could be seen by microscopical examination before the experiment, gave electrical responses to light everywhere, and appeared on subsequent examination of stained sections to be undamaged except for many bent and a few evidently broken receptors, provides fairly good evidence that the R membrane is not a structure lying in front of the rods and cones. In removing the retina from an opened dark-adapted frog's eye only the outer surface is exposed to direct risk of mechanical injury, and the presence of an intact pattern of rods makes it very unlikely that any such injury can have extended to structures internal to them and protected by them. This argument, and the histological appearance in the one retina of which sections were cut after the experiment, indicate that the external limiting membrane itself probably remained undamaged in these experiments. This can readily be reconciled with the hypothesis of the identity of the R membrane and the external limiting membrane if it is assumed that the surface membranes of the rods and cones act as extensions of the R membrane, accounting for its otherwise astonishingly high capacity of about $100\mu F/cm^2$; rupture of the surface membranes of any substantial number of receptors (which almost certainly occurs) will short-circuit the R membrane so that its effect on the distribution of potential across the retina disappears.

If, as here suggested, the surface membrane of the rods and cones, as well as the external limiting membrane itself, forms part of the R membrane, the event electrically identified as the crossing of the R membrane by a micro-

electrode inserted into the retina from its inner surface may not always represent the penetration of the external limiting membrane by the electrode; sometimes the tip of the electrode may be within a rod or cone when it reaches the level of the external limiting membrane, so that the electrical barrier is not crossed until later, when it leaves the rod or cone. When neighbouring regions of one retina were tested, the apparent depth of the R membrane from the inner surface of the retina did not often vary from one impalement to another by more than about 30μ. This suggests either that such penetration of the level of the external limiting membrane within a rod or cone is uncommon, or that when it happens the electrode usually leaves the rod or cone in which it lay, or disrupts its surface membrane, before it has travelled more than about 30μ beyond the external limiting membrane.

Failure to detect an R membrane in isolated retinae does not of course provide evidence against the hypothesis that it is some structure *behind* the external limiting membrane, for example, the pigment epithelium. This hypothesis, however, is made very unlikely by the measurements of its distance from the anterior surface of the retina and from the sclera (Brindley, 1956*a*). Further evidence that the R membrane is not the pigment epithelium is that in the simple pattern of response the e.r.g. appears across the R membrane, and hence must be generated by structures which penetrate it; for origin of the e.r.g. from the pigment epithelium can only with great difficulty be reconciled with the close similarity between the spectral sensitivity curve of the e.r.g. and the absorption spectrum of rhodopsin (Chaffee & Hampson, 1924; Granit & Munsterhjelm, 1937), and with the Purkinje shift which the e.r.g. shows on light-adaptation (Granit & Wrede, 1937).

The source of slow electrical activity in the frog's retina

The possibility of sampling as many as 25 points scattered all over a preparation and finding the simple (Ottoson–Svaetichin) pattern of responses at all of them, though the e.r.g. is completely normal in shape and of not much less than normal size, provides very strong evidence that the intraretinal responses of the complex (Tomita) pattern make no contribution to the e.r.g., or at least none that substantially affects its shape. The simplicity of the effect of light intensity on the e.r.g. and the complexity of its effect on the intraretinal responses of the Tomita pattern provide further support for the same conclusion. In an eye which gives the Ottoson–Svaetichin pattern of responses everywhere, the R membrane (which we will take to be the external limiting membrane) is polarized with the time course of the e.r.g. The structures which carry the current to do this must either all carry current with the same distribution in time, or, if they are of several kinds in this respect, the carriers of any one temporal distribution of current must have on the average the same spatial distribution of sources and sinks as those of any other temporal distribution of

current; for if not, the response would, contrary to observation, differ in shape for different depths of the recording micro-electrode within the retina.

It was earlier inferred (Brindley, 1956*c*) that carriers of current across the R membrane must necessarily be rods and cones. This inference would be valid for mammalian eyes; but it was made for the frog in ignorance of Landolt's discovery (Landolt, 1871; Cajal, 1894, p. 72 and plate 2) that a large proportion of the frog's bipolar cells have processes which penetrate the external limiting membrane, and could possibly carry current across it. We must also consider, in discussing the structures which carry current across the external limiting membrane, that the potential developed across this membrane, though it may, in the simple pattern of response, be everywhere identical with the e.r.g., may also, in the complex pattern of response, be very different. It was earlier suggested (Brindley, 1956*c*) that the elements active in the complex and inactive in the simple pattern of response might fail to contribute to the e.r.g. because they were oriented tangentially in the retina. If this were the whole explanation of their failure to contribute, the potential developed across the external limiting membrane, much though it might differ from the e.r.g. at any one point investigated, would necessarily be equal to it when averaged over the whole retina. This cannot be so; for in the present experiments, as apparently also in Tomita's work, complex patterns closely resembling those shown in Fig. 7 of Tomita (1950) or Fig. 8 of Brindley (1956*c*) were consistently found at all points of freshly dissected retinae in good condition. Examples of the variability from one eye to another of the complex response patterns and the effect of the intensity of the stimulus on them are shown in Fig. 5. The variability from point to point in the same retina was commonly even less, though the oscillations at about 12/sec often differed in phase and sometimes in frequency between points remote from each other. It is clear that the average of such responses over many points will not differ very much from a sample taken at any one point, though the oscillations are likely to be absent from the average. If the structures which carry the current responsible for the e.r.g. in the Ottoson–Svaetichin state do so also in the Tomita state, in which its time course is the same, there must in the Tomita state be other structures which carry across the external limiting membrane current of time course corresponding (in the sense of being approximately its derivative with respect to time) to the difference between the potential across the membrane and the electroretinographic potential. The carriers of this current should, on the evidence of the spatial distribution of potential in front of the membrane, extend much closer to the inner surface of the retina than the structures which carry the current responsible for the e.r.g. Only the rods and cones are suited anatomically to carry the electroretinographic current, and only the bipolar cells to carry that which corresponds to the difference between the e.r.g. and the potential across the external limiting membrane.

There remains on this hypothesis the problem of why the latter current fails to leave any substantial trace in the e.r.g. A possible explanation, consistent with all the experimental results, is that the bipolar cells act as symmetrical tripoles, a sink lying between two sources. If they are symmetrical in terms of potential, they must be very asymmetrical in terms of current, since the external limiting membrane has a much higher radial resistance than the anterior layers of the retina.

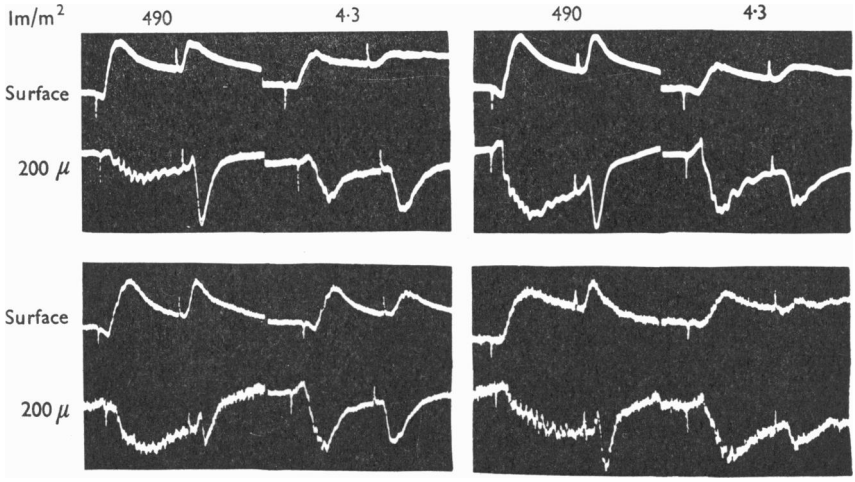

Fig. 5. Responses of four frogs' retinae to uniform illumination at 490 and 4·3 lm/m². In each group, the upper two records are electroretinograms, i.e. responses recorded with the micro-electrode at the inner surface of the retina, and the lower two are the responses to the same stimuli recorded with the micro-electrode at a depth of 200 μ. All records were taken within 15 min of killing the frogs.

The hypothesis of two kinds only of current-carrying conductors—the rods and cones on the one hand, carrying current of time course corresponding to the e.r.g., and the bipolar cells on the other, carrying, as symmetrical tripoles, current of time course corresponding to the difference between the e.r.g. and the potential across the external limiting membrane—accounts very well for the main features of both the simple and the complex patterns of intraretinally recorded responses to spatially uniform illumination. It leaves the origin of the oscillations at about 12/sec uncertain, for they could equally well be a detail of the activity of the bipolar cells or be due to tangentially orientated elements; and there is one feature which it fails to explain, namely that the off-effects at intermediate depths in the neural layers of the retina are often such as cannot be constructed from any sum of multiples of that at the surface of the retina and that immediately in front of the R membrane, or not from the same sum of multiples as is required to construct the on-effect. This is clearly shown in the

records for 490 and 43 lm/m^2 in Fig. 4. Either the internal longitudinal current must differ in time course in different parts of a bipolar cell, or other cells must make some contribution. It is clear that the origins of slow changes of potential within the retina are complex enough to be extremely difficult to analyse with certainty. If two or even three different kinds of simple current-carriers without interaction between one region of the retina and another had been found sufficient to explain all the present results, it might have been possible, after testing a few more consequences of the hypotheses required, to become reasonably sure that they accounted for the whole electrical behaviour of the system; but three kinds of simple carrier are insufficient, for structures resembling the horizontal cells are required to account for the positive-going responses of Fig. 4 of Brindley (1956*c*), and these are inappropriate to explain the change in shape of off-effects with depth. At least four simple carriers or two simple and one complex are needed, and the number of possible explanations of any result is still further increased by the knowledge that one region of the retina certainly interacts with another. The fortunate chance that the complexities can disappear as the preparation ages without change in the e.r.g. allows us to be fairly sure that the rods and cones carry current of time course corresponding to the e.r.g.; the fortunate chance that the bipolar cells are the only other structures which penetrate the external limiting membrane (if we may neglect Müller's fibres) allows us to infer with fair certainty the time course of the current which their outer extremities carry; but detailed analysis of other contributions to the slow electrical activity of the frog's retina is made very uncertain by the multiplicity of probable contributors.

The above argument has been wholly in terms of carriers of current, the assumption being that the retina consists of numerous cells, with cytoplasm of low resistance and a highly resistive surface membrane, embedded in a continuous medium whose resistance is low compared with that of the surface membrane of the cells. A conclusion that the rods and cones *carry* the current responsible for the e.r.g. does not necessarily imply that they *generate* it; but the alternative assumption that they passively carry current generated by other structures is unnecessarily complex, and not very easily reconciled with the failure of separate generating structures to reveal themselves in the electrical records from eyes giving the Ottoson–Svaetichin pattern of responses. The additivity found in the e.r.g., indicating that different regions of the retina act independently of each other in generating it, gives some further support to the hypothesis that the actual generators are the rods and cones, since for them such independence is to be expected, and for elements lying centrally to such extensive tangentially orientated neurones as the horizontal cells it is not.

SUMMARY

1. A membrane of high electrical resistance and capacity (R membrane), previously described for the frog's eye, is present also in the rat and probably in the rabbit. It is absent from frogs' retinae that have been stripped from the underlying pigment epithelium. These findings are consistent with the hypothesis, earlier put forward on the basis of its distance from the anterior surface of the retina and from the sclera, that the R membrane is the external limiting membrane, to which the rods and cones act as extensions, allowing it to have the surprisingly high capacity of about $100 \mu F/cm^2$.

2. Evidence is presented that the complex sequence of responses to diffuse illumination of the whole retina, commonly recorded by a micro-electrode advanced in steps through the retina of a freshly excised frog's eye, is a normal phenomenon, due neither to disturbance of the eye during dissection nor to the presence of the electrode in the region from which the recording is made.

3. The effect of intensity of stimulus on these complex intraretinal responses is very different from its effect on the e.r.g., and the complexities may be absent at all points of a retina without the e.r.g. being abnormal.

4. Different regions of the retina act independently of each other in producing the e.r.g., even when they are so near to each other that such independence is not found in the responses recorded intraretinally.

5. Results 3 and 4 are consistent with the hypothesis that the e.r.g. is generated by rods and cones. The origin of other slow electrical activity of the frog's retina is discussed.

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