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THE PASSIVE ELECTRICAL PROPERTIES OF THE FROG'S RETINA, CHOROID AND SCLERA FOR RADIAL FIELDS AND CURRENTS

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In relating the changes in electrical potential recorded from a living structure to the activity of that structure, or the form and distribution of an externally applied current to the physiological effects that that current may have, it is useful to know something of the passive electrical properties of the structure. The present investigation of the electrical properties of the frog's eye, although it relates only to current flowing radially in the eye, i.e. perpendicularly to the retinal surface, has some value for this purpose. It has also revealed a structure of high radial resistance and capacity and small thickness, here referred to as the 'R membrane', which has proved a useful landmark in investigations of electrical responses of the retina to illumination (Brindley, 1956*c*); and it suggests a new interpretation of the effects, first discovered by Granit & Helme (1939), of steady polarizing currents on the electroretinogram. These effects may be largely due to a decrease during activity in the radial resistance of the retina.

METHODS

Preparation. Opened excised eyes of *Rana temporaria* were used, the cornea, iris, ciliary body and lens being removed together in one piece. In most experiments as much as possible of the vitreous humour filling the cup of the eye was retained, since it was required as an electrical conductor.

Electrodes and manipulators. The preparation was placed in a small porous eathenware cup shaped to fit an average frog's eye. The flat base of the cup rested on a thin plate of porous earthenware which rested in turn over a small chamber filled with Ringer's solution and containing twin spirals of electrolytically chlorided silver wire. One of these spirals was earthed through a resistance of 1200 Ω. The other was available for passing current through the preparation without polarizing the metal-electrolyte junctions of the recording system. The earthenware cup and plate were soaked in Ringer's solution before every experiment. Screw controls allowed the preparation with its underlying cup, plate, chamber and electrodes to be moved in all horizontal directions over the glass-covered floor of a heavy (8 kg.) iron box mounted on three soft rubber pillars.

Glass capillary microelectrodes, filled usually with 3 M-KCl solution but in a few experiments with Ringer's solution, of tip diameter less than 1 μ (resistance, when filled with 3 M-KCl solution, from 3 to 7 MΩ), were attached to a vertically-moving micromanipulator graduated in 10 μ steps, mounted on the fixed part of the roof of the heavy iron box containing the preparation. Electrical contact with the microelectrode was made through a chlorided silver wire entering its shank, and screened from the light used to stimulate the preparation. A second vertical micromanipulator, also mounted on the fixed part of the roof of the heavy iron box, was available to carry when necessary a second upper electrode, usually a chlorided silver wire.

Amplification and recording. The microelectrode was connected to the grid of the first amplifying valve of a single-sided battery-operated preamplifier. The grid current taken by this valve was in most of the experiments about 7×10^{-12} A. In a few early experiments it was as high as 5×10^{-11} A. The stray capacity between the microelectrode and earth was insufficient, even with an electrode of 50 MΩ resistance, to cause appreciable high-frequency loss within the range of frequencies covered by the responses with which these investigations are mainly concerned, though spikes were probably somewhat attenuated. The smallness of the high-frequency loss, even for an electrode of this exceptionally high resistance, can be judged from the square calibrating pulses of Fig. 2.

The preamplifier and its batteries and the heavy iron box containing the preparation were kept within an earthed cage made mainly of sheet iron, which served as electrostatic, electro-magnetic and optical screening. Outside the cage were most of the optical equipment for stimulating the eye, the cathode-ray oscilloscope which was fed by the preamplifier, a camera recording the deflexions of the two spots of the oscilloscope tube, usually on moving film, and a simple thyratron oscillator whose frequency was modulated by the deflecting voltage of one or other of the oscilloscope spots. This oscillator, feeding a loudspeaker, provided convenient auditory indication of changes in potential of the microelectrode, and made it unnecessary to watch the oscilloscope tube whilst manipulating the preparation.

In most experiments both beams of the oscilloscope recorded the potential difference between the microelectrode and earth. One, taken from the second stage of the preamplifier with direct coupling throughout, gave a gain of about 10 mV/cm. The other, taken from the third stage of the preamplifier, with two resistance-capacity couplings of over-all time-constant 0·77 sec, gave between 20 and 200 μV/cm. The auditory presentation was usually used at a gain of about 30 μV/semitone.

Optical equipment. The details of this are of interest only in connexion with the two following papers (Brindley, 1956*b*, *c*), but it was used incidentally in the present experiments, and it is convenient to describe it here. The apparatus, which is shown in Fig. 1, was designed to provide a stimulating spot, variable in size, shape and position, superimposed on a steady uniform illumination of the whole eye, usually at one-tenth of the intensity of the stimulating spot.

The lenses L_1 and L_2 formed coincident images of the ribbon filaments of the lamps S_1 and S_2 on, and almost completely covering, the lens L_3. The light from the two paths was combined by the beam-splitting cube P_1. The right-angled prism P_2, which was mounted on the detachable part of the roof of the iron box containing the preparation, reflected the light down on to the retina, on which images of the stops H_1 and H_2 were formed by the lens L_3, whose mounting allowed focusing adjustments. The image of H_1, providing the steady background, was always larger than the retina. H_2, which provided the stimulating spot, was one of a series of interchangeable drilled plates mounted on a microscope's mechanical stage. Its image on the retina was 1/6·6 of its own diameter, which could be varied between 0·41 and 24 mm. White light was used in all the experiments of this and the following two papers, its intensity being controlled by the colloidal carbon neutral filters F_1 and F_2. A photocell, placed close to L_2, sent a pulse into the third stage of the preamplifier when the shutter was opened or closed, so that the beginning and end of each stimulus was marked on the same trace as the response of the retina to it.

At the back of the iron box containing the preparation was a large opening through which the

electrode and the preparation could be inspected through a specially mounted microscope. Inspection through the microscope was used to focus the stimulating spot and to adjust its position to coincide with the tip of the electrode. It was sometimes used to determine the instant at which the tip of an electrode touched the exposed *posterior* surface of the retina. For determining when the tip of an electrode touched the *anterior* surface of the retina it was found unreliable because of the difficulty of seeing this surface, and an electrical criterion was generally used.

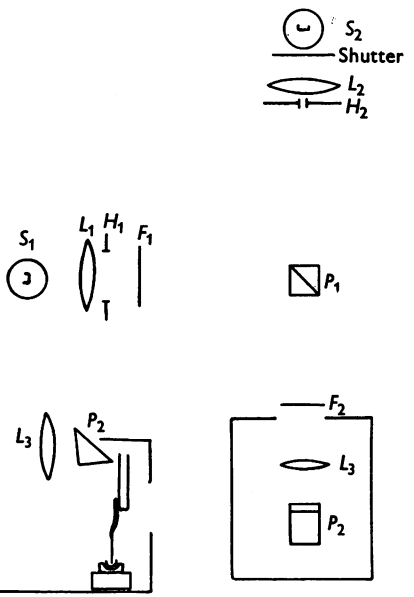

Fig. 1. Optical part of apparatus. S_1, S_2, ribbon filament lamps; L_1, L_2, L_3, achromatic lenses of focal length 10 cm; H_1, H_2, field stops, imaged by L_3 on the retina; F_1, F_2, neutral filters; P_1, non-polarizing beam-splitter.

RESULTS

The radial electrical resistance of the coats of the eye

To measure the radial electrical resistance of the whole thickness of the frog's retina, pigment epithelium, choroid and sclera, an opened excised eye was placed in the apparatus on a cup of porous earthenware which fitted the posterior surface of the sclera, twin upper electrodes of chlorided silver wire were lowered side by side into the vitreous, a square pulse of current was passed between one of them and one of the twin lower electrodes beneath the preparation, and the potential difference developed between the other upper and the other lower electrode was measured. The voltage pulse developed in response to a square current pulse was found to be conspicuously rounded, in the manner to be expected if a substantial part of the resistance of the preparation had a capacity in parallel with it. The steady value of the potential

difference reached was found to be proportional to the current flowing over the range 0·5–18 μA, and over this range of currents there was no rectification: on reversing the direction of a current pulse, the observed voltage pulse was not detectably changed except for the reversal of its polarity.

Since the distance, and hence the resistance, between each upper or lower electrode and its twin was small compared with that between the upper pair and the lower pair, the ratio of the steady potential reached to the current flowing was very nearly equal to the resistance between the upper pair and the lower. To analyse the components of this resistance, the coats of the eye were successively removed. From some eyes, an attempt was first made to remove the retina, without injury to the pigment epithelium. This was possible only in dark-adapted eyes, since in light-adapted eyes the pigment epithelium adhered closely to the retina and came away with it; and only for two of the eyes from which removal of the retina was attempted did it appear on close inspection of the retina that no traces of pigment epithelium had been torn away. From two light-adapted eyes the retina and pigment epithelium were successfully removed together, without any apparent damage to the choroid. After removal of the retina, or of the retina and pigment epithelium together, the eye was replaced in the apparatus, the lost vitreous being replaced if necessary with Ringer's solution, and the resistance again measured with the upper electrodes in the same positions as before. The choroid, or choroid and pigment epithelium, were then removed and the measurement repeated with only the sclera in place. A final measurement was made with no part of the eye in the apparatus, the earthenware cup which had supported it being filled with Ringer's solution. The results of these experiments, expressed as the change in inter-electrode resistance on removing different parts of the preparation, are given in Table 1.

The mean inter-electrode resistance with no eye in the apparatus was 370 Ω. Since this was smaller than the resistance of the eye, and the length of path through the easily conducting Ringer's solution and vitreous was not very different for current passing through different parts of the eye, the distribution of radial current through the retina, pigment epithelium and choroid should be mainly determined by the distribution of their radial resistance. If we make the simple assumption, based on their approximate histological uniformity, that the radial resistance is the same in all parts, then the radial current density should be uniform, and the product of the observed change in inter-electrode resistance on removing a layer and its area in cm^2 should provide a good estimate of its radial resistance in Ω cm^2. With the sclera alone or sclera and choroid in the apparatus, the distribution of current will not be so uniform, but the assumption of exact uniformity should give a rough estimate of the radial resistance, the true value being probably lower than the estimate.

The eyes used in these experiments did not vary much in size, and the mean area of four of them, measured at the end of experiments by making three or four radial incisions, flattening them on graph paper and counting the squares covered, was 0·38 cm². Taking this mean value and those of Table 1, the estimated radial resistance of the retina is 197 Ω cm², of the pigment epithelium and choroid together 78 Ω cm², of the choroid alone 23 Ω cm², and of the sclera 25 Ω cm², the last two estimates being probably too high owing to non-uniformity of current density. For comparison, the resistance of a layer of Ringer's solution of the same thickness as the sclera (160 μ) is about 1·4 Ω cm².

TABLE 1. Change in inter-electrode resistance on removing in succession from between the electrodes the coats of the eye. 1, light-adapted eye; 2, dark-adapted eye; 3, from these eyes some small fragments of the pigment epithelium had been torn away with the retina

Layer removed		Change in resistance (Ω)	Layer removed	Change in resistance (Ω)
Retina + pigment epithelium + choroid	1	1120	Sclera	107
			Sclera	96
Retina + pigment epithelium + choroid	1	690	Sclera	48
			Sclera	83
Retina + pigment epithelium + choroid	2	710	Sclera	63
			Sclera	31
Retina + pigment epithelium + choroid	2	680	Sclera	65
			Sclera	33
Retina + pigment epithelium + choroid	2	730		
Retina only	2	480		
Retina only	2	560		
Choroid only		24		
Choroid only		96		
Pigment epithelium + choroid	2	180		
Pigment epithelium + choroid	2	190		
Pigment epithelium + choroid	2, 3	250		
Pigment epithelium + choroid	2, 3	200		

The rounding of the observed voltage pulse when a square current pulse was passed through an opened excised eye with all its coats intact may be seen in Fig. 2. The degree of rounding varied little from one eye to another provided that the retina was intact; but killing the eye with chloroform or cocaine, or removing the retina with or without the pigment epithelium, always reduced it to a very small amount, indistinguishable from that found when nothing but earthenware soaked with Ringer's solution separated the electrodes. The part of the impedance of the preparation responsible for the rounding resembles in its behaviour a parallel capacity. For the square pulses used in the present experiments, its effect could be adequately matched by roughly 100 μF cm^{-2} in parallel with half of the 200 Ω cm² representing the ohmic part of the impedance loss on removing the retina.

The distribution of resistance, capacity and resting potential through the thickness of the retina

When a microelectrode was lowered gradually, in 25 μ or 50 μ steps, through the vitreous humour of an eye from which cornea, iris and lens had been removed, the thyratron oscillator and loudspeaker having been adjusted to give a gain of about 30 μV/semitone, it was usually found that every advancing of the electrode was electrically uneventful, causing no change in the pitch of the note produced by the loudspeaker, until suddenly, at a point where the

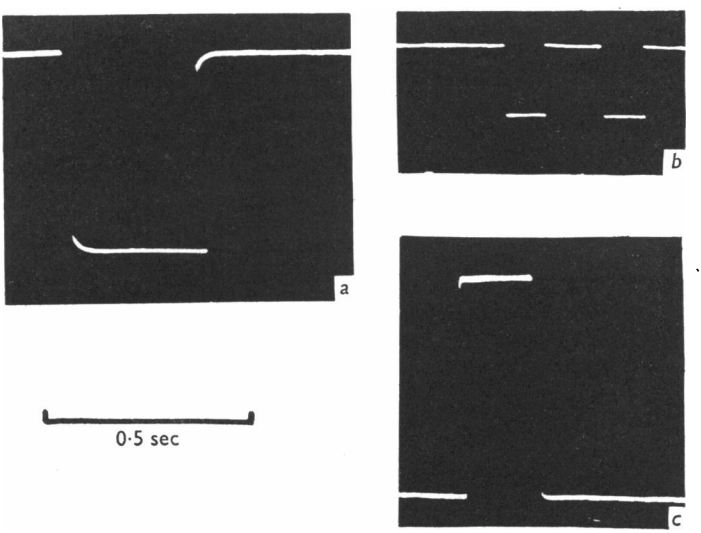

Fig. 2. *a*, voltage pulse recorded across the coats of an opened excised eye and its supporting dish when a square current pulse of 18·0 μA was passed through it—the steady level reached corresponds to a resistance of 1090 Ω. *b*, the same after removal of retina, pigment epithelium and choroid. The steady level reached corresponds to 380 Ω. *c*, square pulse of 21·6 mV recorded through a KCl-filled electrode of resistance 50 MΩ.

tip of the electrode seemed on examination through the microscope to be close to the surface of the retina, a large change in pitch was heard. On subsequent inspection of the photographic record of the low-gain direct-coupled channel, this was seen to correspond to a negative-going potential step of between 0·4 and 11 mV. This negative-going potential step was generally taken as the criterion that the tip of the electrode had reached the surface of the retina. The justification for this was the complete electrical uneventfulness of all or nearly all movements of the electrode before it was reached (suggesting that the tip was then in a homogeneous medium), the fairly regular sequence of changes in potential and resistance as the electrode was advanced beyond it, and the approximately constant distance (varying between 305 and 450 μ in the

fifteen eyes for which this was satisfactorily measured) between it and the point at which the electrode began to bend against a firm obstacle, evidently the cartilaginous sclera.

At the point where the potential showed its first negative-going step, the amount of random activity visible on the high-gain record usually increased. Such an increase in noise was found also by Ottoson & Svaetichin (1953). In the present experiments it was not, as in those of Barlow (1953) with a 20 μ platinum electrode, mainly due to an increase in impedance at the junction between the electrode and the preparation; it could easily be shown to be due largely to microphonicity of the electrode. While the tip of the electrode was in the vitreous or, in a physical experiment, immersed in Ringer's solution, its microphonicity was less than that of the preamplifier, which was small. As soon as the tip entered the retina, it became very microphonic, so that, despite the anti-microphonic mounting of the heavy iron box containing the preparation and electrode, vibrations transmitted from the camera motor often caused a visible electrical disturbance. The degree of microphonicity varied from point to point as the electrode was advanced through the retina. It was usually greater in a freshly excised eye than in one that had been in the apparatus for an hour or more, and very much less in a dead eye.

In all the experiments of this section, between each 25 μ or 50 μ movement of the microelectrode, a square current pulse, usually of 16·8 μA, was passed between a silver wire electrode in the vitreous and one of the twin silver wire electrodes below the eye, and the voltage pulse recorded by the microelectrode was measured. This gave a measure of the radial resistance between the layer of the retina or choroid in which the tip of the electrode lay and the twin electrodes below the eye. In every experiment a very conspicuous landmark was found, to which other phenomena, less conspicuous or less consistently obtained, could conveniently be referred. At a depth from the surface of the retina varying from 150 to 300 μ, but most often 225 μ or 250 μ, one step of 25 μ or 50 μ caused a sudden diminution in the size of the voltage pulse produced by a given square current pulse, and at the same time a complete or very nearly complete disappearance of rounding, as if the electrode had in that step passed through a membrane of high resistance with a large capacity in parallel. This hypothetical membrane is referred to as the 'R membrane'. Its probable anatomical nature will be discussed later. In the same 25 μ or 50 μ step which produced the large fall in resistance, a fall in potential was found, usually of between 10 and 30 mV, but once (in thirty-five experiments) as large as 41 mV, once as small as 2 mV, and once a *rise* of 4 mV.

In many experiments, when the large fall in resistance had been found, the electrode was withdrawn by the same amount (50 or 25 μ) that had been required to produce it; and this single step backwards usually sufficed to restore the resistance, the rounding of the voltage pulse, and approximately the

initial steady potential level. This provides good evidence that the retina had not been much distorted by the electrode, so that the true distance between the retinal surface and the structure responsible for the large change in resistance and capacity cannot be very different from the distance moved by the microelectrode between reaching the retinal surface and causing the fall in resistance.

TABLE 2

		Expt. 29a/3	Expt. 30a/3	Mean of all expts.	Range	No. of observations
(1)	Distance from anterior surface of retina to R membrane (down through membrane) (μ)	230	215	233	160–300	32
(2)	Distance from anterior surface of retina to R membrane (up through membrane) (μ)	170	195	—	—	2
(3)	Distance from anterior surface of retina to sclera (by microelectrode) (μ)	350	450	373	305–450	15
(4)	The same distance (3) measured microscopically on frozen sections of the same eye (μ)	278	315	—	—	2
(5)	Change in resistance on crossing R membrane (Ω)	250	410	271	100–560	20
(6)	Time-constant of R membrane (msec)	9·5	7	About 10	6–15 (approx.)	20
(7)	Capacity of R membrane (from 5 and 6) (μF)	38	17	About 40	—	20
(8)	Difference of potential across R membrane (mV)	−19·5	−8·9	−17·2	−41 to +5	35

Measurements relating to the R membrane are collected in Table 2. The first two columns of this table are from two exceptionally complete experiments carried out in the following manner.

The electrode was advanced slowly through the vitreous until the first negative-going potential step was heard, and the micromanipulator reading noted. It was then withdrawn and advanced again to the first negative-going potential step. The two micromanipulator readings were in each experiment found to agree within 5 μ. Photographic records of the electrical response to illuminating the whole retina and of the potential change on passing a square current pulse of 16·8 μA were obtained. The electrode was then advanced quickly by 150 μ, and further records obtained. From this point it was advanced slowly until the large negative-going potential step corresponding to the R membrane was reached, and the responses to illumination and to a square current pulse again recorded. It was then withdrawn slowly until the previous potential level was restored. This occurred suddenly after withdrawals of 60 and 20 μ in the two experiments and was found on subsequent inspection of the photographic record to be accompanied by restoration of the resistance and capacity characteristic of the R membrane. Finally the electrode was advanced until it could be seen, on inspection through the microscope, to begin to bend against a firm obstacle, assumed to be the sclera. The point at which this occurred was in these two experiments (though not in all others) very well-defined, and its measured position is unlikely to be in error by more than 10 μ.

Sections of these two eyes were cut at 20 μ thickness on a freezing microtome and examined and measured unstained in Ringer's solution within half an hour of the end of the experiments.

The other data of Table 2 are collected from many experiments, in most of which the electrode was moved in steps of 25 μ. Each measurement of distance may thus be in error by as much as 25 μ in either direction.

As the microelectrode was moved through the retina each step forward usually produced a change in the steady potential recorded. Two of these changes, that at the surface of the retina and that corresponding to the R membrane, were obtained consistently, and have already been mentioned. The other changes found did not follow a clear pattern that could be identified in every experiment; but in about half the experiments the following sequence was observed. The negative-going step corresponding to the anterior surface of the retina was followed by further negative-going steps of from 0·5 to 10 mV on the next one, two or three advancements of 25 μ, then by one positive-going step of from 6 to 18 mV, then by no further change in potential until the R membrane was reached at about 250 μ depth. Beyond the R membrane there was usually no change in potential until the electrode bent against the sclera.

In one experiment, at a depth of 50 μ from the anterior surface of the retina, a negative potential step of 76 mV was found. The potential was maintained constant for about 15 sec, during which a record of the electrical response to illumination of the whole retina was obtained. This response was of the same shape as the normal electroretinogram, with a-, b- and d-waves, and did not include any spikes. After 15 sec the potential started to rise rapidly, without the electrode having been moved, and within 30 sec more had become steady at almost its initial level. Transient large negative steps were observed on about twenty other occasions during these and similar experiments, though no other was as large as 76 mV or lasted as long as 15 sec. It seems likely that they were due to penetration of a large nerve cell by the tip of the electrode.

Histological measurements

Measurements were made on paraffin sections, stained with haemalum and van Gieson's stain, of nine eyes fixed in Susa's fixative, and on unstained sections cut on a freezing microtome and examined in Ringer's solution of the two eyes to which the first two columns of Table 2 refer. The results are shown in Table 3. It can be seen that there are considerable variations from the mean values. These variations were much greater from one retina to another than from place to place in the same retina, provided that the extreme anterior part (which was in any case not used in any of the microelectrode measurements), and any part in which, from the appearance of the rods and cones, the section could be seen not to be truly radial, were excluded.

TABLE 3. Distances in microns from the anterior surface of the retina in sections of eleven frogs' eyes. Columns 1 to 9 give measurements from stained paraffin sections, 1 to 4 being light-adapted and 5 to 9 dark-adapted. Columns 10 and 11 are from unstained frozen sections of the eyes of experiments 29a/3 and 30a/3, for which other data are given in Table 2. Column 12 gives the mean of columns 1–9, and column 13 these means corrected, assuming uniform shrinkage, for a total thickness of 373 μ

	1	2	3	4	5	6	7	8	9	10	11	12	13
Middle of ganglion cell layer	10	11	15	13	7	9	14	21	13	—	—	12	18
Beginning of inner nuclear layer	71	53	82	84	70	45	60	92	59	—	—	68	101
End of inner nuclear layer	119	90	127	141	111	85	93	137	89	—	—	110	164
Beginning of outer nuclear layer	131	98	140	155	124	91	105	152	101	—	—	122	182
External limiting membrane	146	112	158	178	146	102	120	170	112	139	153	138	206
Posterior boundary of pigment epithelium	214	169	225	259	210	175	194	252	174	233	264	208	310
Posterior boundary of choroid	245	207	266	301	—	224	259	284	217	278	315	250	373

DISCUSSION

The properties and anatomical nature of the R membrane

The name 'R membrane', introduced for brevity in referring to a conspicuous group of phenomena regularly observed when an electrode was advanced through the retina from its anterior surface, assumes that there is an anatomical structure responsible for the phenomena, but does not prejudge the question of what this anatomical structure may be. The clearest evidence on its site comes from the Expts. 29a/3 and 30a/3 of Table 2. The point at which the electrode first penetrated the R membrane must certainly be at or beyond that membrane's position in the undisturbed state, and the point at which it passed through on withdrawal must almost certainly be at or short of the undisturbed position. Allowing $\pm 5\mu$ for the uncertainty in position of the anterior surface of the retina, this fixes the R membrane at between 165 and 235 μ from the anterior surface of the retina in experiment 29a/3 and between 190 and 220 μ in 30a/3. The total thickness of retina and choroid in the frozen sections was in both experiments less than the same thickness measured by microelectrode. Since the microelectrode was certainly not more than 20° from perpendicular to the retina, the sections had presumably shrunk. The agreement of their measurements with those of paraffin sections, for which shrinkage is to be expected, support this conclusion. Assuming provisionally that shrinkage is uniform, the corrected histological distances are, from the anterior surface of the retina to the external limiting membrane, 175 μ in 29a/3 and 217 μ in 30a/3, and from the anterior surface of the retina to the posterior surface of the pigment epithelium, 293 μ in 29a/3 and 378 μ in 30a/3. These distances agree well with the hypothesis that the R membrane is the external limiting membrane or some structure very close to it, and they contradict fairly clearly the hypotheses that it is the pigment epithelium, or

(taking into account the measurements on stained sections) that it lies in or anterior to the inner nuclear layer. The results of the other experiments shown in Table 2, though they are less complete, support these inferences. The observation of p. 343 that the rounding of the voltage pulse due to the passage of a square current pulse was abolished by removing the retina (the pigment epithelium remaining apparently intact) provides further evidence that the R membrane is not the pigment epithelium.

The only structure close to the external limiting membrane which seems likely, from its histological appearance, to be a complete transverse barrier of high electrical resistance and capacity is the external limiting membrane itself. The work of Sjöstrand (1953), who inferred from electron micrographs of ultra-thin sections of guinea-pig retinae that the external limiting membrane is not continuous, but consists of rings or collars around the bases of the inner segments of the rods, does not seem to favour this interpretation, since the high resistance and time-constant of the R membrane preclude its having substantial gaps; but the other layers of the retina (bacillary, outer nuclear and outer synaptic layers) which are admissible on grounds of distance are so barren of any visible transverse barrier that the identity of the R membrane and the external limiting membrane seems the most likely hypothesis.

The mean membrane time-constant of the R membrane (about 10 msec) is a little lower than that of the cell membrane of the frog's muscle fibres (Katz, 1948; Fatt & Katz, 1951) and a little higher than most other cell membranes which have been investigated (e.g. 2·3 msec, *Homarus* axons, Hodgkin & Rushton, 1946; 4 msec, motor neurones of cat spinal cord, Brock, Coombs & Eccles, 1952). The capacity of the R membrane, averaging about $100\,\mu F\ cm^{-2}$, is very much higher than that of most cell membranes; nearly all the values collected by Cole (1942) lie between 0·5 and $3\,\mu F\ cm^{-2}$, though Fatt & Katz (1953) found the very high value of $40\,\mu F\ cm^{-2}$ for crab muscle fibres. If the R membrane is a single membrane with thickness of the order of 100 Å and a reasonable dielectric constant, it must be much folded, either on a microscopic or a submicroscopic scale. If it is in fact the external limiting membrane, the required folds could possibly be the cell membranes of the rods and cones which pierce it.

The retinal resting potential

It has long been known (Bois-Reymond, 1849) that the cornea of the intact vertebrate is electrically positive in relation to the posterior part of the sclera. This resting potential, usually about 6 mV in the frog, may be in part due to the lens (Brindley, 1956*a*). Of that part which is retinal in origin, the present results suggest that it is the sum of potential steps occurring in several layers of the retina. The largest was in these experiments nearly always that at the R membrane. The anterior surface of the retina was associated with a smaller step of the same polarity, and in about half the experiments a well-defined step

of opposite polarity occurred at an apparent depth of from 50 to 125 μ. The latter two steps were never associated with large changes in radial resistance.

Observations on the steady potentials recorded by intraretinal microelectrodes have been published by Tomita (1950) and Ottoson & Svaetichin (1952). Tomita, using electrodes of 7 to 15 μ external diameter, found that the potential changes observed on advancing the electrode were not reversible on withdrawing it, and concluded that they were mainly due to injury. This would not apply to the present experiments, where the change in potential across the R membrane was always, and the other changes usually, approximately reversible on withdrawing the electrode. Ottoson & Svaetichin, using electrodes similar to the present ones, concluded from experiments which are described only very briefly that the retinal resting potential arises wholly from the receptors. They found that the 'steady potential (6–10 mV) did not undergo any changes until the electrode had reached a depth of approximately 150–175 μ. When the electrode was inserted deeper (200–225 μ), the steady potential dropped to zero.' The present experiments never revealed a pattern quite like this; but they agree in that the *largest* change in potential (R membrane) was commonly at a depth of 200–225 μ.

It remains to be considered whether the observed changes represent true differences of potential. Alternative possibilities are:

Change in electrode resistance. In a few experiments the resistance of the electrode was measured at every step as it was advanced through the retina. In no step did it ever change by more than 2 MΩ, corresponding, with a grid current of 7×10^{-12} A, to a potential difference of 14 μV. This was quite negligible in comparison with the changes in potential observed.

Effect of mechanical conditions around the electrode tip. No apparent potential changes exceeding 100 μV were observed when electrodes were advanced through the retinae of eyes killed by immersion in chloroform for an hour and then equilibrated with Ringer's solution for 2–4 hr, though the mechanical disturbances (including contact with the sclera) should have been similar to or more severe than those met in the living retina. These experiments on dead eyes did, however, indicate that mechanical factors may be concerned to some extent in the variations in microphony found as an electrode was advanced through living retina. The *main* cause of this microphony is likely to be the presence of a large electric field around the tip of the electrode; but contact of an electrode tip with dead sclera was found to produce, apparently purely for mechanical reasons, a detectable increase in microphony, though not as large as was commonly found in the living retina.

Effect of chemical conditions around the electrode tip. The effect of variations in ionic composition within physiological ranges on the junctional potential at the tip of an electrode filled with 3 M-KCl solution should be small, and it

seems unlikely that they account for a large part of the present changes; but further physical investigations of the properties of these electrodes will be required before their contribution can be accurately assessed.

The effect of illumination on the radial resistance of the retina

If a constant current is passed between the inside and the outside of the eye, a change in radial resistance will show itself as a change in the potential difference across the eye. If such a change in radial resistance occurs during illumination, the resulting change in potential difference will be added to the electroretinogram. For this to be the explanation of the effects of polarizing currents on the electroretinogram discovered by Granit & Helme (1939), a necessary condition is that the difference between the modified and unmodified electroretinogram be proportional to the polarizing current. Benoit & Cornu (1953) did not find this to be so: in their experiments the effects of currents between 50 and 100 μA were opposite to those of large currents of the same polarity.

In the present programme the effects on the electroretinogram of steady polarizing currents between 33 and 467 μA were tested on three eyes. In all of these, large currents from back to front of the eye greatly increased the height of b- and d-waves without obviously changing their shape, and the same currents in the opposite direction greatly decreased the height of the b-wave and abolished or inverted the d-wave. The effects of the smallest currents (33 and 67 μA) were similar to but much smaller than those of large currents. Accurate measurement of the records was made difficult by the considerable drift of base line caused by the large polarizing currents, but as far as could be detected all effects were proportional to the currents producing them. These experiments thus do not contradict the hypothesis that the phenomena are due to a change due to illumination in the radial resistance of the retina. If this explanation is correct, the change required to explain the present results is a decrease in resistance of similar time-course to the b- and d-waves of the electroretinogram, and amounting at its peak to between 0·3 and 0·8 Ω.

SUMMARY

1. The radial electrical resistance of the coats of a frog's eye from which cornea, iris and lens have been removed is about 800 Ω, or 300 Ω cm^2, of which about 200 Ω cm^2 is lost when the retina is removed.

2. The eye behaves in relation to square pulses of radial current as if it has a capacity of about 100 μF cm^{-2} in parallel with one-third of its radial resistance. All or nearly all of this apparent capacity is lost when the retina is removed.

3. When a microelectrode is advanced through the retina from its anterior surface, the resistance and capacity between the tip of the microelectrode and the sclera show a sudden large decrease, reversible on withdrawing the electrode,

at a mean depth of 233 μ from the surface of the retina. At the same point, the potential of the tip of the electrode falls suddenly, usually by between 10 and 30 mV. A structure, the 'R membrane', is postulated to account for these properties, and is provisionally identified with the external limiting membrane. The resistance of the R membrane is about 270 Ω, or 100 Ω cm^2, and its capacity, as estimated from square current pulses, about 40 μF, or 100 μF cm^{-2}.

4. The changes of potential found as an electrode is advanced through the retina indicate that the largest component of the retinal resting potential is the potential difference across the R membrane.

5. The effects of steady polarizing currents on the electroretinogram may be largely due to a decrease during activity, amounting at its peak to between 0·3 and 0·8 Ω, in the radial resistance of the retina. This is of the order of 0·1 % of the total retinal resistance.

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