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THE PROPERTIES AND NATURE OF THE R MEMBRANE OF THE FROG'S EYE

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One of us (Brindley, 1956, 1957) has already reported that if a rectangular pulse of current (i.e. a pulse beginning and ending instantaneously, and of constant strength while it lasts) is passed through the coats of a frog's eye, and the difference of potential between the sclera and the vitreous is recorded, the pulses of potential caused by the applied current pulses are not rectangular but rounded, approximately as would be expected if part of the electrical resistance of the coats of the eye were shunted by a capacity. If an excised eye with the cornea, iris and lens removed is used, and the potential pulses are recorded between a large electrode in contact with the sclera and a micro-electrode inserted into the retina from its vitreal surface, they are found to remain rounded until the micro-electrode reaches a depth which varies from one experiment to another between 160 and 300 μ from the internal limiting membrane, but is usually near to 230 μ. At this depth the pulses suddenly become smaller and rectangular, as if the tip of the electrode has passed through the structure responsible for the shunt capacity and for a substantial fraction of the resistance. The change can be reversed by withdrawing the micro-electrode a short distance.

The structure postulated to explain the electrical phenomenon that is met at an apparent depth of about 230 μ was given the non-committal name of 'R membrane' by Brindley (1956), and was provisionally identified on indirect evidence with the external limiting membrane. This provisional identification has been opposed by Tomita, Murakami & Hashimoto (1960), but supported by Byzov (1961). Tomita *et al.* found that a resistance comparable with that of the R membrane could be found in the shell of pigment epithelium, choroid and sclera left after removing the pars optica retinae, but could not be found after removing the whole retina including the pigment epithelium. They argued from this, and from observations of Brown & Wiesel (1958) on a similar phenomenon in the cat's eye, that the R membrane is Bruch's membrane, the structure that separates the pig-

ment epithelium from the choroid. Byzov rejected their conclusion on the following grounds:

(1) His measurements and those of Brindley (1956) showed that the distance through which a micro-electrode had to be advanced from the inner surface of the retina to reach the R membrane was often substantially less than the measured distance from the inner surface of the retina to Bruch's membrane.

(2) He was able to detect in isolated pars optica retinae an impedance whose effect on rectangular pulses of current resembled that of the R membrane.

(3) In the paper of Tomita *et al.* only the *resistance* of the R membrane was stated to persist after removal of the retina; capacity was not mentioned, and no experimental records showing rounded pulses of potential were reproduced.

The purpose of the present paper is to show that Tomita and his colleagues were right in concluding that the R membrane of the frog's eye lies outside the pars optica retinae, but to argue that it is not Bruch's membrane, but the inner bounding membrane of the pigment epithelium. The paper also resolves an apparent discrepancy between the estimates by Brindley (1956) and by Byzov (1958) of the time constant of the R membrane.

METHODS

Eyes of *Rana temporaria* were used, excised and with the cornea, iris and lens removed. The apparatus differed only in minor details from that of Brindley (1956) and Byzov (1958). The eye lay in a closely-fitting cup of porous earthenware. Rectangular pulses of current were generated by means of a battery, a large series resistance and a high-speed relay, and were passed between two chlorided silver wires, one lying in the vitreous or in Ringer's solution by which the vitreous had been replaced, and the other in contact with the earthenware cup. Pulses of potential were recorded between another chlorided silver wire in contact with the earthenware cup and a pipette micro-electrode filled with 3M potassium chloride solution inserted from the vitreous. The resistance of every micro-electrode was measured very frequently, and any event that might be interpreted as penetration of the R membrane was considered significant only if the resistance of the electrode lay in the range 5–40 MΩ immediately before it and immediately after it.

RESULTS

The difference between the estimates by Brindley (1956) *and by Byzov* (1958) *of the capacity of the R membrane*

Brindley (1956, 1957) published only records made on a slow time base. He examined records on faster time bases, but considered them uninterpretable because the pulses of current then used did not begin and end sharply enough. The low-speed records were fitted adequately by exponential curves with time constants of about 10 msec. On the simple model that

the R membrane is a capacity with a resistance in parallel, they required the capacity to be about 100 μF per square centimetre of retina. Byzov (1958) published only records made on a fast time base. These records were adequately fitted by exponential curves with time constants of about 0·3 msec, and Byzov's estimate for the capacity of the R membrane, on the same simple model, was 3μF per square centimetre of retina.

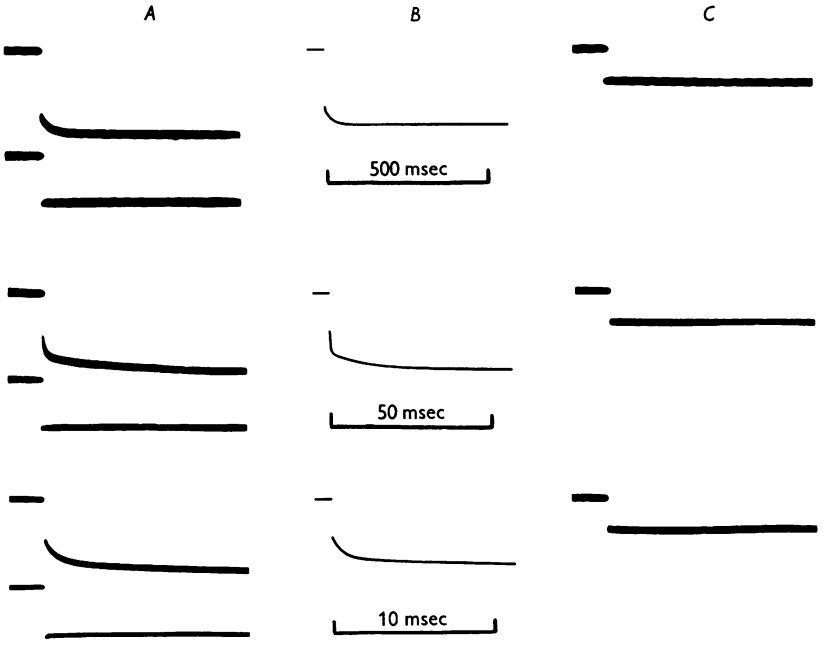

Fig. 1. *A*. Records on three time scales of the difference of potential between a 3 M-KCl-filled capillary micro-electrode inserted into the retina of an opened excised frog's eye and the sclera of the eye. Each pair of records shows the effect of a constant current i suddenly applied between vitreous and sclera; the lower trace of the pair is the potential developed by this current across a resistance of 950 Ω, the upper the potential developed by it between the micro-electrode and the sclera. Descent of either trace corresponds to increase of developed potential.

B. The function $E = a(1-e^{-t/\alpha})+b(1-e^{-t/\beta})+c$, plotted on the same three time scales for $\alpha = 11{\cdot}3$ msec, $\beta = 0{\cdot}32$ msec, $a/i = 344\,\Omega$, $b/i = 434\,\Omega$, $c/i = 788\,\Omega$.

C. Records made in the same way as the upper of each pair in *A*, except that the micro-electrode had been advanced by 12 μ, and had, as the records show, passed through the R membrane.

Figure 1 *A* shows new records of the same kind, but recorded successively on three time scales. It can be seen that the pulse of potential appears approximately exponential both on the fastest and on the slowest record, but with very different time constants. Figure 1 *B* shows the function $E = a(1-e^{-t/\alpha})+b(1-e^{-t/\beta})+c$ plotted on the same three time scales for

$\alpha = 11{\cdot}3$ msec, $\beta = 0{\cdot}32$ msec, $a/i = 344\,\Omega$, $b/i = 434\,\Omega$, $c/i = 788\,\Omega$. This function describes the experimental curves fairly well, and no simple exponential function can do so. Figure 1*C* shows that both the fast and the slow component of the rounding disappear when the micro-electrode is advanced by 12 μ.

Records like those of Fig. 1 could be obtained from at least 70 % of eyes that were taken from lively, healthy-looking frogs and examined within 15 min of excision. For such eyes the records published in Fig. 2 of Brindley (1956), Figs. 1 and 2 of Brindley (1957) and the first record of Fig. 1*A* of this paper are typical of what is seen on a slow time base, and Fig. 2 of Byzov (1958) and the third record of Fig. 1*A* of this paper are typical of what is seen on a fast time base. There is thus no discrepancy between the observations reported in the three early papers, but a simple model of one capacity shunted by one resistance will not account for them.

The R membrane after removal of the pars optica retinae

Unsuccessful attempts to detect an R membrane after removal of the pars optica retinae (i.e. the whole retina excluding the pigment epithelium) were reported by Brindley (1956) as well as by Byzov (1961), but after the report of success by Tomita *et al.* (1960) we were stimulated to try again with more care and patience. We found it difficult to remove the retina without damage to the pigment epithelium, and even when we thought we had done so we sometimes found no R membrane remaining. But in about fifteen eyes from which the whole pars optica retinae had been removed, and no other damage seemed to have been done, we found that the pulses of potential developed by rectangular pulses of current were large and rounded, and became suddenly and reversibly small and rectangular when the recording micro-electrode was advanced a few microns or tens of microns beyond the point at which it first gave the electrical signs (rise of resistance, etc.) of coming into contact with the exposed pigment epithelium. Figure 2 shows records from the best of these, i.e. that in which the pulses obtained before advancing the micro-electrode most nearly resembled those that can be obtained from a fresh healthy eye with intact retina.

Apparent singleness of the R membrane

In many experiments we have advanced a micro-electrode in small steps (usually 12 μ, but sometimes 6 μ) through the retina, and at every step recorded (1) the potential pulse developed between micro-electrode and sclera by a rectangular current pulse; (2) the same between micro-electrode and vitreous; and (3) the resistance of the micro-electrode. Rejecting instances where the resistance of the micro-electrode rose above

40 MΩ, or was already below 5 MΩ, we found no case where a movement that caused pulse (2) to cease to be rectangular did not also cause pulse (1) to become rectangular. Figure 2 of Byzov (1958) beautifully illustrates our finding, which may be expressed by saying that a micro-electrode always behaves as if it has pure resistance (no reactance) *either* between its tip and sclera *or* between its tip and vitreous; it never behaves as if the tip lay between two 'R membranes'.

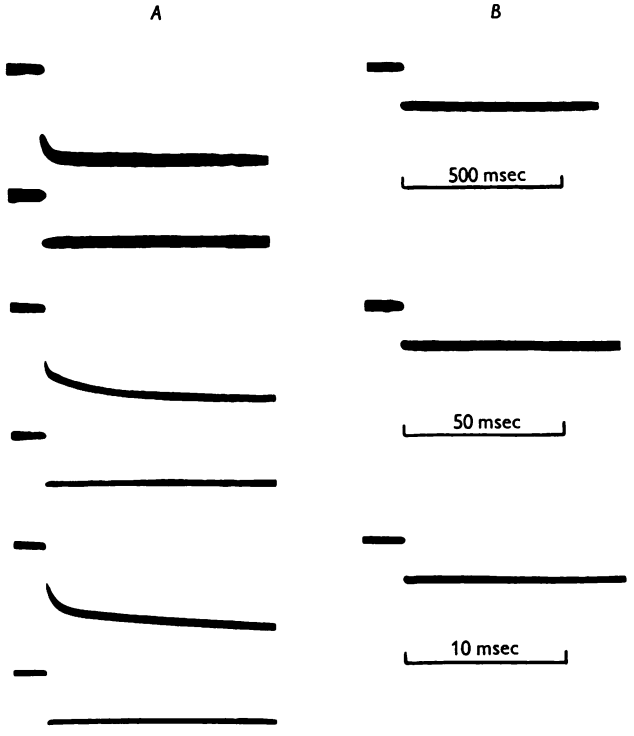

Fig. 2. *A*. Records made in the same way as those of Fig. 1*A* except that from this eye the pars optica retinae had been removed, the vitreous had been replaced with Ringer's solution, and the micro-electrode had been advanced through the Ringer's solution until it just touched the free surface of the pigment epithelium. *B*. The pulses of potential recorded from the micro-electrode (corresponding to the upper traces of each pair in *A*) after advancing it by 12 μ.

Such apparent singleness does not prove that the R membrane is really single, for if it consisted of two membranes close together, local mechanical conditions might ensure that a micro-electrode always penetrated both in the same step. But if there are two (or more) such constituent membranes, they cannot be more than 12 μ apart, for we found many times that when a small advancement had just sent the electrode through the R membrane, withdrawal by 12 μ would bring it back.

DISCUSSION

The nature of the R membrane

We have met the third of Byzov's objections to Tomita *et al.* by showing not only that the capacity of the R membrane can persist after careful removal of the pars optica retinae, but that the whole complex shape of the potential pulse developed by a rectangular current pulse, with its two time constants of about 10 and 0·3 msec, can remain practically unchanged. We would answer his second objection as follows: The complex conditions of his experiment on the isolated retina do not easily permit any estimate in absolute terms of the impedance that he has detected, and he has made no such estimate. Probably the best that can be done is to equate the resistance associated with the instantaneous rise of potential when a step of current is put through the isolated retina with the corresponding resistance of the retina *in situ*, which on Byzov's own measurements is about 74 Ω for the whole retina. From (*e*) and (*z*) of Byzov's Рис. 3, the non-instantaneous rise in potential after a step of current put through the isolated retina appears to be at most 40% of the instantaneous, so it presumably corresponds to less than 30 Ω. This is very far from accounting for the R membrane, for which the resistance corresponding to the non-instantaneous rise of potential is 500 Ω, according to Byzov (1958), and as much as 778 Ω in Fig. 1 of the present paper.

Byzov's first objection is strong evidence against the identity of the R membrane and Bruch's membrane. It need not, however, exclude the possibility that the R membrane is made up of the inner bounding membranes of the cells of the pigment epithelium. This has been suggested by Cohen (1961), who pointed out that the contiguous surfaces of these cells are cemented together by structures of the kind known to electron microscopists as 'terminal bars', which appear appropriate to prevent the movement of ions between the cells, and hence allow the epithelium to have a high electrical resistance. The suggestion is very attractive on three grounds:

(1) Since it implies that the R membrane lies wholly at least 10 μ and in part (long processes of the pigment-epithelial cells) as much as 60 μ in front of Bruch's membrane, it is fully consistent with the distance through which a micro-electrode must be moved to travel from the inner surface of the retina to the R membrane (Brindley, 1956; Byzov, 1958).

(2) By postulating a thin and much folded structure as the R membrane it allows, much more easily than would the thick and foldless Bruch's membrane, the very high electrical capacity, without which it is difficult to explain the longer of the two time constants of the R membrane.

(3) It explains very well why great care and much practice are needed to remove the retina without damage to the R membrane.

Models to explain the two time constants

The most obvious model to account for the R membrane on the equation of Fig. 1 is that shown in Fig. 3. For a square centimetre of retina, the two capacities would be about 86 and 1·9 μF, and their shunt resistances about 131 and 165 Ω respectively.

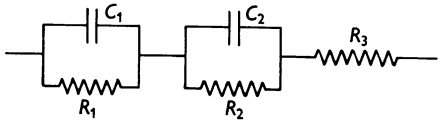

Fig. 3

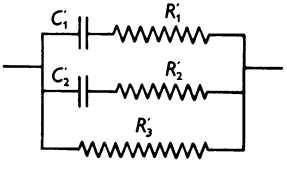

Fig. 4

Fig. 3. Model to account for R membrane. $C_1 = 32{\cdot}6\,\mu$F, $C_2 = 0{\cdot}73\,\mu$F, $R_1 = 344\,\Omega$, $R_2 = 434\,\Omega$, and $R_3 = 152\,\Omega$.

Fig. 4. Alternative model to account for R membrane. $C_1' = 4{\cdot}50\,\mu$F, $C_2' = 0{\cdot}40\,\mu$F, $R_1' = 1585\,\Omega$, $R_2' = 205\,\Omega$, and $R_3' = 930\,\Omega$.

The apparent singleness of the R membrane makes it unlikely, though not impossible, that it in fact contains two leaky condensers, of very different capacities per unit area, connected in series. This raises no difficulty, for there are other models, more easily reconciled with a structure such as the inner bounding membranes of the cells of the pigment epithelium, for which the equation of Fig. 1 would hold exactly, for example that shown in Fig. 4. Between such alternatives, electrical measurements with electrodes outside the system can never distinguish.

It is likely that a full and accurate analysis would show that the system is more complex than the equation of Fig. 1 implies. Deviations from this equation, however, are unlikely to be large. In particular, we were unable, as was Brindley (1956), to detect any non-linearity by using current pulses of different sizes and polarities.

SUMMARY

1. The potential developed across the R membrane by a rectangular pulse of current is not simply exponential, but can be adequately represented by $E = a(1-e^{-t/\alpha})+b(1-e^{-t/\beta})+c$, where α is about 10 msec and β about 0·3 msec.

2. The R membrane can persist, and its complex effect on a rectangular pulse of current remain unchanged, after complete removal of the pars optica retinae.

3. In thorough search we never found an instance where two R membranes in series could be detected. When a small advancement of a microelectrode had sent its tip through the R membrane, withdrawal by as little as 12 μ would often bring it back.

4. The observations support the hypothesis that the R membrane is the inner bounding membrane of the pigment epithelium, and prove that it is not the external limiting membrane or any other structure in the pars optica retinae.

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