COMMUNICATIONS

RESTING POTENTIAL OF THE LENS*

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THE metabolic activity of the lens has been very extensively studied, and something is known of its internal ionic composition (Lebensohn, 1936; Langham and Davson, 1949; Harris, Gehrsitz, and Nordquist, 1953) and its permeability to certain ions (Friedenwald, 1930; Langham and Davson, 1949; Pau and Stüttgen, 1951). But measurements of the difference of electrical potential, which might be expected to be present across the membrane separating its potassium-rich sodium-poor interior from the sodium-rich potassium-poor aqueous and vitreous humours, have not hitherto been reported. Such a resting potential was noticed by the author accidentally during investigations of the electrical activity of the retina, and was found to have the large value of about 70 mV. Further experiments here reported provide some evidence on the relative permeability to sodium, potassium, and chloride of the membrane across which this resting potential appears.

Methods

All the experiments were carried out on eyes excised from pithed frogs or from rabbits killed by a blow on the back of the neck, or on lenses removed from these eyes. The eye or lens was placed in a small unglazed earthenware dish, and a glass pipette micro-electrode, filled with 3M potassium chloride solution or Ringer's solution, was lowered on to it with a micromanipulator under direct observation through a microscope. Electrodes filled with potassium chloride solution were used for the great majority of experiments. The few which were done with Ringer-filled electrodes gave results indistinguishable from those obtained with electrodes filled with potassium chloride.

The unglazed earthenware dish was earthed, and the micro-electrode connected directly to the grid of the first amplifying valve of a one-sided direct-coupled pre-amplifier. The grid current taken by this first valve never exceeded 5×10^{-11} amps, and the electrode resistance, which varied for different electrodes between 2 and 20 megohms, never altered by more than 1 megohm when the electrode was inserted into or withdrawn from a lens, so that apparent changes in potential due to changes in input resistance never exceeded 50 μ V. and can be neglected.

The output from the pre-amplifier was recorded with a cathode-ray oscilloscope whose spot was photographed on slowly moving film. The deflecting voltage of the cathode ray tube was also made to modulate the frequency of an oscillator

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driving a loudspeaker, so that changes in pitch of the note produced indicated changes in potential, and made it unnecessary to watch the oscilloscope screen whilst manipulating the preparation. The changes in potential when the electrode entered or left the lens were measured by comparison of their photographic record with that of a calibrating pulse of 64 mV.

The following solutions were used in experiments on the effects of changing the ionic environment of the lens:

Normal Ringer's Solution.—115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , 2.15 mM Na_2HPO_4 , 0.85 mM NaH_2PO_4 .

Na_2SO_4 Ringer.—Normal Ringer with NaCl replaced by 78 mM Na_2SO_4 .

25 mEq KCl Ringer.—Normal Ringer with NaCl and KCl replaced by 92.5 mM NaCl, 25 mM KCl.

100 mEq KCl Ringer.—Normal Ringer with NaCl and KCl replaced by 17.5 mM NaCl, 100 mM KCl.

156 mEq K_2SO_4 Ringer.—Normal Ringer with NaCl and KCl replaced by 78 mM K_2SO_4 .

Results

Frog.—When a frog's eye was placed with the cornea downwards, a hole having been cut in the sclera, choroid, and retina at the posterior pole, and the recording electrode was lowered through the hole into the vitreous and through the vitreous into the lens, a sudden change in potential was found at the instant when the electrode tip was seen to enter the lens. Over a hundred observations of this kind were made on 42 eyes. For 37 of the eyes the sudden change in potential was between 63 and 88 mV., whatever part of the posterior surface of the lens was entered, the inside of the lens being negative as compared with the vitreous humour. The mean of values lying within this range was 74 mV. Five eyes gave lower values, all less than 45 mV. For two of these the potential difference across the centre of the posterior surface of the lens was much lower (0 and 5 mV.) than that near the equator (36 and 44 mV.). It is thought likely that the five lenses giving very low resting potentials had been mechanically injured during dissection.

While the tip of the electrode was in the vitreous, movement of it never caused any change in the potential recorded. After the lens had been entered, movements often caused changes in potential, usually of less than 10 mV. and gradual, but occasionally sudden and once as large as 20 mV. The large sudden change of potential on entering the lens often varied a little between different points on the posterior surface of the same lens, but never, except for the two very low values quoted in the last paragraph, by more than 10 mV.

When an eye was placed with its anterior surface upwards, a small slit having been made in the cornea, and an electrode was lowered through the slit into the anterior chamber, no change in potential was observed as the

electrode passed through the cornea and aqueous humour, but when its tip entered the lens, the potential fell suddenly by 79 mV. (average of four eyes). The anterior surface of the lens thus appears to have approximately the same difference of potential across it as the posterior.

Several attempts were made to measure the resting potential of lenses removed from frogs' eyes and placed in Ringer's solution. The first few of these gave very low values, between 0 and 40 mV., but it is thought that these low values were due to accidental mechanical damage to the lenses, since the last two tested, which were dissected out exceptionally carefully, gave resting potentials of 55 and 62 mV.

The frog vitreous humour, which is very fluid, could easily be replaced by artificial solutions by injecting these with a pipette through an opening made in the sclera, choroid, and retina at the posterior pole. Replacement of the vitreous by Ringer's solution had very little effect on the resting potential: in four eyes it decreased by an average of 6 mV. Replacement by a solution in which sulphate was substituted for chloride, without change in the amounts of sodium and potassium, likewise had little effect. Replacement by solutions in which potassium was partly or wholly substituted for sodium, however, caused a large fall in the resting potential, which was partly but never quite completely reversed when the potassium-rich solution was again replaced by Ringer's solution. The results of these experiments are shown in Table I. After applying a new solution, the potential attained within about 5 minutes a value which changed very little during the following 20 minutes, and it is these steady values which are shown in Table I.

TABLE I

Test Solution	Resting Potential in Ringer's (R) or Vitreous (v) before applying Test Solution	Resting Potential in Test Solution	Resting Potential in Ringer's after applying Test Solution
Na ₂ SO ₄ Ringer	-70 R -73 R -42 R -70 R -64 v	-62 -61 -40 -64 -64	-65 -60
25 mEq KCl Ringer	-80 R -74 R -60 R -70 R -83 v	-41 -47 -32 -49 -52	-76 -70 -42 -54 -74
100 mEq KCl Ringer	-71 R -80 v	-3 0	-47 -42
156 mEq K ₂ SO ₄ Ringer	-77 R -70 R -72 R -45 R -54 R	+6 -8 +4 +6 +3	-20 -60 0 -15 ..

Rabbit.—The posterior surfaces of the lenses of the six excised eyes of three rabbits, tested after extensive excision of the posterior sclera, choroid, and retina 5, 14, 9, 15, 7, and 19 minutes after killing the animals, gave resting potentials of 69, 63, 68, 66, 72, and 56 mV. respectively. The resting potential was remarkably stable in the excised eye; the Figure shows measurements of it made on one lens during 6½ hours after excision.

FIGURE.—Resting potential of lens of excised eye of a rabbit, measured during 6½ hours after killing the animal and excising the eye. Between the last two measurements the lens was stabbed at about six points with a knife blade. This abolished the resting potential.

The capsule covering the anterior surface of the rabbit's lens was found to be so tough that in all attempts to penetrate it from the front, the tip of the electrode broke without entering the lens; but it was possible in two experiments to penetrate the anterior capsule from behind with an electrode which had passed through the whole thickness of the lens. The potential difference across the anterior surface was found to be almost the same as across the posterior surface: in one case 5 mV. higher, in the other 2 mV. lower.

The rabbit's vitreous humour, in contrast to that of the frog, is a stiff gel very difficult to remove completely without injury to the lens. Attempts to replace it by solutions of different ionic content were inconclusive. In two eyes from which the vitreous was very thoroughly removed, the resting potential of the lens was found to be less than 5 mV., whatever solution was placed in the eye; and if the vitreous were removed less thoroughly, there was no certainty that the solutions inserted into the eye came into contact with the lens.

Discussion

A membrane permeable to one univalent ion and impermeable to all others should have a potential difference across it of $58 \log [A_i^+]/[A_o^+]$ mV., where $[A_i^+]$ and $[A_o^+]$ are the activities on the two sides of the membrane of the ion to which it is permeable. If it is permeable to more than one ion, then, provided that the membrane obeys certain conditions, the chief of which is that the electric field must be constant through its thickness (Goldman, 1943; Hodgkin and Katz, 1949), the potential difference should be a compromise in which the concentration of each ion is weighted with the permeability of the membrane to it:

$$\text{Membrane potential} = 58 \log \frac{[K_i^+]_{P_k} + [Na_i^+]_{P_{Na}} + [Cl_o^-]_{P_{Cl}}}{[K_o^+]_{P_k} + [Na_o^+]_{P_{Na}} + [Cl_i^-]_{P_{Cl}}} \text{ mV.},$$

where P_k , P_{Na} , and P_{Cl} are the permeabilities of the membrane to potassium, sodium, and chloride.

Without making any assumptions about the internal composition of the frog lens, the data of Table I on the effect of replacing the external sodium by potassium indicate that the membrane across which the resting potential appears is much more permeable to potassium than to sodium: if it were more permeable to sodium than to potassium, the interior of the lens would become *more negative* on replacing the external sodium by potassium. The relation of resting potential to the logarithm of the external potassium concentration has between 25 and 156 mEq/litre a gradient not significantly different from 58 (though the scatter of experimental values is too large to fix it very accurately), and the smaller gradient found at low external potassium concentration could readily be explained if the permeability to sodium were not negligibly small, but equal to roughly one-thirtieth of the permeability to potassium.

The lack of substantial effect on the resting potential of total replacement of the external chloride by sulphate is most simply explained by assuming that the membrane across which the resting potential appears is much less permeable to chloride than to potassium; though if, as is likely, this membrane is the cell membrane of the lens fibres, there is the alternative possibility that the lens capsule of the frog, unlike that of cattle (Pau and Stüttgen, 1951) is impermeable to chloride and hence shields the lens fibres from the applied solution.

Consideration of the internal and external ionic composition of the mammalian lens (Table II, overleaf) indicates a similar ratio of potassium to sodium permeability to that inferred for the frog lens from the effect of replacing the external sodium by potassium. If the rabbit's vitreous is similar to that of the horse, and the permeability to chloride negligibly small, the ratio of potassium to sodium permeability required to explain the mean observed resting potential is 32 : 1

TABLE II

Ion	Vitreous (horse) mEq/litre	Lens (rabbit) mEq/litre	Calculated Membrane Potential for a Membrane Permeable to One Ion Only	Mean Observed Potential (rabbit)
Na ⁺	119*	30 †	+ 35 mV.	} - 66 mV.
K ⁺	4.9*	125 †	- 82 mV.	
Cl ⁻	117*	13.8†	- 54 mV.	

* Duke-Elder (1930). † Harris and others (1953). † Langham and Davson (1949), corrected by using the data of Lebensohn (1936) for the water content of the lens.

Experiments with radioactive tracers have shown that the rabbit lens is not absolutely impermeable to sodium (Langham and Davson, 1949). The membrane across which the resting potential appears must therefore be continually leaking sodium, and to maintain the lens in a steady state it must be capable of re-accumulating sodium against both a concentration and a potential gradient. The active re-accumulation must require energy, presumably derived from the glycolysis and respiration of the lens. It seems unlikely that this active process could be a function of the non-cellular capsule, though in view of the finding by Dische and Ehrlich (1955) of glycolytic activity in lens capsules of cattle, this possibility should not be altogether disregarded; it could more plausibly be attributed to the cell membranes of the lens fibres.

Possible Contribution of the Lens to the Resting Potential of the Eye.—It has long been known (Du Bois-Reymond, 1849) that the whole eye has a resting potential, the cornea being about 6 mV. positive to the posterior part of the sclera. This resting potential has been generally attributed to the retina, and certainly part of it at least is still present when the anterior half of the eye has been cut away; but it seems possible from the present experiments that differences between the resting potentials of the anterior and posterior surfaces of the lens may sometimes contribute to it.

Use of the Resting Potential as an Index of Changes in Permeability.—In human cataractous lenses the potassium content is abnormally low and the sodium content abnormally high (Lebensohn, 1936); and similar changes are reversibly induced in rabbit lenses by cooling and by calcium deficiency (Harris and others, 1953). The electrolyte content of the lens must always be determined by the balance between the permeability of its bounding membrane to the ions concerned and the active energy-using process or processes pumping some at least of them against the gradient both of concentration and of electrical potential. The change found in cataractous lenses may be mainly due to an increase in permeability or to a decrease in the active pumping processes. If the alteration is in the pumping mechanisms only, the resting potential should retain the same relation to the ionic composition that it has in the normal eye; but if the first change is in permeability,

and sodium and potassium are differently affected, the resting potential may be altered even if a compensatory increase in active pumping at first keeps the ionic content constant. Thus in experimental studies of cataract formation it is possible that a decrease or increase in the resting potential of the lens, indicating an alteration in the relative permeability to sodium and potassium of the capsule or the cell membrane of the lens fibres, might be the earliest easily observable change.

Summary

The interior of the lens in the frog and rabbit is electrically negative compared with its surroundings. The mean potential difference found between lens and vitreous humour was 74 mV. in the frog and 66 mV. in the rabbit. In the frog, the potential difference was diminished by partial replacement and converted to a small difference of opposite polarity by complete replacement of the external sodium by potassium.

The observations are consistent with the hypothesis that the potential depends on the difference of ionic content between the two sides of a membrane about thirty times as permeable to potassium as to sodium. This membrane may be either the lens capsule or the cell membranes of the lens fibres.

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