J. Physiol. (1977), **272**, *pp.* 167–186
With 1 plate and 5 text-figures
Printed in Great Britain

A COMPARISON OF ION CONCENTRATIONS, POTENTIALS AND CONDUCTANCES OF AMPHIBIAN, BOVINE AND CEPHALOPOD LENSES

BY N. A. DELAMERE* AND G. DUNCAN†

From the School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ

(*Received 19 April 1977*)

SUMMARY

1. The concentrations of sodium, potassium and chloride in frog and bovine lenses showed a normal intracellular ion distribution with the sum of the internal cations approximately equal to the external sum. In the cephalopod lens, however, the sum inside was much lower than that outside.

2. The membrane potentials of frog, *Sepiola* and bovine lenses were −63, −63 and −23 mV respectively. A comparison of the electrical data with the Nernst potentials predicted from ion concentration data indicated that sodium and chloride ions as well as potassium contributed to the membrane potential in frog and bovine. In contrast, the membrane and Nernst potentials for potassium were equal in *Sepiola*.

3. Substituting potassium for sodium in the external medium depolarized lens potentials in all three species. Estimates of the relative permeabilities of sodium, potassium and chloride were obtained by fitting the Goldman-Hodgkin-Katz equation to the potential data.

4. The potassium permeability was determined directly by ^{42}K efflux measurements and values of 2·99, 9·83 and 3·13 ($\times^{-8}$ m sec^{-1}) were obtained for frog, *Sepiola* and bovine lenses respectively.

5. The effect of raising external potassium on the efflux rate constant was determined and there was reasonable agreement between experiment and theory (Kimizuka-Koketsu) in frog and bovine lenses, but the *Sepiola* data indicated that the potassium permeability decreased by a factor of 2·6 when the external potassium was raised from 10 to 120 mM-K^+.

6. The measured specific conductances, obtained using two internal micro-electrodes, were 7·7, 15·9 and 9·9 (Sm^{-2}) for frog, cephalopod and

* Present address: University of Colorado, Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80262, U.S.A.

† Author to whom all correspondence should be addressed.

bovine lenses respectively. These data compare with computed values (Kimizuka-Koketsu theory) of 7·5, 14·1 and 17·2 (Sm^{-2}).

7. The effect of increasing external potassium on the conductance was also tested and there was good agreement between experiment and theory (assuming constant permeabilities) only in the amphibian lens. However, when the cephalopod data were corrected assuming a 2·6-fold decrease in P_K for a twelvefold increase in potassium, then there was excellent agreement between experiment and theory.

8. The bovine measured conductances were much lower than the theoretical values throughout the range of external potassium concentrations and several explanations were proposed to account for the discrepancies.

INTRODUCTION

There have been many studies of the properties of vertebrate lenses using electrophysiological and radiotracer techniques (reviewed by Paterson 1972 and Duncan, 1974) but the investigations have usually been carried out separately and on different systems. For example, the mammalian lens has been most widely used for tracer flux studies (Becker & Cotlier, 1962; Kinsey & Reddy, 1965; Paterson, 1970*a*) while the smaller amphibian lens has been used mainly for electrophysiological investigations (Brindley, 1956; Duncan, 1969*a*; Eisenberg & Rae, 1976). Perhaps because of the different techniques employed, two quite separate ideas concerning the function of lens membranes have emerged. Those working with mammalian lenses have tended to emphasize the role of individual fibre membranes in restricting ion movement (Paterson & Maurice, 1971) while most of those in the amphibian lens field claim an almost negligible resistance to current flow in the interpretation of their electrical data (Duncan & Croghan, 1970; Candia, Bentley & Mills, 1971; Eisenberg & Rae, 1976).

The one study where both approaches have been used, namely on the toad lens, has indicated that the main barriers to diffusion lie at the outer surface of the lens (Duncan, 1969*b*). Later experiments showed that the Kimizuka-Koketsu equations for membrane potential and conductance (Kimizuka & Koketsu, 1964) best fitted the isotope flux and electrical conductance data (Duncan & Croghan, 1970).

The primary aim of this study was to find out if there were basic differences in the membrane properties of amphibian and mammalian lenses and so concomitant flux and electrical measurements were carried out on amphibian (frog) and mammalian (bovine) lenses. The applicability of the Kimizuka-Koketsu equations to both systems was also tested. A preliminary histological study (Pl. 1 and Delamere & Duncan; unpublished) had shown that although the cephalopod lens has a quite different

ontogenetic development (Duke-Elder, 1958), it has a system of internal fibres similar to that found in vertebrate lenses (Duncan, 1974) and so the cephalopod lens has been included in this comparative study.

METHODS

Specimens of the frog (*Rana temporaria*) and marine cephalopod (*Sepiola atlantica*) were decapitated and the eyes removed. Bovine eyes were obtained from approximately 2 yr old animals immediately after slaughter. In each case the eye was equatorially bisected posterior to the ciliary body, the suspensory ligaments cut away, and the lens removed to a dish of the appropriate Ringer solution.

The equatorial diameter and pole-pole distance of each lens was measured using a Zeiss binocular microscope with a calibrated eye-piece. Assuming the lens to comprise two spherical segments, these parameters were used to compute lens surface area (Duncan, 1970).

Solutions

The compositions of the bathing solutions used in the perfusion of each of the lens species are given in Table 1. The frog Ringer was that used by Paterson, Neville, Jenkins & Nordstrom (1974) and the bovine saline was that of Duncan & Bushell (1976). The *Sepiola* solution was based on an analysis of cephalopod aqueous humour (Robertson, 1953; Duncan, 1974).

TABLE 1. Composition of normal perfusing solutions (m-mole/l)

	NaCl	KCl	$CaCl_2$	$MgCl_2$	Tris HCl	NaH_2PO_4	Na_2HPO_4	$MgSO_4$	$NaHCO_3$
Frog	105	2·5	2·0	—	—	2·0	1·2	1·2	5·6
Sepiola	490	10	10	5·0	5·0	—	—	—	—
Bovine	130	6·0	1·0	1·0	5·0	—	—	—	—

All solutions containing glucose, 5 m-mole/l., and the pH was adjusted to 7·4 before use. In experiments where the effect of raising external potassium was tested, potassium was substituted at the expense of sodium.

Water and ion content

The water content and sodium, potassium and chloride concentrations were determined as described in Duncan (1969*a*) and Duncan & Bushell (1975).

Electrophysiological measurements

In each case the lens was seated in a recess at the base of a Perspex (Plexiglas) chamber and was submersed in saline which was constantly flowing through the chamber. The flow rate was such that changes in ion concentrations in the chamber were completed (> 95 % as determined by flame photometry) within 30 sec. The temperature of the solution was held constant to within ± 1 °C by means of a peltier stage beneath the chamber and by water jackets around the saline reservoirs. The temperature was maintained at 18, 10 and 30 °C for frog, *Sepiola* and bovine lenses respectively.

Micro-electrodes formed on a vertical puller (Narashige) were filled with 3M-KCl by capillarity and had tip resistances in the range 20–80 MΩ. The micro-electrodes

were mounted in Perspex holders filled with 3 M-CKl where a scintered silver plug served as a reversible electrode. The holders fitted directly into the probe of a high impedance preamplifier (Model 750, W.P.I. Instruments Inc., U.S.A.) and the electrodes were inserted into the lens by means of a Narashige micromanipulator. A saline-filled agar bridge placed in the chamber served as a reference electrode.

Lens resistance was measured by adding an additional pair of electrodes (one glass micropipette inside the lens and one silver wire in the bath), passing a step pulse of current between them and measuring the voltage response across the first pair (Duncan, 1969*b*). The currents, normally 10 sec in duration, were provided by a specialized amplifier (M 701, W.P.I. Inc., California) controlled by a Tektronix pulse generator, and were in the range $0{\cdot}1–1 \times 10^{-6}$ A. The amplitude and time course of the potentials and currents were monitored on a storage oscilloscope (Tektronix, 564 B) and the latter had essentially zero rise and fall time compared to the voltage response (i.e. < 10 μsec).

^{42}K *fluxes*

After removing the lens from the eye it was incubated (at 18, 10 and 30 °C for frog, *Sepiola* and bovine respectively) for 15 hr in the appropriate Ringer solution (Table 1) in which ^{42}KCl (Code PES 1P, Amersham, U.K.) had been incorporated to an activity of approximately 1 μc/ml. at the time of loading. At the end of the incubation period the lens was briefly washed in non-radioactive saline and placed in a plastic chamber containing 10 ml. of the appropriate solution. Fresh saline could be introduced through a port in the chamber, displacing the original saline through a second port where it was collected in a scintillation vial. The activity in the displaced saline was assayed by monitoring the Cerenkov radiation in a liquid scintillation counter (Intertechnique SL 65 A). At the end of the experiment, the lens was removed from the chamber, homogenized in 20 ml. saline and 1 ml. of the resulting solution was added to 9 ml. normal saline for assay as above. Efflux graphs were constructed as described by Duncan (1969*c*).

RESULTS

Ion content and electrical potential

Transmembrane potentials are a function of ion concentration as well as membrane permeability (Goldman, 1943; Hodgkin & Katz, 1949; Kimizuka & Koketsu, 1964; Kimizuka, 1966) and so basic information on the intra- and extracellular distribution of permeant, charged species is essential to any electrophysiological study. As an *in vitro* preparation was used for this investigation, the extracellular ion concentrations were defined for a particular experiment. The total lens concentrations of sodium, potassium and chloride are given in Table 2 and the intracellular values, computed by assuming a value of 5 % for the extracellular space of the lens (Thoft & Kinoshita, 1965; Paterson, 1970*b*; Duncan, 1970) are given in Table 3. When these corrected values are used in the various membrane equations, it will be assumed that the ion activities for all internal and external species are equal. This is a reasonable assumption for potassium as Paterson *et al.* (1974) have shown that the internal activity coefficient

is near the free solution value. However, Duncan (1974) cites evidence that indicates the sodium activity in the lens to be lower than in free solution. As the internal sodium term (P_{Na} Na_i) in all of the equations used here (Appendix, eqns. (2) and (6)) is likely to be small relative to the other terms, errors arising from assuming free internal sodium are likely

TABLE 2. Ion levels determined in fresh lenses (m-mole/kg lens water)

Lens	Na	K	Cl
Frog	14·7 ± 1·1	90·3 ± 3·1	17·7 ± 2·0
Sepiola	62·4 ± 2·9	124·9 ± 2·8	235·1 ± 5·6
Bovine	30·0 ± 3·1	142·0 ± 3·4	32·8 ± 2·1

The concentrations are given as the mean ± S.E. of thirty lenses in each case.

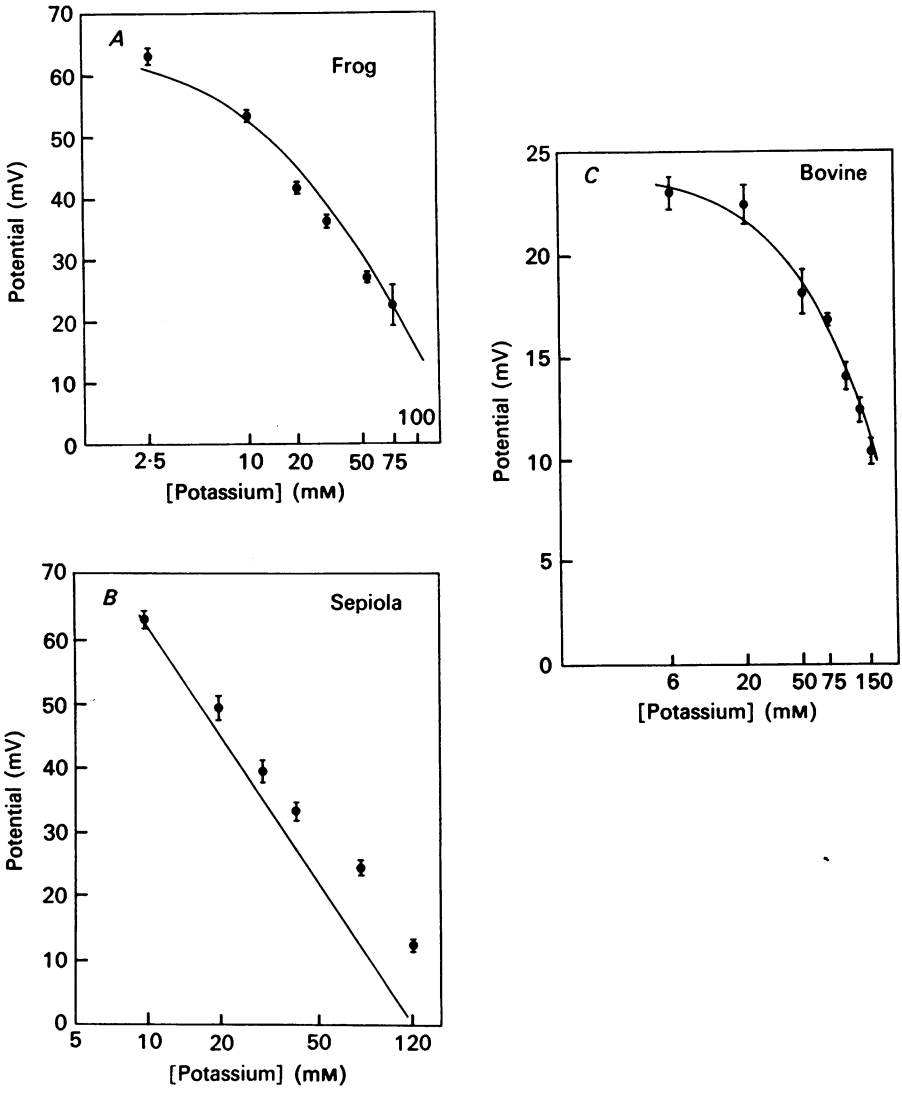
TABLE 3. Computed internal ion concentrations, Nernst potentials and measured membrane potentials

	Na^+	K^+	Cl^-	E_{Na}	E_K	E_{Cl}	E_m (mV)
Frog	9·4	94·9	12·8	+62	−90	−54	−63·0 ± 1·4
Sepiola	39·9	131·0	219·3	+61	−63	−22	−63·3 ± 1·2
Bovine	24·7	149·0	26·9	+43	−84	−44	−23·1 ± 0·9

The ion concentrations (m-mole/kg lens water) were calculated assuming a value of 5% for the extracellular space. The Nernst potentials were computed by substituting the above data, together with the relevant extracellular values from Table 1, into eqn. (1). The measured potentials are given as the mean ± standard error of twenty-five measurements in each case.

to be negligible. Although Paterson & Eck (1971) provide evidence that a relatively small fraction of chloride in the rabbit lens is non-exchangeable in isotope tracer experiments and so is 'bound' to a certain extent, Duncan (1970) has obtained a complete exchange of chloride in the amphibian lens, as have Kinsey & Hightower (1976) in the rabbit. The possible errors introduced by overestimating lens chloride activity are probably also small.

The individual Nernst potentials (eqn. (1)) computed from the asymmetrical distribution of sodium, potassium and chloride are presented in Table 3 together with the measured lens potentials. Often, the potential recorded immediately after insertion of the microelectrode was unstable, but within 10 min would settle to a steady value that was maintained for several hours with less than 1 mV variation. The lens potential was taken as the steady level obtained not less than 10 min after penetration. The measured potential in frog and bovine lenses fell within the range of the predicted Nernst potentials indicating a significant permeability for all the ions. However, the measured potential in *Sepolia* was close to the



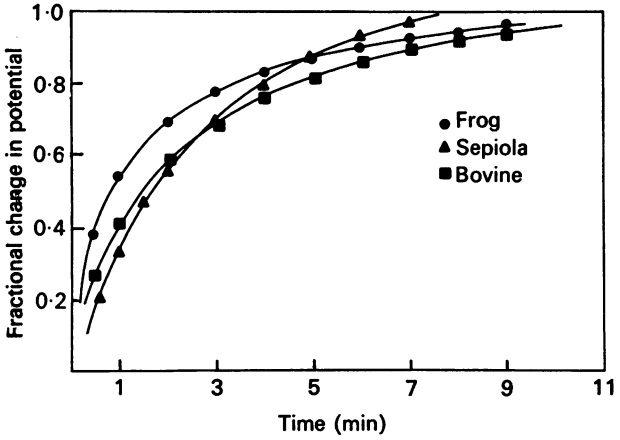
Text-fig. 1. Effect of increasing $[K]_o$ on lens membrane potentials. The data are given as the mean ± S.E. of at least six lenses in each case. The continuous lines were obtained by fitting the Goldman-Hodgkin-Katz eqn. (2) to the data with the values of $\alpha(P_{Na}/P_K)$ and $\beta(P_{Cl}/P_K)$ shown. See also Text-fig. 5. *A*, frog: $\alpha = 0{\cdot}2$, $\beta = 1{\cdot}2$. *B*, *Sepiola*: $\alpha = \beta = 0$. Note that the deviation from the Nernst relation increases with increasing external potassium, which is in contrast to the trend in *A* and *C*. *C*, bovine: $\alpha = 0{\cdot}6$, $\beta = 0{\cdot}5$.

most negative Nernst potential, indicating a relatively low permeability for sodium and chloride.

An estimate of the relative permeabilities can be obtained by fitting the Goldman (and Kimizuka-Koketsu) potential equation to the potential data at different external potassium concentrations.

Permeability ratios

In all preparations investigated, the normal perfusing medium in the bath was exchanged for the test solution in less than 30 sec and a steady value for the lens potential was attained in less than 10 min (see later). After a 15 min exposure to high potassium solutions, normal perfusate was returned and in all of the experiments reported here, recovery of the control value for the transmembrane potential was always observed. Experiments were abandoned where full recovery was not obtained.



Text-fig. 2. Time course of change in potential on increasing external potassium. The half-time for the potential change can be used to estimate the thickness of the 'unstirred' layers between the ion-restricting membranes in the lens and the external bulk solution.

The Goldman eqn. (2) was fitted to potential data from frog and bovine lenses using the ion concentration data from Tables 1 and 3 (Text-fig. 1*A* and *C*). The best fits to the data were obtained by using values for $\alpha(P_{Na}/P_K)$ and $\beta(P_{Cl}/P_K)$ of 0·2 and 1·2 respectively in frog and 0·6 and 0·5 in the bovine preparation. The *Sepiola* data (Text-fig. 1*B*) could not be fitted by any combination of α and β, but the simple Nernst relation for potassium (eqn. (1)) gave a close fit at potassium concentrations near the normal value. The deviations at higher potassium concentrations indicated a decrease in potassium permeability relative to the other ions (i.e. either

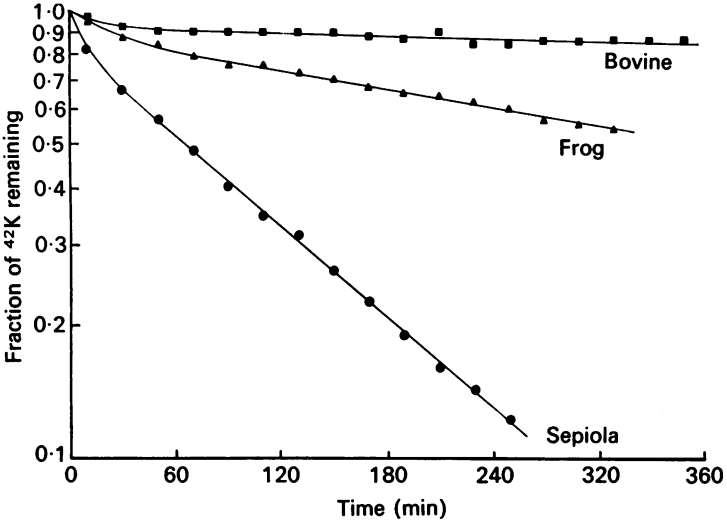
an actual decrease in potassium permeability or an increase in sodium or chloride movement).

The time course of the potential change on increasing the external potassium was significantly slower than the time course for solution change in the bath. The fractional change in potential (F) was plotted against time (Text-fig. 2), where

$$F = \frac{E_t - E_0}{E_\infty - E_0}. \tag{7}$$

E_0 is the potential in the control solution, E_t the potential in the test solution at time t and E_∞ is the final steady-state potential.

Although the lenses varied greatly in size, the three preparations responded similarly with half-times close to the 100–120 sec reported for the toad lens (Duncan, 1969*b*).



Text-fig. 3. Comparison of the rate of loss of potassium from frog, *Sepiola* and bovine lenses. In all three cases the curves are composed of two exponential components, the faster presumably arising largely from fragments of suspensory tissues adhering to the lenses (see also Text-fig. 4).

Potassium permeability

Having now obtained reasonable estimates of the relative permeabilities of sodium, potassium and chloride, values for the actual permeabilities of all three ions can be calculated if the absolute permeability of any one is known. Efflux measurements provide the most direct means of determining actual permeabilities and a comparison of the relative ion

distributions and Nernst potentials (Table 3) indicate that the effluxes of potassium and chloride are both passive in nature. Duncan (1970) has previously found that a significant fraction of the ^{36}Cl efflux from *Bufo* is due to an exchange diffusion mechanism and so estimates of P_{Cl} are greatly overestimated in ^{36}Cl efflux experiments. Tracer effluxes of potassium comprise only a small exchange diffusion component (Duncan & Croghan, 1970) and so permeability estimates in this study were obtained using ^{42}K.

Duncan (1969*c*) and Paterson (1970*a*) have previously shown that the potassium efflux kinetics of toad and rabbit lenses are relatively simple. After an initial, fast component of efflux, the time course of exchange of the bulk of the internal potassium can be fitted by a single exponential. The efflux graphs for frog, *Sepiola* and bovine lenses show similar simple kinetics (Text-fig. 3) and so the efflux rate constant (k) can be calculated at any time during the efflux from the expression

$$k = \frac{\ln (N_{t_1}) - \ln (N_{t_2})}{t_2 - t_1},$$

where N_{t_1} and N_{t_2} give the fraction of the initial radioactivity in the lens remaining at times t_1 and t_2 respectively.

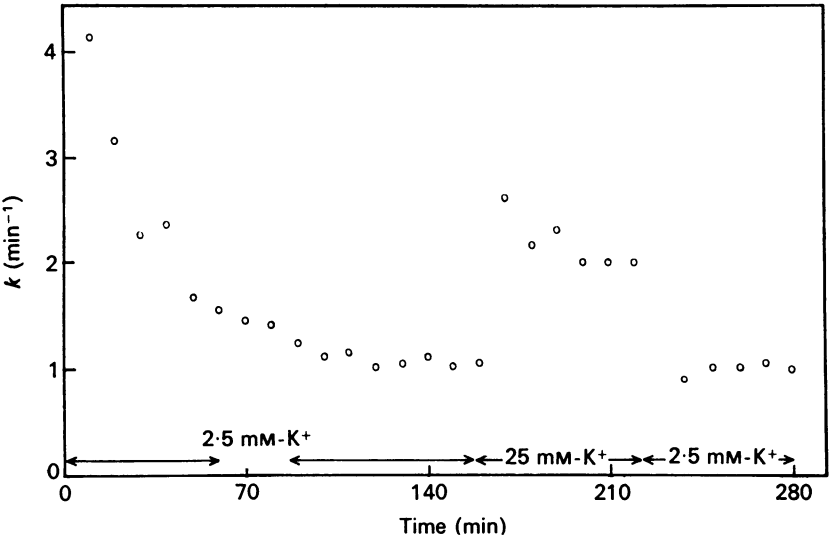
A typical rate constant graph (in this case for the frog) is given in Text-fig. 4 and it can be seen that the simple one-component behaviour is achieved after about 75 min. The relatively long time course of the initial component presumably reflects the loss of potassium from the capsule and suspensory ligaments still attached to the lens. The response to a change in external potassium is, however, rapid as indeed is the change in transmembrane potential (Text-fig. 2) and in this case reflects the time course of diffusion of potassium from the bulk solution to the ion-restricting membranes.

Estimates of potassium permeability (Table 4) were obtained by substituting the values for rate constant, area, water content (V) and membrane potential into eqn. (4). If it is assumed that the change in efflux rate-constant in high potassium solutions is due to the change in membrane potential alone, then predicted values for the rate constant ratio can be obtained from eqn. (5) provided that the permeability is assumed to be independent of the external potassium concentration. The predicted and experimental ratios are given in Table 5. There was good agreement between experiment and theory in frog preparations, reasonable agreement with bovine lenses (see Discussion section), while the measured rate constant increase in *Sepiola* was much less than predicted. If it is assumed that the potassium permeability in the *Sepiola* lens is a function of external potassium, then the permeability ratios in the control and test

solutions can be obtained from eqn. (5). A permeability ratio ($P_K/P_{K'}$) of 2·6 is obtained for a twelve-fold increase in external potassium.

Resistance measurements

In all lenses tested, the relationship between current and voltage was ohmic as had previously been reported by Duncan (1969*b*) and Eisenberg & Rae (1976) for amphibian lenses. Specific resistances (Ω m^2) were calculated from the slope of the current–voltage graphs and from measurements of the diameter and pole to pole distances of the lenses. The data from the three species are given in Table 6 and represent the means of thirty measurements in each case.



Text-fig. 4. Effect of increasing external potassium on the rate constant of loss from the amphibian lens. Note the relatively long time for the rate constant to settle down to a steady value initially and the rapid response to changing external potassium.

TABLE 4. ^{42}K Efflux parameters

	Area ($m^2 \times 10^{-4}$)	Water volume ($m^3 \times 10^{-6}$)	Rate constant ($min^{-1} \times 10^{-3}$)	Permeability ($m\,sec^{-1} \times 10^{-3}$)
Frog	0·276	0·0088	1·6	2·99
Sepiola	0·168	0·0075	4·47	9·83
Bovine	3·63	1·797	0·24	3·13

The areas, volumes and rate constants are the mean values of measurements from eight lenses of each species. The potassium permeability was obtained by substituting the above data together with the measured membrane potentials (Table 3) into eqn. (4).

The reciprocal of resistance, the conductance (G in units of S m^{-2}), is a convenient alternative way of expressing the data and experimental values may be directly compared with those predicted from theory. Duncan & Croghan (1970) found that the conductances predicted from the Kimizuka-Koketsu equations showed good agreement with the measured

TABLE 5. Comparison between predicted and measured rate constant ratios

	Control [K]	Test [K]	ΔE (mV)	Predicted k/k'	Observed k/k'
Frog	2·5	25	+22·0	0·64	0·625 ± 0·203
Sepiola	10	120	+51·5	0·35	0·895 ± 0·041
Bovine	6	60	+5·3	0·90	0·798 ± 0·018

The rate constant ratios (k/k') were obtained by dividing the mean of the last four values in the control solution (before perfusing with high potassium solution) by the mean of the last four values in the test solution obtained before immersing in the control solution again (see Text-fig. 4). The experimental values presented in the Table are the means and S.E. of data from four lenses of each species. The potential changes (ΔE) obtained on changing solutions were obtained in a separate series of experiments (Text-fig. 1. *A*, *B* and *C*) and a positive value indicates a depolarization in the test solution. The predicted values were obtained by substituting the values for ΔE into eqn. (5) and assuming a constant potassium permeability in the control and test solutions.

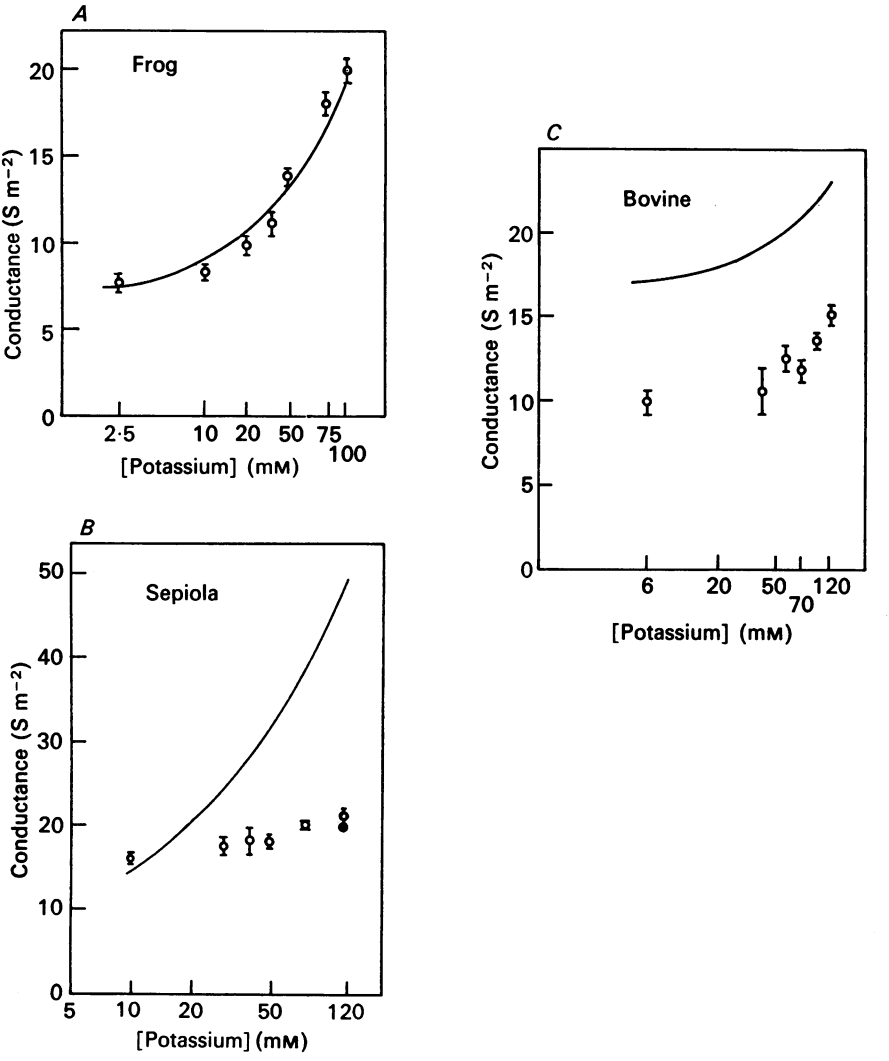
TABLE 6. Specific resistance and conductance of lens membranes

	Resistance (Ω m^2)	Measured conductance (S m^{-2})	Computed conductance (S m^{-2})
Frog	0·13 ± 0·007	7·7 ± 0·4	7·5
Sepiola	0·063 ± 0·004	15·9 ± 1·0	14·1
Bovine	0·101 ± 0·009	9·9 ± 1·0	17·2

The measured resistance and conductance are given as the mean ± S.E. of thirty lenses in each case. The computed values were obtained from eqn. (6) using the ion concentrations in Tables 1 and 3, the potassium permeability values in Table 4 and the relative permeabilities (α and β) from Text-fig. 1*A* and 1*C*.

lens conductance in the toad. Using this approach (eqn. (6)) together with the values of P_K from Table 4 and values of α and β from Text-fig 1., theoretical predictions of conductance were made and are also given in Table 6. The Kimizuka-Koketsu equations predict, as indeed will the Goldman-Hodgkin-Katz equations (Hope & Walker, 1961), that as the external potassium concentration increases so will the membrane conductance.

The measured conductance changed to a new steady value within 10



Text-fig. 5. Effect of increasing $[K]_o$ on the lens conductances. The data are given as the mean ± S.E. of at least six lenses in each case. The solid lines were obtained by fitting the Kimizuka-Koketsu conductance eqn. (6) to the data with the values of α and β shown. See also Text-fig. 1. *A*, frog $\alpha = 0{\cdot}2$, $\beta = 1{\cdot}2$. Note the relatively good fit throughout the range tested. *B*, *Sepiola* $\alpha = \beta = 0$. The filled circle value was computed from eqn. (6) assuming that P_K decreased 2·6-fold on increasing $[K]_o$ from 10 to 120 mM (Table 5). *C*, bovine: $\alpha = 0{\cdot}6$, $\beta = 0{\cdot}5$. Note that although the computed and experimental data follow the same trend there is a constant discrepancy between the two sets.

min after changing the external potassium concentration and the time course of the conductance change was in all cases similar to that of the potential change (Text-fig. 2). The experimentally measured conductances as a function of external potassium are given in Text-fig. 5. The solid lines give the predicted conductances. Results from the frog (Text-fig. 5*A*) are in excellent agreement with theory over the potassium range tested and although the measured bovine conductance (Text-fig. 5*C*) varied with external potassium, the measured values were consistently much lower than the predicted curve and this markedly significant deviation will be discussed later. The experimentally measured conductance of the *Sepiola* lens in the normal solution was slightly greater than the predicted value, suggesting perhaps that the contributions of other ions to the conductance is not negligible as had been assumed. However, at higher external potassium concentrations, the measured conductance was much less than predicted by the Kimizuka-Koketsu equation, and the difference between experiment and theory increased with increasing external potassium. These conductance experiments provide further evidence that the potassium permeability of *Sepiola* lens membranes decreases with increasing external potassium. The permeability does not seem to be a function of the potential difference across the membrane (which also changed on increasing external potassium) as the current-voltage graphs for *Sepiola* were always ohmic.

DISCUSSION

Ion concentrations

The lens consists essentially of a mass of tightly packed fibre cells with a layer of epithelium on the anterior surface bounded by a collagen-based capsule, and although there are relatively small gradients of concentration running from cortex to nucleus (Amoore, Bartley & van Heyningen, 1959) and from anterior to posterior (Paterson, 1969*a*), the over-all ion concentrations of all lenses studied so far show a normal intracellular pattern (Duncan, 1974). The lens of *Sepiola* is different from the others, however, in that the total sum of the cations inside does not equal that outside. This ionic imbalance is similar to that found in molluscan muscle where the osmotic deficit is made up with a high internal concentration of free amino acids (Potts & Parry, 1964).

Permeability properties determined by potential measurements

The potentials of frog and *Sepiola* lenses, both −63 mV, were similar to previously published values for frog (Brindley, 1956; Patterson & Rae, 1974) toad (Duncan, 1969*a*) and trout lenses (Rae, Hoffert & Fromm,

1970). The present value of -23 mV for the bovine lens is significantly lower than that quoted by Sperelakis & Potts (1959). However, Sperelakis & Potts arrived at their estimate by a combination of measurements involving questionable theory. They observed a p.d. of -23 mV on inserting a micro-electrode into the bovine lens which they ascribed to the potential across individual fibres. On withdrawing the microelectrode slightly the potential fell to -10 mV which was suggested to be the p.d. between the extracellular space and the bathing medium. A third potential of -30mV, taken to be that across the outer membranes, was measured by inserting an agar-wick electrode into the lens. They assumed these potentials to be in series and so presented the lens potential as the sum of the three (-63 mV). It is only relevant to note therefore that their 'fibre potential' is in close agreement with the present value.

The lens potentials of all three species depolarized when the external potassium was raised and the frog and bovine lens potential approached the Nernst relationship at high external potassium values (Text-fig. 1*A* and *C*). In contrast, the *Sepiola* lens potential only deviated from this relationship as the external potassium increased (Text-fig. 1*B*), indicating that the potassium permeability decreases with increasing external potassium, or that the sodium and chloride permeabilities increase. Flux and conductance measurements (Text-fig. 5*B* and Table 5) indicate that the former actually occurs. No other lens yet investigated has shown the relatively simple potassium Nernst potential behaviour found in *Sepiola*. The values for the relative permeabilities, α and β, of 0·2 and 1·2 respectively for the frog lens agree well with those previously reported by Duncan (1969*a*) for the toad lens. The bovine values of 0·6 and 0·5 are almost identical to the mean values for the anterior and posterior membranes recently found by Duncan, Juett & Croghan (1977) using an oil chamber technique to isolate the two surfaces of the lens.

The time taken for the potential to change to a new value on increasing the external potassium can be used to estimate the magnitude of the 'unstirred layers' (Dainty & House, 1966) between the bathing solution and the ion-restricting membranes. If the important membranes are near the surface, then the unstirred layers would be expected to be small. If, on the other hand, each fibre membrane controls the membrane potential, the unstirred layers would be large and, considering the paucity of the extracellular space between the fibres, the half-times for the potential change would be expected to be extremely long. Duncan (1969*b*) has reported half-times of the order of 60 sec for the toad lens. The values obtained in the present study were similar in all three species and ranged from 0·8 min in *Sepiola* to 1·7 min in the bovine lens (Text-fig. 2). Assuming the surface of the lens can be approximated mathematically by a plane

sheet, then the relationship between unstirred layer thickness (δ) and diffusion half-time ($t_{\frac{1}{2}}$) is given by (Crank, 1957; Dainty & House, 1966):

$$\delta = \sqrt{\frac{D_K t_{\frac{1}{2}}}{0{\cdot}38}},$$

where D_K is the free solution value for potassium (4×10^{-10} m^2 s^{-1}). Estimates of δ, therefore ranged from 220 μm for *Sepiola* to 310 μm for the bovine lens. As unstirred layers extend outwards approximately 100 μm from the surface of any tissue in relatively unstirred conditions (Dainty & House, 1966) and as the thickness of the capsule in the bovine lens, for example, is of the order of 100 μm, the ion-restricting membranes must be located near the surface in all three species.

Permeability properties as revealed by flux measurements

Transmembrane ion fluxes that are predominantly passive in nature are influenced by the potential difference across the membrane. As the Nernst potentials for potassium indicate a passive efflux in all three types of lenses (Table 3), the changes in efflux rate-constant should be predicted by flux equations that assume a passive movement. Agreement between experiment and theory is excellent in the frog lens, as it is in the toad (Duncan & Croghan, 1970). In the bovine lens, the change in efflux rate constant is greater than predicted by the Kimizuka-Koketsu equations indicating either that the potassium permeability increases with increasing external potassium or that a potassium exchange-diffusion mechanism exists in the bovine lens similar to that reported in the toad lens (Duncan & Croghan, 1970). Further experiments over a wide range of external potassium concentrations (0–120 mM) will be required to resolve this question.

The rate constant increase of the *Sepiola* lens ^{42}K was much less than predicted, and indicated a decrease of potassium permeability in high external potassium concentration. This phenomenon has been observed in crayfish giant axon (Strikholm & Wallin, 1967; Wallin, 1967) where the ratio P_{Cl}/P_K was seen to change from 0·13 at 5 mM-$[K]_o$ to 0·85 at 25 mM-$[K]_o$, and also in frog muscle fibre membranes (Hodgkin & Horowicz, 1959; Adrian, 1958). Adrian suggested that outward movement of potassium is restricted in muscle membranes, and Hodgkin and Horowicz drew from this a model for electrical rectification in the membrane, and suggested that inward conductance for potassium may be 100 times the outward conductance.

Permeability properties as revealed by resistance measurements

The measured resistances of the lens membranes in all three species were found to be independent of the position of the voltage and current

electrodes and values ranged from 0·13 Ω m^2 in the frog to 0·063 Ω m^2 in *Sepiola*. These data confirm the observations of Sperelakis & Potts (1959) and Duncan (1969*b*) who found resistances of the order of 10^{-1} Ω m^2 for bovine and toad lenses respectively. Rae and his colleagues (Rae & Blankenship, 1973; Rae, 1974) originally found resistances of the order of 10^2 Ω m^2 for the frog lens but have more recently reported values of the order of 10^{-1} Ω m^2 (Eisenberg & Rae, 1976). The discrepancy arose because in the earlier work either single or double-barrelled electrodes were used to measure resistance and with these techniques only the bulk resistance of the lens was measured. Eisenberg & Rae (1976) have in fact shown that the internal electrodes have to be spaced at least 10° of arc apart before the membrane resistance contributes to the major part of the measured resistance. In the present study the two electrodes were always at least 20° apart and usually considerably more.

The resistances were sensitive to changes in external potassium and the time-course of change in resistance was similar to the time course of the potential change (Text-fig. 2), again indicating that the main permeability barriers lie near the outer surface of the lens.

The measured specific conductance of the frog lens membranes was in close agreement with that predicted from eqn. (6) over a wide range of external potassium levels (Text-fig. 5*A*), again confirming Duncan & Croghan's (1970) observations on the toad lens. The *Sepicla* lens conductance was slightly greater than predicted at normal (10 mM) external potassium (Table 6) which indicates that the other ions present probably make a small contribution to the resting conductance. However, as the external potassium was raised, the measured conductance was much lower than predicted (Text-fig. 5*B*). Data from electrical potential (Text-fig. 1*B*) and isotope flux experiments (Table 5) had previously indicated a decrease in P_K with increasing external potassium. Substituting the flux rate-constant data into eqn. (5) gave a value for P_K at 120 mM-K that was a factor of 2·6 lower than the value at 10 mM-K. On substituting the adjusted value for P_K at 120 mM-K, there was close agreement between the predicted and experimental values (Text-fig. 5*B*).

The measured conductance of the bovine lens varied as a function of the external potassium in a similar manner to the predicted conductance, but the measured values were consistently lower, being only about 0·6 of the computed values throughout the potassium range tested (Text. fig 5*C*). There are two basic reasons why this discrepancy could occur. Firstly, an overestimation of the potassium permeability and secondly, an underestimation of the electrical conductance. The former would occur if a fraction of the internal potassium were non-exchangeable (V in eqn. (4) was taken as the total water volume of the lens and tacitly assumes single-

compartment efflux kinetics). Duncan (1969*c*) and Paterson (1972) have in fact previously shown that all of the potassium in amphibian and mammalian lenses is readily exchangeable. The presence of a potassium exchange diffusion mechanism in the lens membranes would lead to an overestimation of the potassium permeability. Such a mechanism could be present as raising the external potassium leads to a greater efflux of potassium than predicted by eqn. (5). However, the difference between experiment and theory (Table 5) was not sufficient to account for the discrepancy in the conductance data (Text-fig. 5*C*). Another, more likely, explanation is that the measured resistance contains contributions from sources other than the surface membranes. N.A. Delamere & G. Duncan (unpublished data) have recently found using both transient and alternating currents that the total resistances of all three types of lenses are the sum of at least two components. The first component has a characteristic time-constant of 10^{-3} sec, which is normally associated with biological membranes, whereas the other value is of the order of 10^{-1} sec. The fast (membrane) component is by far the larger in frog and *Sepiola*, whereas the slower component contributes over 50 % to the resistance measurements in the bovine lens. It is not clear at present if the different relative magnitudes of the two components in the different lenses are due to better intercellular coupling in frog and *Sepiola* or whether it simply reflects the much lower surface to volume ratio in the bovine lens.

APPENDIX

A detailed summary of the relevant membrane flux and potential equations are given in Duncan (1974) and they will simply be summarized here for convenience. If an ion is in equilibrium, the Nernst equation holds:

$$E_r = \frac{-RT}{zF} \ln \left(\frac{C_{ri}}{C_{ro}}\right) \text{mV}, \tag{1}$$

where C_{ri} and C_{ro} are the internal and external concentrations of the species r, and E_r is the Nernst equilibrium potential (the outside solution is taken as the arbitrary zero of potential).

Assuming that only potassium, sodium and chloride ions carry significant currents across the membrane and also assuming that there is no net current passing through the membrane, the following equation can be derived using either the approach of Goldman (1943) or of Kimizuka & Koketsu (1964):

$$E = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}. \tag{2}$$

In isotope experiments, where the efflux of the species r ($J_{r,out}$) is observed, then the Kimizuka & Koketsu approach gives

$$J_{r,out} = P_r C_{ri} \exp(FE/2RT) \quad (3)$$

and the permeability of the species r can be obtained from the efflux rate constant k:

$$P_r = \frac{kV}{A} \exp(-zFE/2RT), \quad (4)$$

where V/A is the ratio of the volume of water in the lens to the surface area of the ion-restricting membranes.

The relationship between efflux rate constant (k) and membrane potential is given by

$$\frac{k}{k'} = \frac{P_r}{P_{r'}} \exp\left[\frac{Z_r F}{2RT}(E-E')\right], \quad (5)$$

where k and k' are the rate constants in normal Ringer solution and the test solution respectively, and E and E' are the membrane potentials in the two solutions. The Kimizuka-Koketsu permeabilities need not be independent of membrane potential, whereas the Goldman permeabilities are so defined.

The Kimizuka-Koketsu equation to describe membrane conductance measured by passing a pulse of current is

$$G = \frac{F^2}{RT}\sqrt{(AB)}, \quad (6)$$

where $A = P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i$ and $B = P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o$.

We wish to thank Dr Peter Croghan for many stimulating discussions. We are also indebted to Mr Norman Baker for carrying out the electron microscope studies, to Mr Andrew Bushell for producing the Figures. N.A.D. was in receipt of an S.R.C. studentship during the course of this work and The Royal Society and the National Institute for the Blind kindly provided funds for most of the electrophysiological equipment used. Bovine eyes were kindly supplied by the Harford Bridges Slaughterhouse, Norwich, and we are indebted to Mr Frank Moore for obtaining live specimens of *Sepiola*.

REFERENCES

ADRIAN, R. H. (1958). The effects of membrane potential and external potassium concentration on the potassium permeability of muscle fibres. *J. Physiol.* **143**, 59*P*.

AMOORE, J. E., BARTLEY, W. & VAN HEYNINGEN, R. (1959). Distribution of sodium and potassium within the cattle lens. *Biochem. J.* **72**, 126–133.

BECKER, B. & COTLIER, E. (1962). Distribution of ^{86}Rb accumulated in the rabbit lens. *Investve Ophth.* **1**, 642–649.

BRINDLEY, G. S. (1956). Resting potential of the lens. *Br. J. Ophthal.* **40**, 385–391.

CANDIA, O. A., BENTLEY, P. J. & MILLS, C. D. (1971). Short-circuit current and active Na transport across isolated lens of the toad. *Am. J. Physiol.* **220**, 558–564.

CRANK, J. (1957). *The Mathematics of Diffusion.* Oxford: Oxford University Press.

DAINTY, J. & HOUSE, C. R. (1966). Unstirred layers in frog skin. *J. Physiol.* **182**, 66–78.

DUKE-ELDER, S. (1958). *System of Ophthalmology*, vol. 1. London: H. Kimpton.

DUNCAN, G. (1969*a*). Relative permeabilities of the lens membranes to sodium and potassium. *Expl Eye Res.* **8**, 315–325.

DUNCAN, G. (1969*b*). The site of the ion restricting membranes in the toad lens. *Expl Eye Res.* **8**, 406–412.

DUNCAN, G. (1969*c*). Kinetics of potassium movements across amphibian lens membranes. *Expl Eye Res.* **8**, 413–420.

DUNCAN, G. (1970). Movement of Na and Cl across amphibian lens membranes. *Expl Eye Res.* **10**, 117–128.

DUNCAN, G. (1974). Comparative physiology of the lens membranes. In *The Eye*, vol. 5, pp. 357–398, ed. H. Davson and L. T. Graham. London: Academic.

DUNCAN, G. & BUSHELL, A. R. (1975). Ion analyses of human cataractous lenses. *Expl Eye Res.* **20**, 223–230.

DUNCAN, G. & BUSHELL, A. R. (1976). The bovine lens as an ion-exchanger: a comparison with ion levels in human cataractous lenses. *Expl Eye Res.* **23**, 341–353.

DUNCAN, G. & CROGHAN, P. C. (1970). Effect of changes in external ion concentrations and 2,4-dinitrophenol on the conductance of toad lens membranes. *Expl Eye Res.* **10**, 192–200.

DUNCAN, G., JUETT, J. R. & CROGHAN, P. C. (1977). A simple chamber for measuring lens asymmetry potentials. *Expl Eye Res.* **25**. (In the Press.)

EISENBERG, R. S. & RAE, R. L. (1976). Current-voltage relations in the crystalline lens. *J. Physiol.* **262**, 285–299.

GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **2**, 37–49.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of K and Cl ions on the potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

HOPE, A. B. & WALKER, N. A. (1961). Ionic relations of cells of *Chara australis*. *Aust. J. biol. Sci.* **14**, 26–34.

KIMIZUKA, K. (1966). Ion current and potential across membranes. *J. theor. Biol.* **13**, 145–163.

KIMIZUKA, H. & KOKETSU, K. (1964). Ion transport through cell membranes. *J. theor. Biol.* **6**, 290–305.

KINSEY, V. E. & HIGHTOWER, K. R. (1976). Studies on the crystalline lens XXII. Characterization of chloride movement based on the pump-leak model *Expl Eye Res.* **23**, 425–434.

KINSEY, V. E. & REDDY, D. V. N. (1965). Studies on the crystalline lens XI: The Relative role of the epithelium and capsule in transport. *Investve Ophthal.* **4**, 104–116.

PATERSON, C. A. (1969). Distribution of sodium and potassium in ox lenses. *Expl Eye Res.* **8**, 442–6.

PATERSON, C. A. (1970*a*). Efflux of ^{22}Na and ^{86}Rb from the crystalline lens. *Expl Eye Res.* **10**, 331–339.

PATERSON, C. A. (1970*b*). Extracellular space of the crystalline lens. *Am. J. Physiol.* **218**, 797–801.

PATERSON, C. A. (1972). Distribution and movement of ions in the ocular lens. *Documenta Ophth.* **31**, 1–28.

PATERSON, C. A. & ECK, B. A. (1971). Chloride concentration and exchange in rabbit lens. *Expl Eye Res.* **11**, 207–213.

PATERSON, C. A. & MAURCIE, D. M. (1971). Diffusion of sodium in extracellular space of crystalline lens. *Am. J. Physiol.* **220**, 256–263.

PATERSON, C. A., NEVILLE, M. C., JENKINS, R. M. & NORDSTROM, D. K. (1974). Intracellular potassium activity in the frog lens determined using ion specific liquid ion-exchange filled microelectrodes. *Expl Eye Res.* **19**, 43–48.

PATERSON, C. A. & RAE, J. L. (1974). The potential difference of the rabbit lens. *Expl Eye Res.* **18**, 507–508.

POTTS, W. T. W. & PARRY, G. (1964). *Osmotic and Ionic Regulation in Animals.* London: Pergamon Press.

RAE, J. L. (1974). Voltage compartments in the lens. *Expl Eye Res.* **19**, 235–242.

RAE, J. L. & BLANKENSHIP, J. E. (1973). Bioelectric measurements in the frog lens. *Expl Eye Res.* **15**, 209–217.

RAE, J. L., HOFFERT, J. R. & FROMM, P. O. (1970). Studies on the normal lens potential of the rainbow trout (*Salmo gairdneri*). *Expl Eye Res.* **10**, 93–102.

ROBERTSON, J. D. (1953). Further studies on ionic regulation in marine invertebrates. *J. exp. Biol.* **30**, 277–294.

SPERELAKIS, N. & POTTS, A. M. (1959). Additional observations of the bioelectric potential of the lens. *Am. J. Ophthal.* **47**, 395–409.

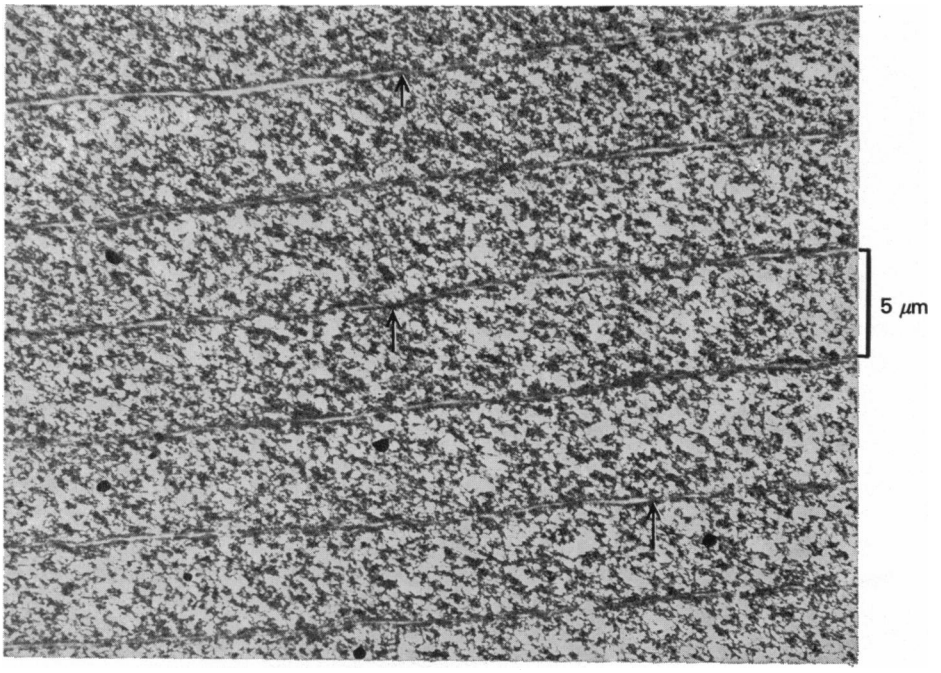
STRICKHOLM, A. & WALLIN, B. G. (1967). Relative ion permeabilities in the crayfish giant axon determined from rapid external ion changes. *J. gen. Physiol.* **50**, 1929–1953.

THOFT, R. A. & KINOSHITA, J. H. (1965). The extracellular space of the lens. *Expl Eye Res.* **4**, 207–214.

WALLIN, B. G. (1967). The relation between external potassium concentration membrane potential and internal ion concentrations in crayfish axons. *Acta physiol. scand.* **70**, 431–448.

EXPLANATION OF PLATE

Low magnification electron micrograph of *Sepiola* lens fibres. The tissue was fixed in glutaraldehyde, post-fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. The section shows a regular array of cortical fibres which are remarkably similar in size and appearance to cortical fibres in vertebrate lenses (Duncan, 1974). There is little extracellular space between fibres and there appear to be regions of membrane fusion between adjacent fibres (arrows) again similar to the regions found in vertebrate fibres.


5 μm