J. mar. biol. Ass. U.K. (1970) 50, 349–363
Printed in Great Britain

ELECTRO-OSMOSIS IN SQUID AXONS

By W. B. STALLWORTHY
Mount Allison University, Sackville, New Brunswick, Canada.

(Text-figs. 1–4)

Electro-osmosis occurs when a current of 5–50 μA is passed lengthwise through a segment of a giant axon of the squid, *Loligo forbesi* Steenstrap, with an average electro-osmotic efficiency of 16 ± 5 molecules H_2O per positive charge.

The zeta potential is negative. In artificial sea water containing 100 mM-KCl, the electro-osmotic efficiency increases to 28 ± 4 molecules H_2O per positive charge.

Although electro-osmosis does occur in the axoplasm, the flow observed was considered to be due chiefly to the dominating properties of the axon membrane. Changes in electro-osmotic efficiency caused by a tenfold increase in KCl probably reflect changes in the pore structure of the cell membrane.

There is also a tendency for electro-osmotic efficiency to rise with currents below 5 μA, or approximately 400 μA/cm^2.

INTRODUCTION

When electro-osmosis is observed through a substance (or tissue) certain inferences can be made about the fine structure of the substance. These include the presence of a zeta potential[1] whose sign is the same as that of the electrode towards which water moves, and the presence of interstices or channels large enough to allow ions to move through them and to sweep along more water molecules than those carried as hydration shells. Channels too large may permit both positive and negative ions to move or at least allow a counter flow of water to reduce the net (observable) movement of water molecules.

Electro-osmosis has been demonstrated in *Nitella* by Fensom & Dainty (1963), and it has been related to pore size by Fensom & Wanless (1967) and by Fensom, Ursino & Nelson (1967).

In animals electro-osmosis has been reported in the serous membranes of cat and dog by Mudd (1926) and in the aorta and vena cava of the dog by Sawyer & Harshaw (1966). Mudd was interested in the influences of current strength, buffer system, and buffer concentration on water transport. Sawyer & Harshaw wished to evaluate zeta potentials and charges per pore in their tissues. The data of both have in the present paper been used to derive comparative current densities and electro-osmotic efficiencies.[2] Table 1 shows

[1] Zeta potential is the potential difference across any solid–liquid interface.

[2] Electro-osmotic efficiency is defined as J/I or volume flow divided by the current, and hence may be expressed as cm^3 per coulomb, water molecules per ion, moles per Faraday, etc. The approximation is made here that volume flow is the same as flow of free water; and the assumption is made that all the current is carried by positive ions.

the resulting calculated values. The current densities were 2–50 times those required to depolarize a squid nerve membrane (Hodgkin, Huxley & Katz, 1952) and may therefore have caused some physiological change or even damage in the tissue cells. In any case the location of the 'pores', whose charge was reported by Sawyer & Harshaw, and the exact paths in these complex tissues are uncertain.

TABLE 1. CALCULATED CURRENT DENSITIES AND ELECTRO-OSMOTIC EFFICIENCIES

Currents		Electro-osmotic efficiency		
As reported by author(s)	As current densities ($mA.cm^{-2}$)	Author's datum used in calculation of e-o	Moles H_2O $Faraday^{-1}$	Author(s) and tissues used
1–25 mA	3–75	0·25 mm^3/min/mA in serum	23	Mudd (1926), serous membranes
10 mA	3–15	Zeta P.D. of 9·0 mV in Ringer	26	Sawyer & Harshaw (1966), aorta and vena cava
50–100 $\mu A\ cm^2$	0·05–0·1	In sulphate Ringer	40*	House (1964), frog skin

* e-o value given by House.

On a much firmer basis is the work of House (1964) where irreversible thermodynamics were applied to frog skin to explain non-osmotic water flows across the skin, the conclusion being that they are partially electro-osmotic. There was in fact a very poor correlation between the short circuit current across the skin and the net water flux observed. Only when the skin was placed in sulphate Ringer, and the intrinsic water flow eliminated, did the observed flow become proportional to externally applied currents. Under these circumstances an electro-osmotic efficiency of about 40 molecules H_2O per positive charge was obtained.

There has been no attempt to look for electro-osmosis in animal cells in simpler conditions analogous to those used in the work on *Nitella*. For such an attempt the giant axon of squid (or other invertebrates) probably offers a unique opportunity in the animal kingdom. Its size and shape make possible the use of the equipment developed by Dainty & Hope (1959) (see Fig. 1). Preliminary observations by Stallworthy & Fensom (1966) were successful in detecting electro-osmosis and an average value of 28 molecules H_2O per Faraday was reported for axons of *Illex illecebrosus* Lesueur. It is the purpose of the present paper to confirm this observation and to discuss its interpretation.

METHOD

Technical details

The electro-osmometer used is shown in Fig. 1. It was the same as that used by Fensom & Dainty (1963) and by Stallworthy & Fensom (1966), and consisted essentially of two sea-water-filled glass chambers, A and B, separated by a rubber stopper with a small hole in it. When the axon occupied this hole it formed the only electrical path between the two Ag–AgCl electrodes placed one in each chamber. Chamber A was open to the air and chamber B was sealed, except for a horizontal capillary tube (radius 0·1 mm) in which a bubble formed a meniscus which could be followed by a travelling microscope reading to $\frac{1}{50}$ mm. The capillary opened into a third chamber, C, which served merely to equalize hydrostatic pressure.

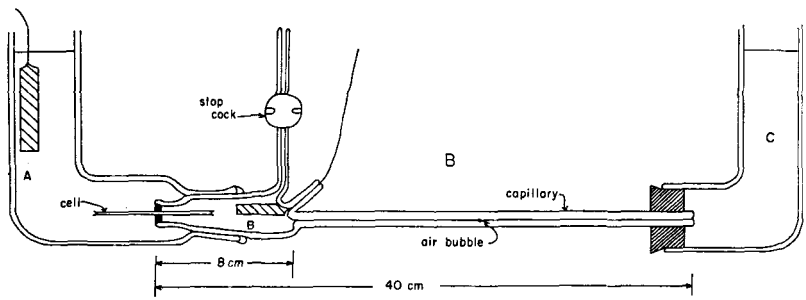

Fig. 1. Electro-osmometer. Chamber B is closed, except for glass capillary. Chambers A and C are open. If water moves when a current is passed between electrodes in A and B, it can be detected by a change in the motion of the bubble in the capillary.

The giant axons were dissected from freshly killed squid, *Loligo forbesi* Steenstrap, cleaned of other nerve fibres and tied by cotton ligatures 30–60 mm apart. Such axon segments were used only if capable of conducting throughout. An external electrical stimulus was introduced at one end of the prepared axon segment and a spike demonstrated at the other end on an oscilloscope. Complete conduction was only assumed when any intermediate portion could be allowed to dip into a dish of earthed sea water without eliminating the spike.

To permit placing the axon, the stopper was cut in half, each half was covered with a film of 'Vaseline' on its cut surface including the groove for the axon. The axon was blotted by laying on filter paper, then dipped in one molar (isotonic) sucrose which also contained 10 mM-$CaCl_2$/l., and blotted again. It was laid in the groove of the stopper. The two halves were pressed gently together and firmly inserted in the opening of chamber B. This must be done so as to leave the chamber full of sea water with no bubble whatever. Chambers A and C were then joined to B and the whole assembly placed in a water bath. Until temperature equilibrium was nearly reached the equalizing stopcock leading to the surface from B remained open. The capillary bubble must be introduced by pressure on C so as not to risk spoiling the seal around the axon.

The water bath had a stirrer, but no regulator. By adding ice or hot water, bath temperature was adjusted to about 3 °C below that of the room. This caused a steady small thermal drift of the meniscus which was evaluated by readings taken while no current was flowing both before and after a period of current flow. Generally readings were taken at 1 min intervals in groups of six. Average drift rate (before and after) was

subtracted from the average rate during current passage to give the average electro-osmotic effect in mm/min.[1] Finally, the flow was expressed as water molecules per positive charge, or electro-osmotic efficiency. Figure 2 shows a sample of the sequence of rates obtained during two tests, each at 30 μA. Each test was always followed by a second at the same current and reversed polarity after a 6 min rest period. From two to twelve 6 min test observations were carried out on each preparation. At the end of the experiment the axon was tested again for its ability to conduct and was measured for length and diameter.

Currents were drawn from a 2·0 V accumulator and measured by a Unipivot microammeter and were set at definite values from 2·5 to 30 μA. Voltage, read on a vacuum tube voltmeter (Electronic Testmeter), varied from 50 to 500 mV across the electrodes.

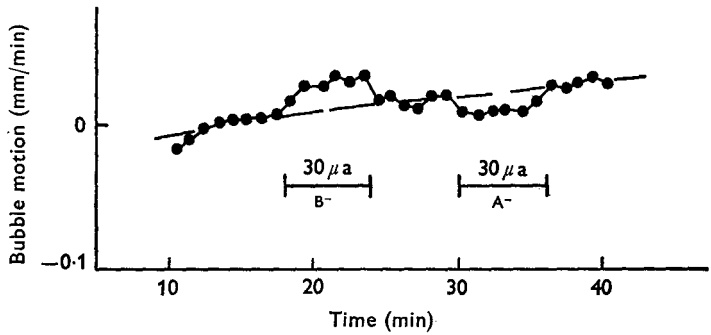

Fig. 2. Time-course of two electro-osmotic observations on the same axon in sea water. Dotted line shows general thermal trend or drift. Average electro-osmotic efficiencies for the two intervals are 23 and 16 water molecules per positive charge. A and B refer to chambers and their contained electrodes, shown in Fig. 1. Note the change in direction of the electro-osmotic effect with change in applied polarity.

Solutions in the chambers A and B could be changed at will by sucking out with polyethylene tubing and hypodermic syringe. Commonly, filtered sea water was used (S.W.).[2] Artificial sea water (A.S.W.) was also used and was made up according to Caldwell (1962). When KCl was to be increased or decreased, NaCl was changed in the opposite sense to keep the total molarity constant.

A total of 33 axon preparations were made, of which three were tested for electro-osmosis with their protruding ends cut about 3 mm from the stopper.

Some observations were also made on agar gel (two preparations) and axoplasm (five preparations), both in glass tubes. Tubing drawn to about 1 mm was used but measurements of radius and length were taken after the observations in all cases. Agar was 1% solution in 0·5 M-NaCl stained with methylene blue to distinguish it easily from axoplasm. Axoplasm was forced into the tube by inserting it as a cannula (drawn down if necessary) into the axon which had been dissected out of the squid but not cleaned of connective tissue and other fibres. With the axon laid on filter paper

[1] Occasionally the experiment was commenced before the thermal drift rate had assumed a steady value, the assumption being made that the drift rate (r) was moving exponentially to an asymptotic value (a). A plot of ln $(a-r)$ against time gave roughly a straight line. From this the actual drift rate at any time during any treatment could be derived, and this was subtracted from the observed rate to get the effect of the treatment.

[2] Recently refrigerated sea water must be avoided.

the axoplasm could be forced into the cannula by rolling with a glass roller. The finer insertion tip was then broken off and hot (fluid) agar drawn into the tip for about 1 cm. The tube was then broken at the other end of the column of axoplasm so as to discard the top centimetre of possibly sea water contaminated axoplasm. Then 5 mm of agar were drawn into that end, thus leaving a 5 mm plug of agar at each end. This was necessary because it was found that axoplasm alone would not stay in the capillary tube during the taking of observations. Dipping each end in the hot agar after completion of the mount made a stronger bond. Storage even for less than 1 h must be in 0·5 M-NaCl solution to avoid shrinkage of the agar plugs and the introduction of air. The mounting of axoplasm, or agar-filled tubes is done directly into the vaselined stopper with no sucrose rinse.

ANALYSIS AND CRITIQUE

Reliability of observations

The 'resolution' of this method is limited by the scale readings ($\frac{1}{50}$ mm) and by the smoothness of motion of the bubble in the capillary. Rejection of readings because of the development of a leak (usually between chambers A and B at the axon) is sometimes necessary. Air entrapped at the stopper will cause very erratic motion of the bubble. Such motion is taken as an indication of technical imperfections and on this ground readings on seven axons were rejected, as well as all readings taken at 2·5 μA currents.

The average electro-osmosis efficiency (e-o) of all rejected observations (44) was 18 moles H_2O per Faraday and ranged from -99 to $+308$. This very high variation is further indication of previously unnoticed technical imperfections and justifies their rejection. Table 2 is based on the remaining 58 observations.

TABLE 2. ELECTRO-OSMOTIC EFFICIENCIES (MOLES H_2O PER FARADAY) $\mathcal{J}/I$ ($\Delta P = 0$).

	Axon segments			Glass capillary preparations	
	Ends tied				
	A.S.W. or S.W.	A.S.W. + 100 K	Ends open A.S.W.	Agar in 0·5 M-NaCl	Axoplasm with agar plugs
Ranges of currents (μA)	10–30	5–20	20–30	5–50	5–30
No. of specimens prepared	11	4	3	2	5
No. of observations	36	14	8	13	23
Average e-o, moles per Faraday	16	28	14	9	14
Standard error of mean	1	4	2	1	2

Probable current paths

An estimate of the resistance of the various paths open to the electric current is necessary to decide where electro-osmosis might be occurring and what structures therefore are responsible for it. Fig. 3 shows diagrammatically the relation between stopper and axon as well as a simplified equivalent electric circuit, on which the symbols correspond with those of Cole & Hodgkin (1939). Membrane capacity may be ignored when dealing with

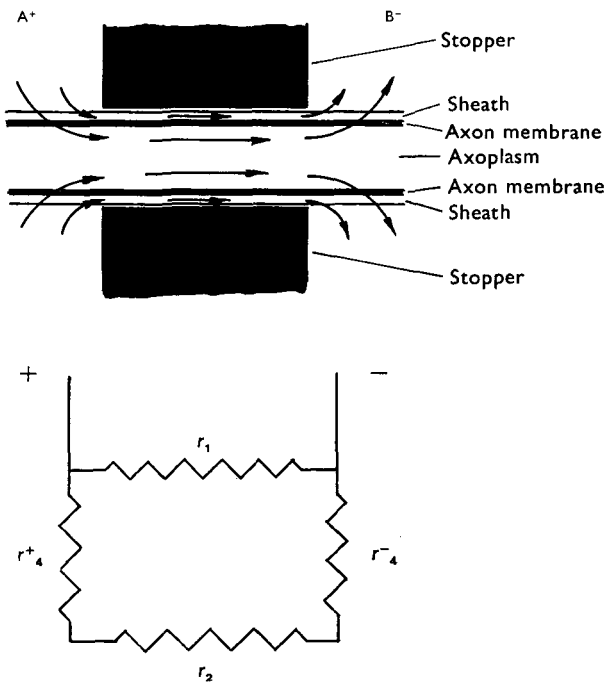

Fig. 3. The axon and the stopper as paths for the electric current. r_1 = resistance of sheath which includes Schwann cells, adhering connective tissue or small nerve fibres and a film of sea water and sucrose. Vaseline in the stopper is not shown. r_2 = resistance of axoplasm within the stopper. r_4^+ and r_4^- = the effective resistances of the protruding anodal and cathodal ends of the axon. Total resistance of loop

$$R = \frac{r_1(r_4^+ + r_4^- + r_2)}{r_1 + r_4^+ + r_4^- + r}.$$

direct current. Also we may assume that no current flows through stopper or 'Vaseline' since a stopper with 'Vaseline'-filled channel passes less than 0·2 μA at 2 V. This leaves the following possible current paths in series and parallel: (*a*) the surface membrane of the axon, assumed to be a structure of high longitudinal resistance but with an effective transverse resistance,

r_4^+ and r_4^-, at either end of the stopper; (*b*) in series with this the axoplasm of longitudinal resistance r_2; (*c*) in parallel with (*a*) and (*b*) the sheath, by which is meant the connective tissue sheath, any unremoved nerve fibres, the cells of the sheath of Schwann and any residual film of sea water between the axon and the vaselined stopper. Its resistance is designated r_1 in the diagram. The total effective resistance is then

$$R = \frac{r_1(r_4^+ + r_4^- + r_2)}{r_1 + r_4^+ + r_4^- + r_2}. \tag{1}$$

The notation is that of Cole & Hodgkin (1939). For approximate values of these resistances the measurements taken by these authors may be used with some adjustment to suit the present circumstances. Hence, if stopper length is 1 cm, we may take

$$r_2 = 16{,}000\ \Omega. \tag{2}$$

For the resistances at each end, r_4, the formula provided by Cole & Hodgkin (1939, p. 676, equation 1) takes into consideration the spread of the current through some length of the protruding axon and, according to these authors, applies satisfactorily as long as the axon extends into the sea-water medium a distance of 1 cm or more. This provides an average value of

$$r_4 = 9000\ \Omega. \tag{3}$$

We assume as a first approximation that

$$r_4^+ + r_4^- = 2 \times 9000 = 18{,}000\ \Omega.$$

The experimental value of r_1 given by Cole & Hodgkin must be modified in the present case to allow for the difference between an axon mounted in mineral oil as theirs was, and our mount in a vaselined stopper. To do this, the axoplasm was removed from two axons and was replaced by air. When mounted, the total resistances were as shown in Table 3.

TABLE 3

	Resistance of sheath		Electro-osmotic efficiency	
Axon	At start	Later 30–40 min	Start	Later
19	100,000 Ω	69,000 Ω	3·4 (B-)	10·2 (A-)
21	155,000 Ω	127,000 Ω	1·7 (A-)	27·2 (B-)
		Average e-o	10·6	

As a representative figure, 100,000 seems reasonable for this total resistance, R, although it is obviously rather variable. To obtain a value for r_1, assume r_2 to have been increased tenfold by the removal of probably 90% of the

https://doi.org/10.1017/S0025315400004574 Published online by Cambridge University Press

axoplasm, i.e. $r_2 = 160{,}000\ \Omega$. From Ohm's Law and the above values we have, for the air-filled axon,

$$R = \frac{r_1(2r_4+r_2)}{r_1+2r_4+r_2},$$

giving

$$r_1 = 230{,}000\ \Omega. \tag{4}$$

The proportion of the current passing through the core and both end membranes will therefore be (see (2), (3), (4) above), in the normal axon,

$$\frac{r_1}{r_1+r_2+2r_4} = 0{\cdot}87.$$

This figure drops to 0·6 if r_1 is taken as 62,000 Ω, the value found by Cole & Hodgkin for an axon in mineral oil.

By further reference to the calculations of Cole & Hodgkin (1939), $2r_4$ may be apportioned to the anodal and cathodal ends of the axon and the above ratio (0·87) used to calculate expected voltage drops across the membrane at either end, the cathodal end where depolarization exists having, of course, a much lower drop than the anodal end which is hyperpolarized. For example, at 10 μA current the voltage drop across the anodal nerve membrane is of the order of 140 mV, while that at the cathodal end is only about 15 mV. These values are very close to the voltages employed in the present series of experiments and the cathodal end is already above the probable depolarization threshold. At less than 2 μA current, voltages may be expected to remain below the threshold of the cathodal end and hence the voltage drops at anodal and cathodal membranes begin to approach equality at that value which corresponds to the resistance of the resting membrane. This current value (2 μA) is below any values observed in the present series. We therefore believe that the observations reported here are within the physiological range, although they probably all involve large depolarization and hyperpolarization of the membranes. Indeed, observations made at 30 μA may have reached the damaging level.

RESULTS

Fig. 2 (p. 352) shows two typical observations on the same preparation each at 30 μA current. Quantities plotted are volume change during each minute against time. The rate of thermal drift is shown by the dotted line. In Fig. 4 averages of all observations, expressed as $cm^3\ min^{-1}$ ($\mathcal{J}$), are shown. plotted against current (I).

The volume flow was always increased towards the cathode when a current was applied. Therefore the zeta potential was negative. When calculated as molecules of water per ion (= electro-osmotic efficiency or e-o) all values are significantly greater than 2·0, which is assumed by Spiegler (1958) to be the hydration number for Na^+. This is taken to indicate true electro-osmosis.

Electro-osmosis in axon segments with open ends and even in glass tubes filled with axoplasm (Table 2) is not significantly less than that observed in normal axons in S.W. + A.S.W., when calculated on the basis given. Glass tubes filled with agar also show some electro-osmosis, and this fact indicates that the agar plugs used to keep the axoplasm in place may have contributed to the effect observed. This matter will be treated again in the discussion.

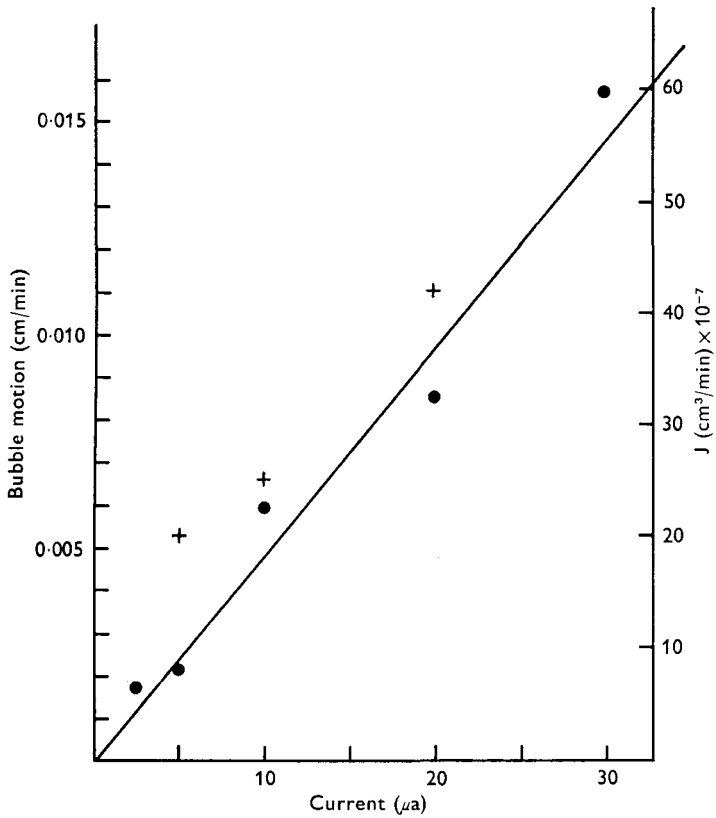

Fig. 4. Average rates of motion of water after elimination of thermal drift (cm/min) *vs.* current (μA). The right-hand scale also shows volume changes in cm^3 min^{-1}. The straight line is drawn to correspond with the average electro-osmotic efficiency (16) of all observations in sea water and artificial sea water. These points are shown ●, while points shown + are averages from artificial sea water in which K^+ had been increased tenfold.

Axon segments in artificial sea water (A.S.W.) show no significant difference from those in normal sea water (S.W.). In A.S.W. with the K^+ concentration raised to 100 mM (A.S.W. + 100 K), however, the electro-osmotic values are much higher. The difference between preparations in S.W. and A.S.W. and those in A.S.W. + 100 K is 12 molecules per charge which yields $t = 6{\cdot}5$ and

$P < 0{\cdot}001$. The points plotted in Fig. 4 as + are representative of this increased e-o. They all fall well above the average straight line.

Reduction of the current to its lowest value in the presence of 100 K^+ caused still higher electro-osmotic efficiencies. At the lowest currents used (5 μA) the average e-o was 36, while the average at 10 and 20 μA combined was 22. The difference, 14, is clearly significant and suggests that e-o might be expected to increase further at still lower currents. Similar high e-o values at low currents have been observed in *Nitella* cells (MacRobbie & Fensom, 1969).

DISCUSSION

Electro-osmosis may be inferred if the observed volume flow is consistently towards the same electrode in the absence of a pressure differential ($\Delta P = 0$) and exceeds the quantity (2·0 molecules/charge) associated with the ionic hydration shells. Fig. 4 and Table 2 demonstrate that these criteria are met by all average values. Electro-osmosis has therefore been observed in isolated axons of *Loligo* and the preliminary observations of Stallworthy & Fensom (1966) on *Illex* thereby substantiated.

The e-o values are of the same order as those which can be derived from the work of Mudd (1926) and Sawyer & Harshaw (1966) (see Table 1). The value given by House (1964), 40 H_2O per positive ion, is significantly higher than those of Tables 1 and 2, but represents e-o when the chloride ion was eliminated from the surrounding medium.

It is now appropriate to examine the outer sheath, the axoplasm, and the membranes for their possible parts in the observed electro-osmosis.

The sheath and electro-osmosis

It is unlikely that the sheath was contributing much to the observed electro-osmosis for the following reasons: (1) As shown above (p. 355) the proportion of the current traversing this path is probably only one-tenth of the total current. (2) Two attempts to observe electro-osmosis in air-filled axons showed initially, at least, none (see above). More replications were not carried out because the flexibility of the air-filled axon made good contact with the stopper hard to obtain.

The axoplasm and electro-osmosis

The axoplasm proteins are probably negatively charged and it, therefore, would qualify as a possible site for the electro-osmosis observed. This is confirmed by the observations of electro-osmosis in axon segments with open ends and in axoplasm contained in glass tubes (Table 2). The e-o values are not significantly different from those of segments with tied ends. Under these conditions therefore the axoplasm seems to be able to account for all of the observed electro-osmosis.

The membrane and electro-osmosis

Although the contribution of the membrane to electro-osmosis seemed undetectable in S.W. and in A.S.W., the increase observed in A.S.W. + 100 K may be attributed to the membrane. This raises the question of the probable effect of two electro-osmotically active materials placed in series. An indication of the relationship may be obtained by considering the particle movements (flows, currents) as independent variables and expressing the coefficients relating motion to gradients (voltage, pressure) as resistances. This yields a series of expressions analogous to those given by Kedem & Katchalsky (1963), p. 1921, as follows:

$$\Delta P^m = R_p^m \mathcal{J} + R_x^m I, \tag{1}$$

$$\Delta E^m = R_x^m \mathcal{J} + R_E^m I, \tag{2}$$

$$\Delta P^A = R_p^A \mathcal{J} + R_x^A I, \tag{3}$$

$$\Delta E^A = R_x^A \mathcal{J} + R_E^A I, \tag{4}$$

where ΔP and ΔE are pressure difference and voltage difference respectively and superscripts refer to membrane (^{m}) and axoplasm (^{A}), $\mathcal{J}$ is volume flow and I is electric current, R_p is an hydraulic resistance, and R_E an electrical resistance.

R_x has the typical Onsager reciprocal symmetry and represents the effect of pressure on current or voltage on flow. Note that $\mathcal{J}/I$ is electro-osmosis when $\Delta P = 0$. If $\Delta P^m = 0$, (1) yields

$$\mathcal{J}^m/I^m = -R_x^m/R_p^m \text{ for the membrane,} \tag{5}$$

and when $\Delta P^A = 0$, (3) yields

$$\mathcal{J}^A/I^A = -R_x^A/R_p^A \text{ for the axoplasm.} \tag{6}$$

If membranes and axoplasm are in series (1) and (3) may be added, since

$$\Delta P = \Delta P^m + \Delta P^A = (R_p^m + R_p^A)\mathcal{J} + (R_x^m + R_x^A)I.$$

When $\Delta P = 0$, i.e. in the case of electro-osmosis

$$\frac{\mathcal{J}}{I} = \frac{R_x^m + R_x^A}{R_p^m + R_p^A}. \tag{7}$$

In the case where the hydraulic resistance of the membrane is much greater than that of the axoplasm, i.e. $R_p^m \gg R_p^A$, (7) becomes

$$\frac{\mathcal{J}}{I} = -\frac{R_x^m + R_x^A}{R_p^m} = -\frac{R_x^m}{R_p^m} - \frac{R_x^A}{R_p^m}, \tag{8}$$

substituting from (5) and (6) gives

$$\frac{\mathcal{J}}{I} = \frac{\mathcal{J}^m}{I^m} + \frac{\mathcal{J}^A}{I^A}\,\frac{R_p^A}{R_p^m}. \tag{9}$$

https://doi.org/10.1017/S0025315400004574 Published online by Cambridge University Press

This states that as long as the hydraulic resistance of the membrane is much greater than that of the axoplasm the electro-osmotic efficiency of the system is equal to that of the membrane plus the electro-osmotic efficiency of the axoplasm $\times R_p^A/R_p^m$.

Since R_p^m is assumed to be much greater than R_p^A, then the fraction R_p^A/P_p^m is also very small making the e-o contribution of the axoplasm small too, unless $\mathcal{J}^A/I^A$ be very large. But we know from Table 2 that $\mathcal{J}^A/I^A$ is not very different from $\mathcal{J}/I$. Therefore the contribution of the axoplasm must be small compared with that of the membrane.

Accepting this conclusion and that we are dealing with true electro-osmosis, other properties of the membrane may be calculated. For instance, the minimum concentration of ions in the pore fluid ($\bar{x}$) is (Fensom & Wanless, 1967)

$$\bar{x} = \frac{1}{F}\frac{I}{\bar{\mathcal{J}}} \text{ where F is the Faraday constant,}$$

$$= 3 \times 10^{-3} \text{ equivalents cm}^{-3}.$$

Since sea water contains only 5×10^{-4} equiv. cm^{-3} this does support the finding of Hodgkin & Keynes (1955) that electrical resistance measurements of permeation do not agree with diffusion measurements by a factor of about 4.

While highly speculative, it is perhaps worth noting that if pore size be assumed, pore number can also be calculated (Fensom & Wanless, 1967) and hence number of ions per pore. If their values for *Nitella* are used the number of ions per pore is 6, which is surprisingly close to the factor of 4 above.

Anodal and cathodal effects

The current (and the water) must enter at the anodal end and leave at the cathodal end of the axon segment. When the very different permeabilities of these areas are considered, it is possible that the axon might shrink somewhat since water leaves it more easily than it can enter. This would cause a slight error which would augment readings taken when chamber A is negative and diminish these taken when B is negative (see Fig. 1). Since all observations were paired with regard to electrical polarity, this will not influence our average results.

The differing resistances of the two ends give reason to assume that the change in the membrane caused by a tenfold increase in K^+ has occurred at the anodal, hyperpolarized end, if only because this end was offering the greatest resistance at low K^+ and was therefore capable of the greatest modification by the depolarizing influence of the increased K^+. This assumption is strengthened by the observation by Hodgkin & Keynes (1955) that at constant potential a tenfold increase in external K^+ caused a 20- to 40-fold increase in K^+ influx through the axon membrane. Moreover, these authors

point out that at low outside K^+ concentrations other ions than K^+ are carrying current whereas at high K^+ only the K^+ is involved. This would be very likely to alter the electro-osmotic efficiency. This observation of an increase in electro-osmotic efficiency at high external K^+ concentrations lends support to the hypothesis that the membrane contains pores as suggested by these authors to account for the observed differences in influxes and effluxes. In fact their discussion postulates as one possible hypothesis an electro-osmotic effect. 'Even in a relatively large tube, movement of one potassium ion might tend to sweep along a column of water, making it easier for other potassium ions to move in the same direction and hindering movements in the opposite directions' (p. 85). Evidently a tenfold increase in external K^+ increases both K^+ influx and electro-osmotic efficiency, which lends credance to the above suggestion. Dainty, Croghan & Fensom (1963) also suggested (p. 963) on theoretical grounds that electro-osmosis may be involved in disproportionately high conductance values like those of K^+ influxes observed by Hodgkin & Keynes.

Electro-osmosis of various currents

Electro-osmotic efficiency at 5 μA in A.S.W. + 100 K is significantly greater than at any higher currents. This suggests that currents lower than 5 μA might cause a further increase in e-o and also that there may be a minimum for e-o values at moderate currents of about 20 μA (Fig. 4). With the accompanying rise in resistance caused by hyperpolarization (Hodgkin *et al.* 1952) it seems unlikely that the lower electro-osmotic efficiency at higher currents is due to counter currents of ions or water, but it seems more probable that increased frictional effects are preventing maximum water flows from accompanying the movement of ions. As the current increases still further the frictional forces should be reduced again as the membrane structure begins to fail. Whether this level of current has been reached in the results of Fig. 4 is not certain, although the e-o efficiency at 30 μA does seem to be a little higher (not statistically significant).

If it be granted that the rise in e-o in high K^+ concentrations is due to the effect on the membrane this constitutes evidence that pores or channels exist in the membrane and that the area of pores of a suitable size (4–8 Å, see Fensom & Wanless, 1967) has increased in high K^+ either by a change in overall number of pores, or in their size, or both.

In any case, the detection of electro-osmosis in the system would seem to imply channels through all parts of it where water and ions can flow and this must include the membrane.

CONCLUSIONS

Electro-osmosis occurs when a current of 5–50 μA is passed lengthwise through a segment of the giant axon of the squid, *Loligo forbesi*. In sea water

the average electro-osmotic efficiency is 16 ± 1 moles H_2O per positive charge. The Zeta potential is negative.

In extruded axoplasm in glass tubes current can also cause electro-osmosis and at a similar electro-osmotic efficiency (14 ± 2).

In artificial sea water containing 100 mM-KCl the electro-osmotic efficiency of squid axons is increased to 28 ± 4 moles H_2O per positive charge.

At the smallest currents (5 μA) in sea water containing 100 mM-KCl there is a significant increase in electro-osmotic efficiency over the average at currents of 10 and 20 μA, which raises the possibility that electro-osmotic efficiency may further increase at very low currents.

Since electro-osmosis was detected in the system, channels or pores for simultaneous ion and solution flow must exist.

Electro-osmosis through axoplasm could account for the effects observed in normal sea water, but calculations suggest that it is the axon membrane which is contributing the dominating effect. In high potassium concentrations the contribution would seem to be greater than in normal sea water and this suggests that pores have increased either in number or in size.

This work was supported by the Marjorie Young Bell Fund of Mount Allison University, and by a travel grant from the Nuffield Foundation of Great Britain.

The author's obligations to these organizations for their support are cheerfully acknowledged. Furthermore, the helpfulness of the entire staff, both technical and professional, of the Marine Biological Station has added greatly to the accomplishment of the task. I appreciate particularly the critical suggestions of my colleague, Prof. D. S. Fensom.

REFERENCES

CALDWELL, P. C., 1960. The phosphorus metabolism of squid axons and its relationship to the active transport of sodium. *J. Physiol., Lond.*, Vol. 152, pp. 545–60.

COLE, K. S. & HODGKIN, A. L., 1939. Membrane and protoplasm resistance in the squid giant axon. *J. gen. Physiol.*, Vol. 22, pp. 671–87.

DAINTY, J., CROGHAN, P. C. & FENSOM, D. S., 1963. Electro-osmosis, with some applications to plant physiology. *Can. J. Bot.*, Vol. 41, pp. 953–66.

DAINTY, J. & HOPE, A. B., 1959. The water permeability of cells of *Chara australis* R. Br. *Aust. J. biol. Sci.*, Vol. 12, pp. 136–45.

FENSOM, D. S. & DAINTY, J., 1963. Electro-osmosis in *Nitella*. *Can. J. Bot.*, Vol. 41, pp. 685–91.

FENSOM, D. S., URSINO, D. J. & NELSON, C. D., 1967. Determination of relative pore size in living membranes of *Nitella* by the techniques of electro-osmosis and radioactive tracers. *Can. J. Bot.*, Vol. 45, pp. 1267–75.

FENSOM, D. S. & WANLESS, I. R., 1967. Further studies of electro-osmosis in *Nitella* in relation to pores in membranes. *J. exp. Bot.*, Vol. 18, pp. 563–77.

HODGKIN, A. L., HUXLEY, A. F. & KATZ, B., 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol., Lond.*, Vol. 116, pp. 424–48.

HODGKIN, A. L. & KEYNES, R. D., 1955. Potassium permeability of a giant nerve fiber. *J. Physiol., Lond.*, Vol. 128, pp. 61–88.

HOUSE, C. R., 1964. The nature of water transport across frog skin. *Biophys. J.*, Vol. 4, pp. 401–16.

KEDEM, O. & KATCHALSKY, A., 1963. The permeability of composite membranes, Part I—Electric current, volume flow and flow of solute through membranes. *Trans. Faraday Soc.*, Vol. 59, pp. 1918–30.

MACROBBIE, E. A. C. & FENSOM, D. S., 1969. Measurements of electro-osmosis in *Nitella translucens*. *J. exp. Bot.*, Vol. 20, pp. 466–84.

MUDD, S., 1926. Electroendosmosis through mammalian serous membranes. III. The relation of current strength and specific resistance to rate of liquid transport. Transport rate with serum. *J. gen. Physiol.*, Vol. 9, pp. 361–73.

SAWYER, P. N. & HARSHAW, D. H., 1966. Electroosmotic characteristics of canine aorta and vena cava wall. *Biophys. J.*, Vol. 6, pp. 653–63.

SOLOMON, A. K., 1960. Red cell membrane structure and ion transport. *J. gen. Physiol.*, Vol. 43, Suppl. I, pp. 1–15.

SPIEGLER, K. S., 1958. Transport processes in ionic membranes. *Trans. Faraday Soc.*, pp. 1408–28.

STÄMPFLI, R., 1959. Is the resting potential of ranvier nodes a potassium potential? *Ann. N.Y. Acad. Sci.*, Vol. 81, pp. 265–84.

STALLWORTHY, W. B. & FENSOM, D. S., 1966. Electro-osmosis in axons of freshly killed squid. *Can. J. Physiol. Pharmac.*, Vol. 44, pp. 866–70.