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AXONAL ADAPTATION TO OSMOTIC AND IONIC STRESS IN AN INVERTEBRATE OSMOCONFORMER (*MERCIERELLA ENIGMATICA* FAUVEL)

III. ADAPTATIONS TO HYPOSMOTIC DILUTION

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SUMMARY

The giant axons of this extreme osmoconformer were adapted, *in vitro*, to progressive hyposmotic dilution of the bathing medium (from 1024 m-Osmol to concentrations as low as 76·8 m-Osmol). Hyposmotic adaptation is associated with reductions in the intracellular concentrations of both sodium and potassium ions. These reductions do not appear to result from appreciable axonal swelling. The different electrical responses to isosmotic and hyposmotic dilution suggest that reduction in $[Na^+]_i$ results from ouabain-dependent sodium extrusion, in response to ionic dilution, and that reduction in $[K^+]_i$ is induced by a combination of ionic *and* osmotic dilution. The reduced level of intracellular potassium achieved during hyposmotic adaptation represents a balance between the necessity to contribute to osmotic equilibration and to maintain a potassium gradient across the axon membrane sufficient to produce appreciable axonal hyperpolarization during dilution of the bathing medium. This hyperpolarization tends to maintain the amplitude of the action potential, by compensating for reduction in overshoot (with decline in E_{Na}), and by reducing sodium inactivation. This, together with the reduction in $[Na^+]_i$, enables overshooting action potentials of relatively large amplitude and rapid rise time to be maintained during more than tenfold dilution of the ionic and osmotic concentration of the bathing medium.

INTRODUCTION

In the previous paper we analysed the electrical responses of the giant axons of an extreme osmoconformer, the serpulid polychaete *Mercierella enigmatica* Fauvel, to ionic dilution of the bathing medium in isosmotic conditions (Benson & Treherne, 1978). We showed that the axon of sea-water-adapted individuals were able to produce action potentials of large amplitude over a wide range of ionic dilutions when the osmotic concentration was maintained by the addition of mannitol to the bathing medium. This unusual ability appears to result largely from the axonal hyperpolarization associated with the dilution of the relatively high external potassium

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concentration which is a characteristic of the blood of sea-water-adapted *Mercierella* (cf. Skaer, 1974*b*). The axonal hyperpolarization observed during isosmotic dilution has two important consequences. First, it tends to maintain the amplitude of the action potential, the decline in the extent of the overshoot (resulting largely from reduction in $[Na^+]_o$) being compensated by the increase in resting potential. Secondly, this hyperpolarization reduces the apparent inactivation of the sodium channels and, thus, maintains the rapid rising phase of the action potential and enables the peak of the overshoot to approach E_{Na} over a wide range of isosmotic dilutions. An apparent reduction in intracellular sodium during prolonged external ion dilution also occurs, increasing the inward sodium gradient across the axon membrane.

Mercierella giant axons are therefore able to function effectively over an unusually wide range of ionic dilutions in isosmotic conditions (i.e. when constant osmotic concentration is maintained by the addition of mannitol to the bathing medium). In its natural estuarine habitats, *Mercierella* is exposed to considerable daily variation in the ionic concentrations of the surrounding water, and so is subjected to osmotic as well as ionic stress. In this paper we examine the dual effects of both ionic and osmotic stress on the electrical responses of these axons during hyposmotic dilution of the bathing medium.

METHODS AND MATERIALS

The electrophysiological apparatus and techniques for intracellular recording from the giant axon were similar to those used in previous investigations (Carlson & Treherne, 1977; Benson & Treherne, 1978; Skaer *et al.* 1978). Changes of solution were made by vigorously syringing directly on to the axon to avoid fluid stratification in the vicinity of the axon surface during hyposmotic dilution of the bathing solution (Treherne, Benson & Skaer, 1977). The normal physiological saline had the following composition: Na^+, 482·3; K^+, 30; Mg^{2+}, 77; Ca^{2+}, 31; SO_4^{2-}, 26; Cl^-, 663·8; OH^-, 12·5; Pipes, 7·5 mM (pH, 6·9; osmotic concentration, 1024 m-Osmol). Hyposmotic dilution was made by dilution with distilled water; isosmotic dilution was achieved by appropriate substitution with mannitol or choline chloride. The osmotic concentration of the blood was measured using the method of Ramsay & Brown (1955).

Measurements of axonal diameter were made using Zeiss-Nomarski differential interference equipment for transmitted-light microscopy (Allen, David & Nomarski, 1969). The dissected worms were pinned flat across two pieces of Sylgard separated by a glass strip and mounted between microscope cover glasses. A portion of the body wall containing a giant axon was photographed using time-lapse cinematography. Solutions of varying salinity were perfused through the experimental chamber.

RESULTS

Effects of abrupt hyposmotic dilution on axonal resting and action potentials

The axonal responses to abrupt hyposmotic dilution (i.e. when both the ionic and osmotic concentrations were reduced) were compared with those observed during isosmotic dilution of the bathing medium (i.e. when the osmotic concentration

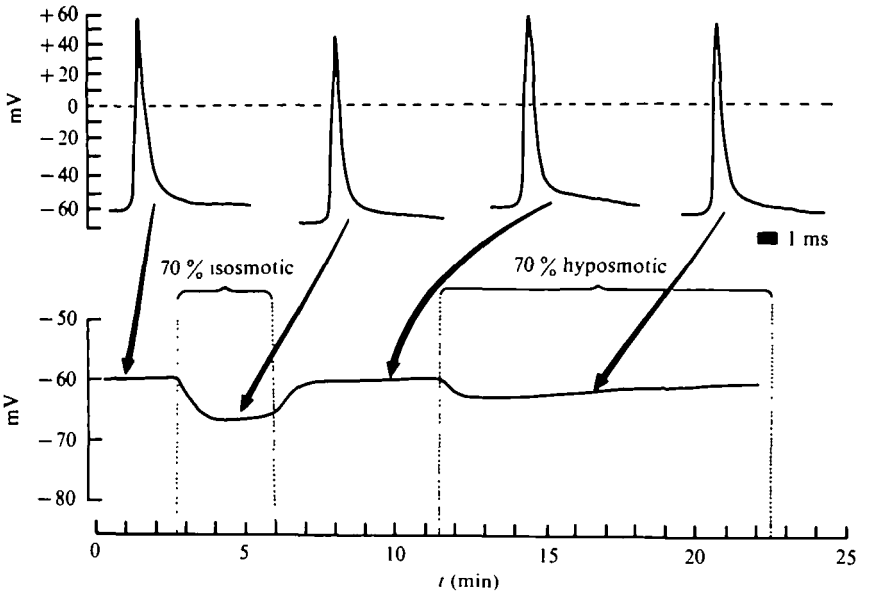

Fig. 1. The effects of step change of 70%, isosmotic, dilution and of 70%, hyposmotic, dilution on the intracellularly recorded resting and action potentials in the giant axon of *Mercierella*. The action potentials were displayed on a chart recorder using a transient signal processor. The lower tracing shows the continuous potentiometric recording of the resting potential.

was maintained by addition of mannitol during ionic dilution). As previously observed, isosmotic dilution of the bathing medium resulted in a rapid and sustained hyperpolarization of the axons from sea-water-adapted animals (Fig. 1): an effect which is postulated to result primarily from reduction in $[K^+]_o$ (Benson & Treherne, 1978). In contrast, equivalent hyposmotic dilution induced a smaller hyperpolarization which declined relatively slowly (Fig. 1).

Abrupt exposure of impaled sea-water-adapted axons to 60% hyposmotic saline occasionally resulted in axonal damage in the form of a rapid depolarization and conduction block. This damage appeared to be an artifact of microelectrode impalement during hyposmotic stress, since it was possible to reimpale and to record action potentials from the adjacent giant axon. Essentially similar results were obtained on exposure to 50% hyposmotic saline. However, abrupt exposure to 40%, hyposmotic, saline appears to cause irreversible axonal damage (Skaer *et al.* 1978).

The results of these experiments indicate that rapid, hyposmotic, dilution of the medium bathing sea-water-adapted axons induces a transient hyperpolarization of the axonal membrane which contrasts with the larger, sustained, hyperpolarization recorded during isosmotic dilution. Sea-water-adapted axons can survive abrupt dilutions as low as 50%, but appear to suffer irreversible damage at 40%, hyposmotic, dilution.

Effects of progressive hyposmotic dilution

Attempts were made to measure, *in vitro*, the axonal responses to dilution regimes equivalent to those of the body fluids, *in vivo*, during extreme osmotic stress. In

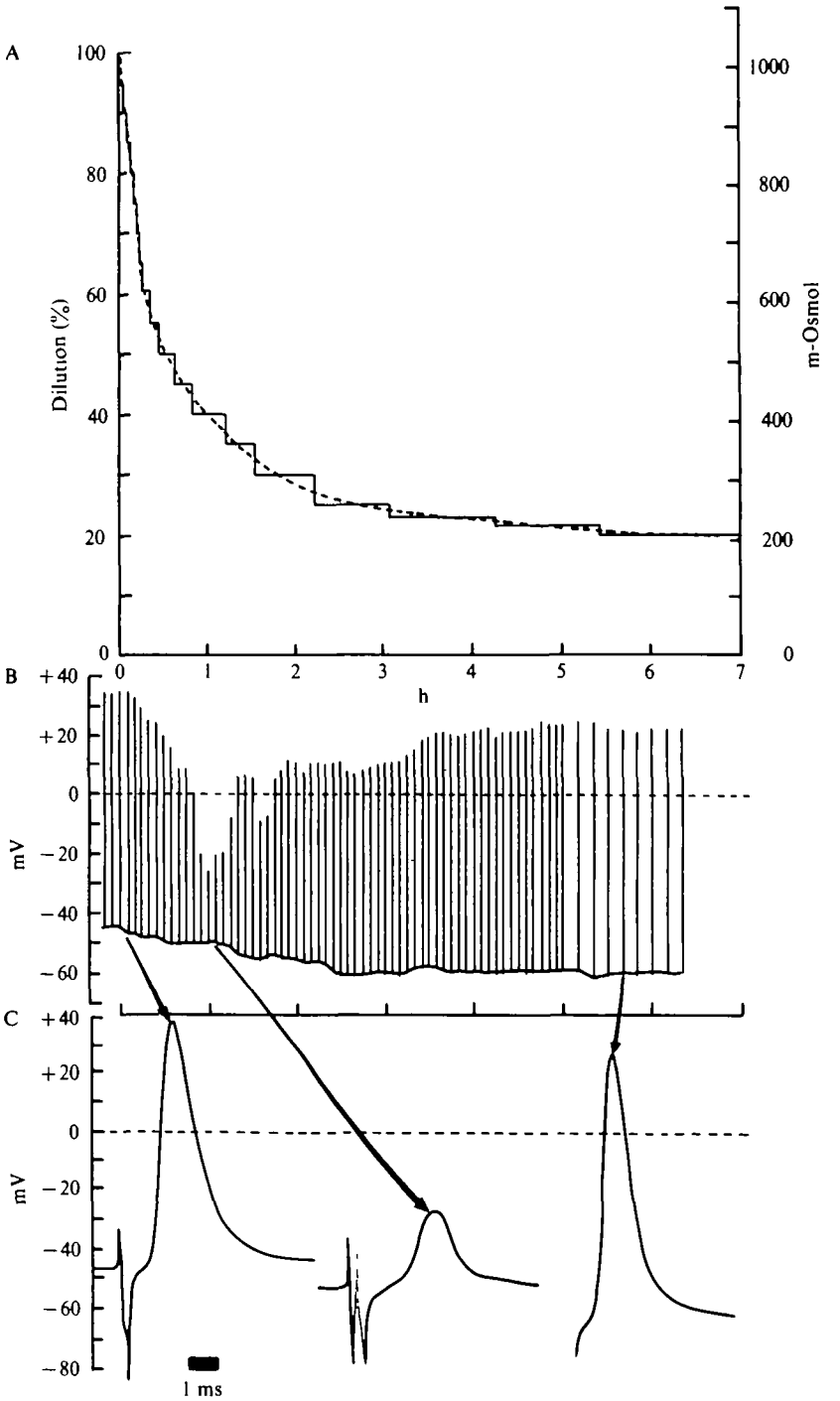

Fig. 2. The effects of an experimental hyposmotic dilution regime on axonal resting and action potentials recorded using an intracellularly located microelectrode. (A) The experimental dilution regime, shown as a series of step changes, mimics the rate of reduction in blood concentration (broken line) observed on transfer of a colony of *Mercierella* (in their tubes) to distilled water (data from Skaer, 1974). (B) Continuous recording of the resting and action potentials during the above experimental dilution regime. (C) Action potentials recorded at successive stages of hyposmotic adaptation.

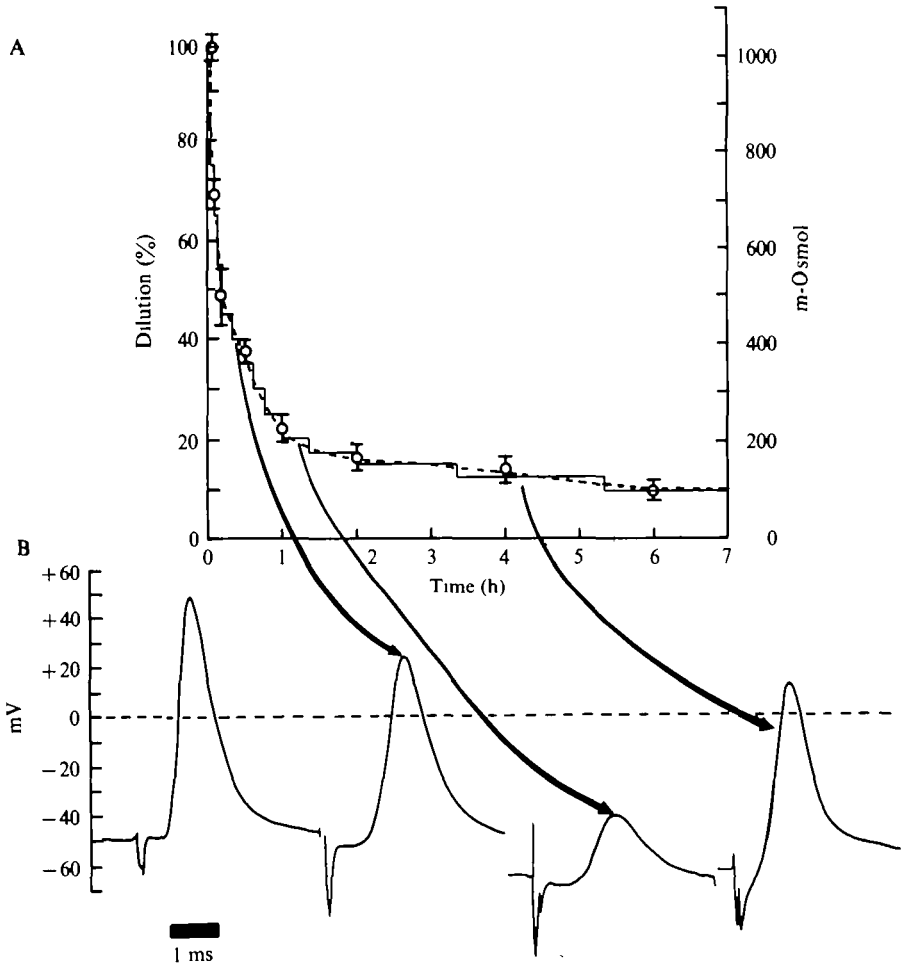

Fig. 3. The effects of an extreme hyposmotic dilution regime (equivalent to that of the blood of *Mercierella* on transfer of isolated individuals to distilled water) on the action potentials from the giant axon. (A) The decline in osmotic concentration of the blood is shown by the curved broken line. The open circles represent the mean values of the osmotic concentration of the blood (n = 5) and the vertical lines the extent of twice the standard error of the mean. The experimental dilution regime is shown as a series of step changes. (B) Action potentials recorded on impalement of the giant axon with an intracellular microelectrode at different times during exposure to the experimental dilution regime.

these experiments the rates of dilution mimicked those measured in the body fluids by a series of appropriate step dilutions of the normal bathing medium (Fig. 2).

The experimental dilution regime illustrated in Fig. 2 is based on the changes in osmotic concentration of the blood measured in individuals, in their tubes, in a colony transferred from sea water (1024 m-Osmol) to distilled water (effectively 32 m-Osmol) (Skaer, 1974*a*).

The hyposmotic dilution regime illustrated in Fig. 2 was associated with a progressive hyperpolarization of the axonal membrane, the resting potential of which had increased by some −15 mV after 2½ h. The rapid, initial, phase of dilution induced a decline in amplitude of the recorded action potentials. At the apparently

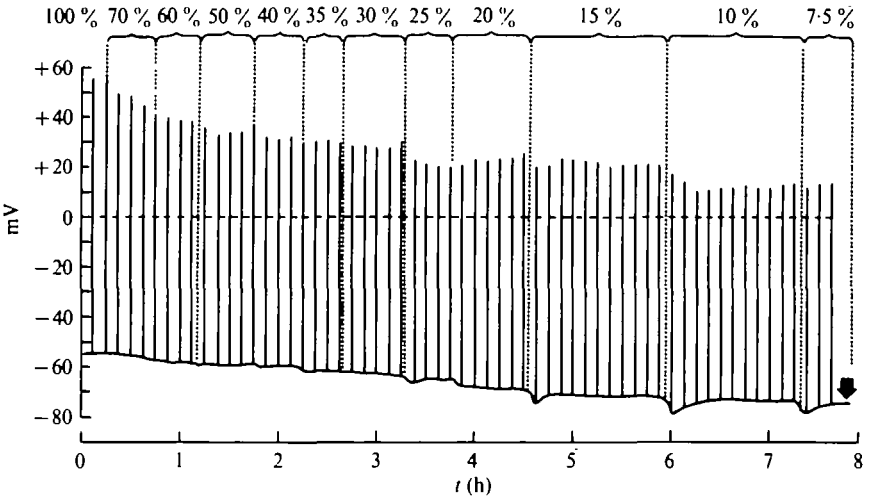

Fig. 4. Continuous recording of the resting and action potentials during gradual hyposmotic dilution of the bathing medium.

critical dilution of 40% there was an abrupt decline in action potential amplitude. This phase of hyposmotic dilution was followed by a progressive increase in the amplitude of the action potentials. After $4\frac{1}{2}$ h the action potentials were of larger amplitude and of shorter duration than initially recorded in full strength saline.

Attempts were also made to follow the axonal responses to an extreme rate of dilution equivalent to that which occurs in the blood on direct transfer of individuals (removed from their tubes) from sea water to distilled water. The rate of decline in osmotic concentration of the blood measured under these circumstances and the equivalent experimental regime are illustrated in Fig. 3. These data show that the blood concentration dropped precipitately from 1028 ($\pm$ 16·3; n = 8) to 220 ($\pm$ 8·8; n = 5) after 1 h and to 99·6 ($\pm$ 6·3; n = 5) m-Osmol after 6 h. After 6 days the osmotic concentration declined further to 84·2 ($\pm$ 7·4; n = 5) m-Osmol. Many, but not all worms were prostrated by this extreme change in osmotic concentration of the bathing medium and the withdrawal reflex responses sometimes disappeared shortly after exposure to distilled water. Such worms subsequently recovered, within 2–4 h, and the characteristic rapid withdrawal response to stimulation of the crown was re-established.

It was difficult to maintain continuous intracellular recordings from the axon with the extreme dilution regime illustrated in Fig. 3. In these experiments, therefore, the electrical responses of the axons were monitored during hyposmotic adaptation by successive microelectrode impalements. As can be seen from Fig. 3, axonal function persisted throughout the experiment, although action potentials of greatly reduced amplitude were recorded on transfer from 20 to 17·5% dilution. Subsequently, overshooting action potentials of larger amplitude were recorded. These were associated with a 10 mV hyperpolarization of the axonal membrane.

Routine hyposmotic adaptation was achieved using the less extreme rate of dilution illustrated in Fig. 4. With this regime overshooting action potentials were maintained

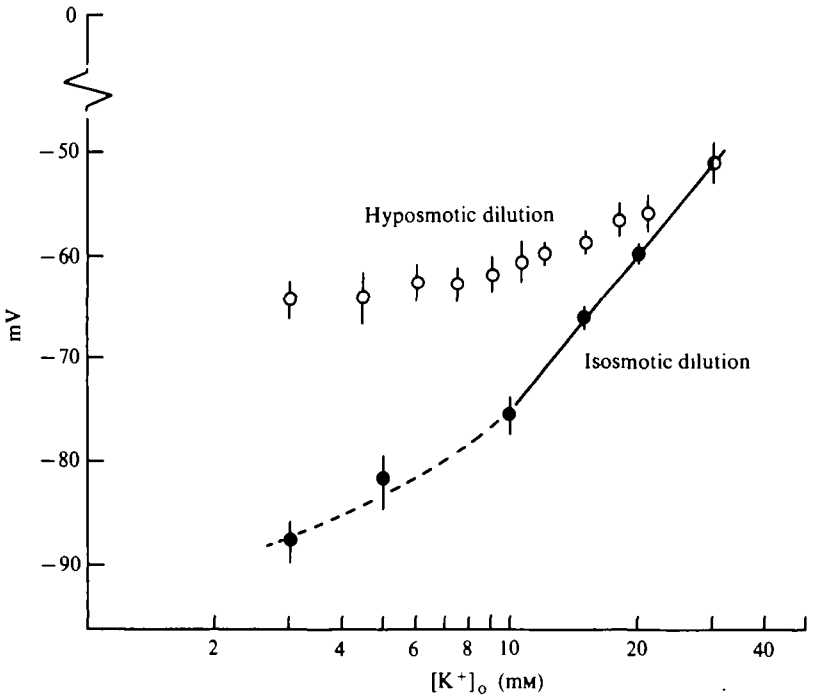

Fig. 5. The relation between resting potential (open circles) and external potassium concentration during progressive hyposmotic dilution regimes such as illustrated in Fig. 4. The closed circles show the resting potentials measured during equivalent isosmotic dilution (i.e. when constant osmotic concentration was maintained with mannitol). The vertical lines indicate the extent of twice the standard error of the mean (n = 6–10).

down to 10% dilution, conduction block developing at 7·5%. The adaptation process was associated with a continuous increase in the axonal resting potential which, at the termination of the experiment, had increased by −20 mV.

Relation between $[K^+]_o$ and axonal resting potential during progressive hyposmotic dilution

Fig. 5 illustrates the resting potentials, relative to the external potassium concentration, during *in vitro* hyposmotic adaptation as shown in Fig. 4. The values for membrane potential plotted in Fig. 5 were those measured following stabilization at each successive dilution. There was a marked difference in the relation between resting potential and $[K^+]_o$ during progressive hyposmotic and isosmotic dilution of the bathing medium. In the latter case ionic dilution (which was compensated by addition of mannitol) was accompanied by a massive hyperpolarization, primarily as a result of reduction in $[K^+]_o$ (Benson & Treherne, 1978). Hyposmotic dilution, on the other hand, produced a smaller hyperpolarization of the axonal membrane (Fig. 5).

The relatively small effect of reduced $[K^+]_o$ on axonal resting potential during progressive hyposmotic dilution could result either from decreased potassium permeability of the axon membrane or from a decline in $[K^+]_i$. The former possibility

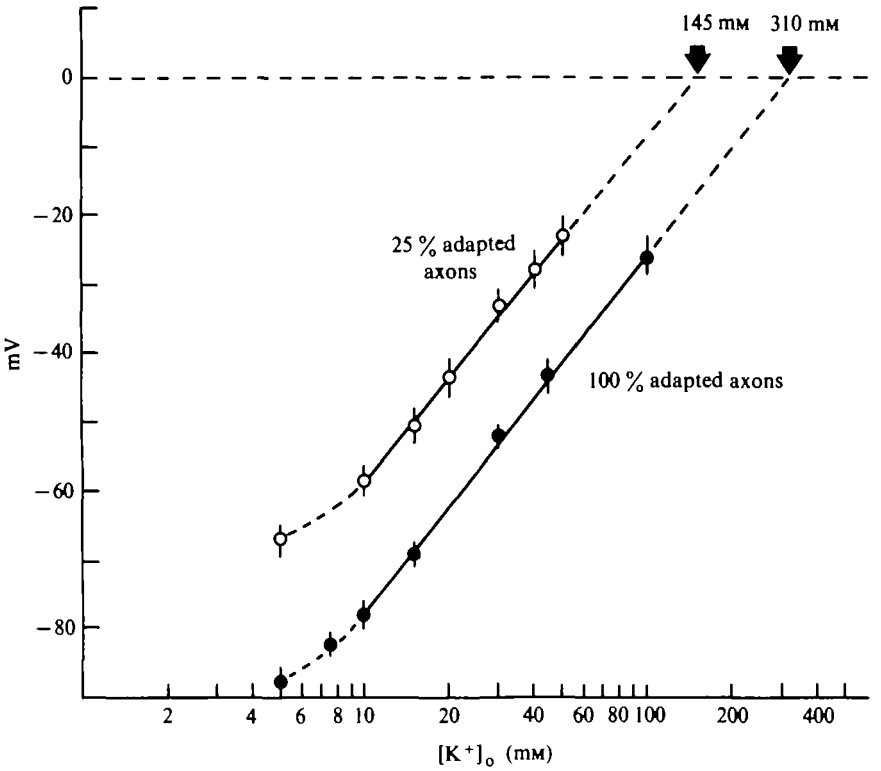

Fig. 6. Relation between resting potential and external potassium concentration for axons in 100% saline (closed circles) and in those adapted to 25%, hyposmotic, saline (open circles). The calculated regression line (continuous line) for the unadapted axons has a slope of 51·2 mV for decade change in $[K^+]_o$ ($r = 0{\cdot}982$; $n = 30$) and for 25% adapted axons a slope of 50·7 mV for decade change in $[K^+]_o$ ($r = 0{\cdot}956$; $n = 44$). The values of $[K^+]_i$ were estimated by extrapolation of the regression lines to zero potential. The variations in potassium concentration were made by appropriate substitution with choline chloride. The symbols indicate the mean and twice the standard error of the mean.

is eliminated by the similarity of the slopes relating potential to $[K^+]_o$ for 100% and 25% (hyposmotically) adapted axons (Fig. 6). A change in intracellular potassium concentration (from around 310 to 145 mM) is therefore indicated by the data illustrated in Fig. 6.

Relation between $[Na^+]_o$ and action potential overshoot during progressive hyposmotic dilution

The decline in overshoot relative to $[Na^+]_o$ during progressive hyposmotic dilution was rather close to that observed during isosmotic dilution of the external ion in the higher concentration range (Fig. 7). A marked departure was seen, however, at lower dilutions. As with potassium, this departure does not appear to result from large changes in the selective permeability of the axonal membrane to sodium ions. This is shown by the approximate similarity of the slopes relating $[Na^+]_o$ to the extent of the overshoot of axons adapted to 100% and to 25% diluted media (i.e. 56·3 as compared with 52·5 for decade change in $[Na^+]_o$ for 100 and 25% adapted

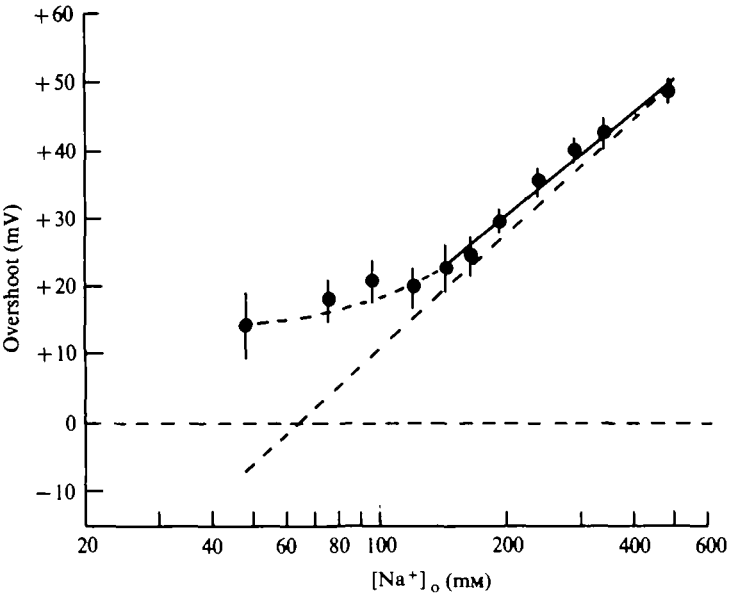

Fig. 7. The relation between the extent of the overshoot of the action potentials recorded during progressive hyposmotic dilution such as is illustrated in Fig. 4. The continuous line is the calculated regression line which has a slope of 50·5 mV for decade change in $[Na^+]_o$ (r = 0·954; n = 7). The broken line indicates the relation between overshoot and $[Na^+]_o$ (55·8 mV change for decade alteration in $[Na^+]_o$) when sodium concentration was varied in axons from sea water adapted animals in normal saline (from Carlson & Treherne, 1977).

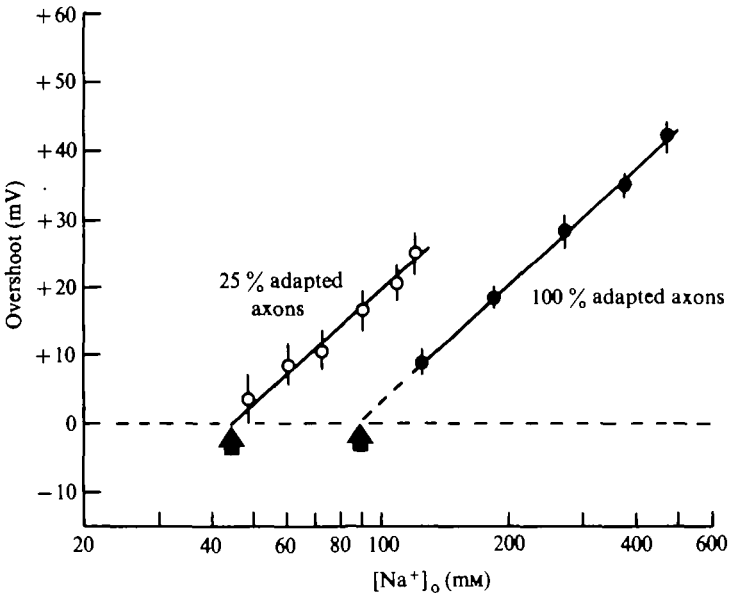

Fig. 8. The relation between $[Na^+]_o$ the extent of the overshoot measured in the axons of sea-water-adapted animals in normal saline in axons adapted, *in vitro*, to 25 %, hyposmotic saline. The calculated regression line for 100 % adapted axons had a slope of 56·3 mV (r = 0·9741; n = 27) and for 25 % adapted axons a slope of 52·2 mV for decade change in $[Na^+]_o$ (r = 0·9459; n = 37). The values of $[Na^+]_i$ were estimated by extrapolating the regression lines to zero potential. Variation in $[Na^+]_o$ were made by substitution with choline chloride. The symbols represent the mean and extent of twice the standard error of the mean.

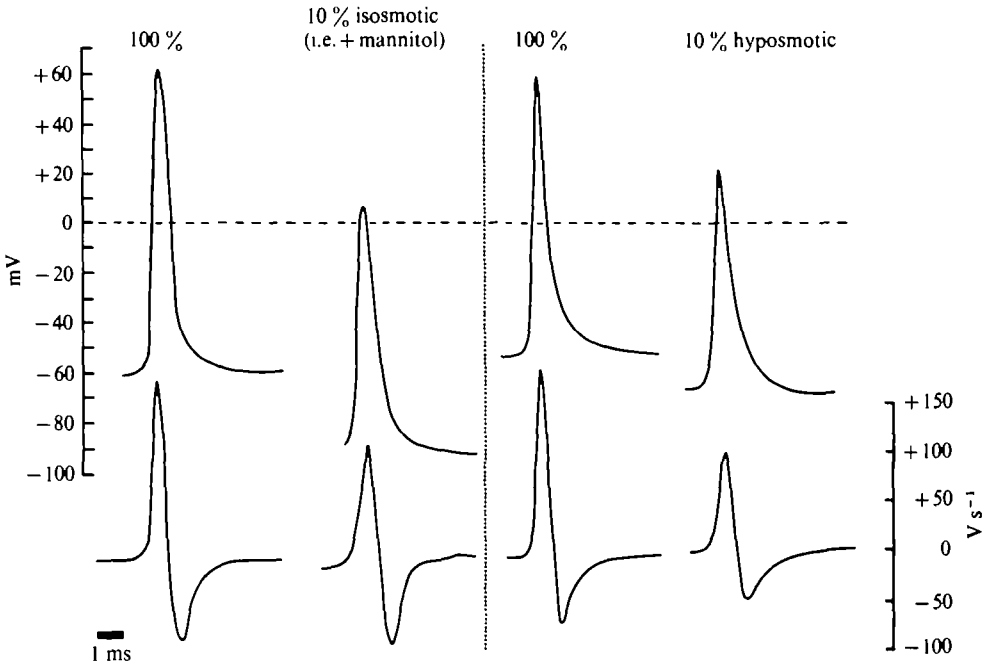

Fig. 9. Action potentials and rates of change of potential (V_{max}) recorded in axons before and after isosmotic and hyposmotic adaptation to 10% ionic dilution of the bathing medium.

axons) (Fig. 8). As can be seen from Fig. 8, the estimated intracellular sodium concentration is substantially reduced, from 87 to 44 mM, in the 25% adapted axons.

Effects of hyposmotic adaptation on the rates of rise and fall of the action potential

The preceding results indicate that hyposmotic adaptation, *in vitro*, involves reduction in the intracellular concentrations of both sodium and potassium ions. These alterations will influence the extent of the overshoot, the magnitude of the resting potential and, consequently, the kinetics of the change of membrane potential during the action potential. The effects of hyposmotic dilution on the rising and falling phases were measured by differentiating the recorded action potentials. The maximum rates of rise ($\dot{V}_{max}$) and fall provide measures of the net inward and outward ionic currents of the action potential (cf. Hodgkin & Katz, 1949).

Fig. 9 compares the action potentials and rates of change of membrane potential in axons before and after adaptation to 10% isosmotic (mannitol substituted) and 10% hyposmotic media. Typically, $\dot{V}_{max}$ of the hyposmotically adapted axon shows a more pronounced decline, to approximately one half, as compared with that of the isosmotically adapted axon. This can be correlated with the smaller axonal hyperpolarization observed during hyposmotic adaptation (an effect of reduction in $[K^+]_I$) and is probably due to the consequently higher degree of sodium inactivation as compared with that of the isosmotically-adapated axon (Benson & Treherne, 1978).

The maximal rate of fall of the action potential showed a slight increase during isosmotic adaptation, as compared with the decline observed with hyposmotic adaptation (Fig. 9). This can be attributed to a decrease in the outward, potassium, current which will clearly be related to the intracellular potassium concentration which has been shown to decrease in hyposmotically-adapted axons (Figs. 5, 6).

Measurement of axonal diameter during osmotic stress

Observations were made to determine the extent of axonal swelling during *in vitro* hyposmotic adaptation, using Zeiss–Nomarski differential interference optics. In these experiments the preparations were exposed to the dilution regime illustrated in Fig. 4. No increase in axonal diameter could be detected (with 5% limits of accuracy) during hyposmotic adaptation.

DISCUSSION

The experiments described above show that the giant axon of *Mercierella* can adapt, *in vitro*, to rapid dilution regimes equivalent to the most extreme rates of dilution that can occur in the body fluids (i.e. from 1024 to 84 m-Osmol on transfer from sea to distilled water). It is possible that short-term anomalous decreases in action potential amplitude observed during the most extreme dilution regimes (Figs. 2, 3) were due to electrode damage of the axonal membrane, since solutions were syringed in rapid succession directly on to the axons. The more gradual dilution of the bathing medium to equally low concentrations of external ions did not produce such abrupt decline in action potential amplitude (Fig. 4).

Axonal adaptation to progressive hyposmotic dilution involves a continuous hyperpolarization of the axon membrane. This hyperpolarization is, however, much less pronounced than that observed during isosmotic dilution of the ions in the bathing medium (i.e. when constant osmotic concentration is maintained with mannitol) (Fig. 5). In the latter case axonal hyperpolarization results primarily from the dilution of $[K^+]_o$ (Benson & Treherne, 1978). As previously shown, the potassium concentration of the blood of sea-water-adapted animals is unusually high (Skaer, 1974), *ca.* 30 mM-K^+, which maintains the axonal resting potential (*ca.* 54 mV) in a steep portion of the slope relating membrane potential to $[K^+]_o$ (Carlson & Treherne, 1977). Thus, in isosmotic conditions, the reduction in $[K^+]_o$ during ionic dilution produces a large axonal hyperpolarization.

The present results indicate that the smaller hyperpolarization observed during hyposmotic dilution does not result from appreciable decline in the relative potassium permeability of the axon membrane, but from a substantial reduction in intracellular potassium concentration. In axons adapted to 25% hyposmotic dilution, $[K^+]_i$ declined from 310 mM to 145 mM. This decline reduces the axonal hyperpolarization resulting from dilution of $[K^+]_o$ to about half (*ca.* 15 mV) of that observed during equivalent isosmotic dilution of the external ions. The effect of the reduced intracellular potassium concentration is also seen in the reduction in the apparent outward, potassium, current of the action potential (as judged from maximal rate of fall of the active membrane potential) during hyposmotic dilution (Fig. 9). This contrasts with the lack of effect of isosmotic ionic dilution on the apparent outward

current, an effect which can be attributed to the relatively high intracellular potassium level maintained during ionic dilution at constant osmotic concentration.

Unlike the *Mercierella* axon the giant axon of a sabellid worm, *Sabella penicillus*, showed no appreciable hyperpolarization during hyposmotic adaptation, either *in vivo* or *in vitro* (Treherne & Pichon, 1978). In this modest osmoconformer, however, hyposmotic adaptation of the giant axon is associated with an approximately proportional reduction in intracellular potassium concentration during dilution of the external ions. In contrast, hyposmotic adaptation of the muscle fibres of crustacean osmoconformers involves marked hyperpolarization caused, as in the *Mercierella* axon, by increase in the potassium gradient across the membrane resulting from dilution of $[K^+]_o$ (Freel, 1978*b*). In these crustacean muscle fibres the myoplasmic potassium is maintained as a relatively constant fraction of the cell dry weight during hyposmotic adaptation (Freel, 1978*a*) and changes in E_m are solely determined by alterations in $(a_K)/(a_{K_o})$ brought about by changes in cellular and haemolymph hydration (Freel, 1978*b*).

Hyposmotic adaptation of the *Mercierella* axon is associated with a reduction in the effective intracellular sodium concentration. This is particularly evident at lower dilutions, the relative sodium gradient thus maintained causing appreciable departure from the approximately linear relation between $[Na^+]_o$ and membrane potential during progressive dilution below *ca.* 25% (Fig. 7).

In axons adapted to 25%, hyposmotic, dilution the reductions in $[Na^+]_i$ and $[K^+]_i$ were of approximately similar proportions (i.e. from 310 to 145 mM for potassium and 87 to 44 mM for sodium ions). Thus reduction of the external concentration to one quarter resulted in an approximate halving of the intracellular concentrations of both sodium and potassium ions. Such an effect could reasonably be supposed to result, in part at least, merely from axonal swelling during hyposmotic stress. However, as in the *Sabella* giant axon (Treherne & Pichon, 1978), the measurements with Nomarski optics revealed no appreciable increase in axonal diameter during prolonged hyposmotic adaptation. Furthermore, the reduction in $[Na^+]_i$ during isosmotic dilution is inhibited by ouabain and is presumed to be mediated by the sodium pump (Benson & Treherne, 1978). It appears, in fact, that the reductions in $[Na^+]_i$ and $[K^+]_i$ in response to ionic dilution may be achieved by separate mechanisms. This is indicated by the differential responses to isosmotic and hyposmotic dilution. In the former case ionic dilution results in reduction in $[Na^+]_i$, but no equivalent change in $[K^+]_i$ (Benson & Treherne, 1978), whereas in hyposmotic conditions ionic dilution results in decline in both $[Na^+]_i$ and $[K^+]_i$. This suggests the possibility that reduced intracellular sodium results from increased sodium extrusion which, as in the squid axon (Hodgkin & Keynes, 1955), is stimulated by the change in external sodium concentration and that the net efflux of potassium ions is induced by a combination of ionic and osmotic dilution.

Whatever the mechanism responsible for eliminating potassium ions from the *Mercierella* axon during hyposmotic stress it is clear that it is singularly effective in rapidly reducing the internal concentration of this cation. This can be seen, for example, by comparing the responses of the axon membrane to step change in external potassium concentration in isosmotic and hyposmotic conditions. Abrupt isosmotic dilution resulted in rapid and sustained hyperpolarization; equivalent

Hyposmotic dilution, on the other hand, produced only a transient hyperpolarization which decayed relatively rapidly (Fig. 1).

It seems reasonable to suppose that the net loss of sodium and potassium ions from the *Mercierella* axon during hyposmotic stress might be accompanied by an equivalent escape of anions (either organic or inorganic). In this case axonal adaptation from 100 to 25% (hyposmotic) dilution would involve a net decline in internal concentration of 416 m-Osmol (i.e. a reduction in $[K^+]_i$ of 165 mM and $[Na^+]_i$ of 43 mM with equivalent reduction in monovalent anions). If the axon is in isosmotic and hydrostatic equilibrium with the bathing medium then adaptation from 100 to 25% (hyposmotic) saline would involve a reduction from 1024 to 256 m-Osmol (i.e. a maximum net loss of 768 m-Osmol). In this case hyposmotic adaptation could involve a maximal additional loss equivalent to up to 352 m-Osmol if osmotic equilibrium between the axoplasm and the bathing medium is to be maintained. However, the *Mercierella* axon possesses structural specializations (hemidesmosome-like structures which link the membrane to a system of internal fibrils) which could support some increase in hydrostatic pressure resulting from excess in intracellular osmotic concentration. Theoretical calculations show, for example, that an excess osmotic concentration of 100 m-Osmol would, within this specialized structural arrangement, lead to local membrane tensions of only about 0·03 N m^{-1} for 10% membrane extension (Skaer *et al.* 1978), which is of a similar order of magnitude tolerated by other cell membranes (cf. Rand, 1964; MacKnight & Leaf, 1977). It is therefore impossible to predict the extent of additional solute loss occurring during hyposmotic adaptation of the *Mercierella* axon. If such additional loss occurs then it would be necessary to invoke changes in intracellular organic particles, such as the amino acids which have been shown to be involved in maintaining osmotic equilibrium in crustacean nerve and muscle cells (cf. Schoffeniels, 1976). In the nerves of the *Eriocheior sinensis* the changes in intracellular amino acid concentrations are mediated not only by changes in their metabolism, but also by regulation of the permeability of the axon membrane (Gilles & Schoffeniels, 1969). The well-documented involvement of intracellular amino compounds in cell volume regulation in crustacean muscle cells (cf. Schoffeniels, 1976) is likely to differ from that of nerve cells. As emphasized by Freel (1978*b*), the major myoplasmic amino acids (glycine, alanine and proline) will be neutral at normal cellular pH whereas the axoplasmic ones (e.g. aspartate and glutamate) are largely anionic. In the latter case decrease in negatively charged amino acids is likely to be accompanied by an equivalent decline in intracellular potassium concentration whereas in muscle cells of crustacean osmoconformers the reduction in neutral amino acids during hyposmotic adaptation is not accompanied by an appreciable net loss of intracellular potassium (Freel, 1978*a*). As shown here, hyposmotic adaptation of the *Mercierella* axon, unlike that of the *Sabella* giant axon (Treherne & Pichon, 1978), does not involve proportional reduction of $[K^+]_i$ with decreasing $[K^+]_o$. It is conceivable that cell volume regulation in the *Mercierella* axon could be partly achieved by a release of neutral amino acids to reduce the intracellular osmolarity and, consequently, the membrane tension during hyposmotic stress.

The regulation of the internal potassium concentration during hyposmotic adaptation of the *Mercierella* axon seems to represent a balance between the osmotic

necessity to reduce the concentration of this major internal cation and the necessity to maintain a potassium gradient across the axon membrane sufficient to produce axonal hyperpolarization during hyposmotic dilution. The response of this axon to hyposmotic stress contrasts with that of the *Sabella* giant axon in which there is a near proportional reduction of $[K^+]_I$, in response to 50% dilution of the external medium, and no appreciable axonal hyperpolarization (Treherne & Pichon, 1978). As has been previously shown, the hyperpolarization of the *Mercierella* axon is of primary importance in maintaining the amplitude of the action potential by compensating for the reduction in overshoot (due to reduction in E_{Na}) during ionic dilution in isosmotic conditions (Benson & Treherne, 1978). In addition, this increase in resting potential reduces sodium inactivation and, thus, tends to maintain a rapid rise of the action potential during extreme isosmotic dilution of the bathing medium. The degree of reduction of $[K^+]_I$ during hyposmotic adaptation can, therefore, be regarded as a physiological compromise which, while contributing to osmotic equilibration, also enables appreciable axonal hyperpolarization to occur at extreme hyposmotic dilutions. This, together with the demonstrated reduction in $[Na^+]_I$, enables overshooting action potentials of relatively large amplitude to be maintained in axons exposed to more than tenfold dilution of the ionic and osmotic concentration of the bathing medium.

The results of this and preceding investigations (Treherne, Benson & Skaer, 1977; Skaer *et al.* 1978; Benson & Treherne, 1978) have shown that the *Mercierella* giant axon can adapt to massive changes in the osmotic and ionic composition of the bathing medium. It has also been shown that the giant axon of the more limited euryhaline osmoconformer, *Sabella penicillus*, can adapt both *in vivo* and *in vitro* to more modest hyposmotic stress (Carlson, Pichon & Treherne, 1978; Treherne & Pichon, 1978). These observations demonstrate that nerve cells may not necessarily be at the mercy of the composition of their body fluids and establish the important physiological principle that neurones can adapt relatively rapidly to massive changes in the osmotic and ionic composition of their immediate fluid environment.

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