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EFFECTS OF OSMOTIC STRESS ON THE ELECTRICAL ACTIVITIES OF THE GIANT AXON OF A MARINE OSMOCONFORMER, *SABELLA PENICILLUS*

BY A. D. CARLSON,* Y. PICHON† AND J. E. TREHERNE‡§
The Laboratory, Marine Biological Association, Citadel Hill, Plymouth, U.K.

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SUMMARY

The giant axons of the polychaete, *Sabella penicillus*, can withstand, *in vitro*, abrupt changes in osmotic and ionic concentration of the bathing medium in the range measured in the blood of this osmoconformer (543–1236 m-osmol) at different external salinities. Isosmotic dilution of the external ions (i.e. when osmotic concentration was maintained by sucrose) induced a modest hyperpolarization of the axonal membrane and a rapid decline in the overshoot of the action potential. In contrast, abrupt hyposmotic dilution resulted in a relatively slow and complex decline in overshoot in the absence of axonal hyperpolarization. A slow potassium depolarization and rate of decrease in overshoot in sodium-free conditions suggests that there is a reduced intercellular access to the axon surfaces following exposure to hyposmotic media. It is suggested that this restricted access could provide short-term protection from fluctuations in blood osmotic concentration.

INTRODUCTION

The body fluids of some invertebrates are inconstant media. Despite Claude Bernard's dictum concerning 'la fixité du milieu intérieur' the composition of the blood of some estuarine species can fluctuate widely following changes in the salinity of the external medium. This is seen, most spectacularly, in some euryhaline polychaete worms and lamellibranch molluscs in which the osmotic and ionic concentrations of the blood approximate to those of the external medium over wide ranges of external salinities (cf. Potts & Parry, 1964). The most extreme example appears to be the serpulid worm, *Mercierella enigmatica*, which tolerates relatively rapid changes in the osmotic concentration of the blood of between 2304 and 94 m-osmol (Skaer, 1974).

Such massive changes in blood composition are difficult to reconcile with current concepts of the ionic basis of the electrical activity of nerve cells which are generally supposed to be critically dependent upon the ionic and osmotic concentration of their immediate fluid environment. This dependency is well exemplified by the irreversible

* Department of Cellular and Comparative Biology, State University of New York at Stony Brook, Stony Brook, N.Y. 11790, U.S.A.
† Laboratoire de Neurobiologie Cellulaire, Centre Nationale de la Recherche Scientifique, 91190 Gif-sur-Yvette, France.
‡ Department of Zoology, University of Cambridge, U.K.
§ Reprint requests should be addressed to Dr Treherne.

change caused to the spike generating system in the axons of a stenohaline osmoconformer, the spider crab (*Maia squinado*), by relatively modest dilution, of below 70% of the normal concentration of the bathing medium (Pichon & Treherne, 1976).

Previous research on neuronal adaptations to osmotic stress has been largely confined to biochemical studies on the regulation of the intracellular constituents of crustacean axons during osmotic stress. In particular, the role of amino acid metabolism and release, during the limited osmotic stress to which the nerve cells of euryhaline crustacean osmoregulators are subjected, has been convincingly demonstrated (cf. Gilles & Schoffeniels, 1969; Gérard & Gilles, 1972; Schoffeniels, 1976). We remain, however, largely ignorant of the effects of osmotic stress on the ionic mechanisms of nervous conduction in euryhaline osmoconformers. In this paper we describe the effects of ionic and osmotic stress on the electrical responses of the giant axon of the polychaete, *Sabella penicillus* (L.), a species which provides a convenient experimental preparation and which can be exposed to fluctuating environmental salinity in natural conditions.

METHODS AND MATERIALS

Sabella penicillus L. were trawled from the Tamar estuary and kept in the laboratory in fresh circulating sea water. Adaptation to hyposmotic and hyperosmotic media was carried out by placing freshly caught individuals in 20 l aquaria at 10 °C for 6 h. To adapt to 50% sea water the individuals were successively exposed, each day, to 70, 60 and 55% sea water. The osmotic concentration of freshly collected sea water averaged 1040 m-osmol, during the period of these experiments. Hyposmotic sea water was made by dilution with glass distilled water and hyperosmotic media by addition of NaCl to normal sea water.

The osmotic concentration of the blood was estimated using the freezing point method of Ramsay & Brown (1955). Blood samples were collected from the tentacular crown by cutting and squeezing.

For electrophysiological experiments, a 2·5 cm long segment was severed from the animal. A 0·5 mm wide strip of the body wall was excised from the medioventral part of the segment and pinned, ventral side down, onto the 'Sylgard' bottom of the experimental chamber. After removal of the gut and the blood vessels, the two giant axons (100–200 μm in diameter) could be seen, running almost side by side parallel to the midline.

The experimental chamber consisted of a 'Sylgard' platform on top of which was sealed with petroleum jelly a 2 mm thick and 4 mm high circular perspex wall (diameter 30 mm). Rapid solution changes were achieved by syringing solutions into the chamber while the solutions were pumped out by continuous aspiration. A complete change of the solution was achieved within 10–30 s.

Giant axons were stimulated by short (20–200 μs) rectangular current pulses from a Farnell pulse generator via an RF isolating unit and a pair of silver electrodes. KCl filled glass microelectrodes with resistances ranging from 5 to 20 MΩ were used to impale one of the giant axons. Resting and action potentials were recorded through these electrodes via a high impedance negative capacitance convertor (W.P. Instruments Inc.), an oscilloscope (Tektronix 561) and stored in a 1024 bit Datalab transient

recorder. The output of the transient recorder was connected to a pen recorder (Tekman) on which were recorded both resting and action potentials. Action potentials were also photographed using a Nihon Kohden PC2A oscilloscope camera.

For most experiments, an artificial sea water (ASW) was used. It had the following ionic composition: Na^+, 470 mM; K^+, 10 mM; Ca^{2+}, 11 mM; Mg^{2+}, 55 mM; Cl^-, 609·7 mM and HCO_3^-, 2·3 mM. Variations in osmotic concentration were achieved either by dilution with distilled water (hyposmotic solution) or by adding NaCl (hypertonic solution). Changes in ionic concentration under given osmotic conditions were obtained by appropriate addition of either sucrose (solutions of low ionic strength), choline or potassium (high K^+ solutions).

Axonal swelling was either estimated under binocular microscope *in situ* in the experimental chamber or measured using a Watson binocular microscope and a calibrated eyepiece micrometer on surgically isolated axons.

All experiments were carried out at room temperature (20–25 °C). Values are quoted as mean ± S.E. × 2 (number of observations).

RESULTS

Relation between osmotic concentration of the blood and bathing medium

The osmotic concentration of the blood of individuals equilibrated to varying external salinities showed a linear relation with the osmotic concentration of the bathing medium which did not differ significantly from the isosmotic line shown in Fig. 1.

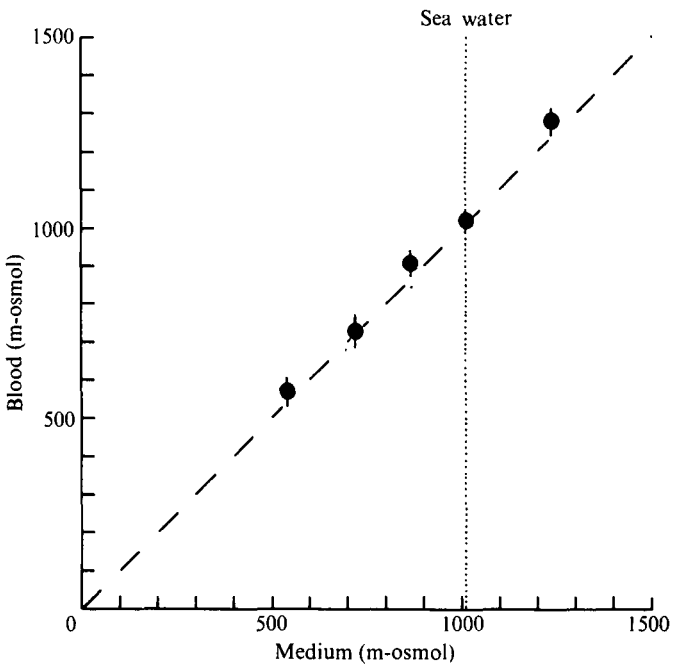

Fig. 1. Relation between the osmotic concentration of the blood and bathing medium. The broken line is the isosmotic line. The vertical lines through the points show the extent of twice the standard error of the mean.

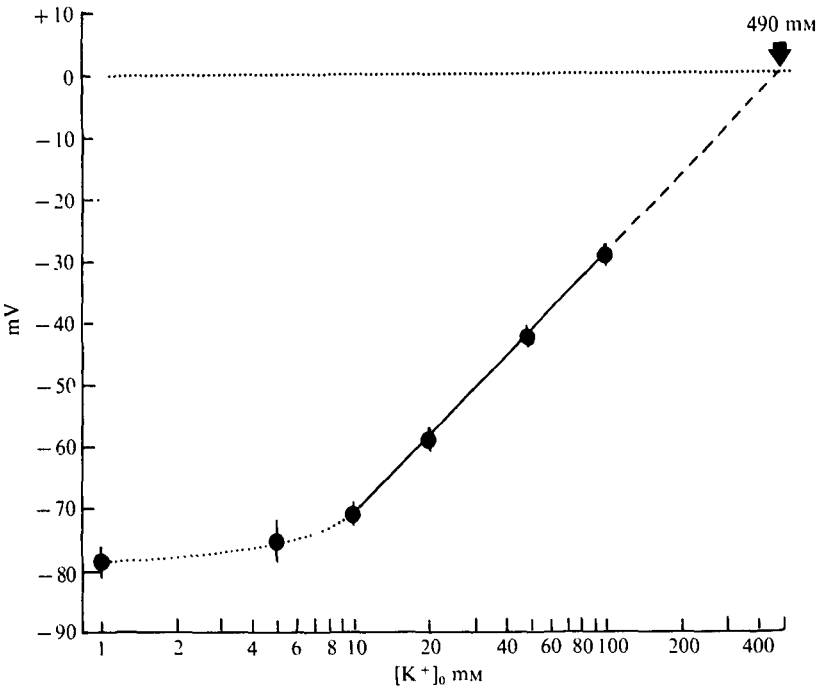

Fig. 2. Relation between external potassium concentration and the axonal resting potential. The continuous line drawn through the point is the calculated regression line (r = 0·9914). Each point is the mean of 5–6 observations on different axons. The calculated line has a slope of 41·8 mV for decade change in $[K^+]_o$. Extrapolation to zero potential yields a value of 49 mM for $[K^+]_i$.

The data in Fig. 1 establishes that *Sabella* is an osmoconformer in which the nervous system is exposed to variation to osmotic concentrations in the range of 543–1236 m-osmol. In subsequent sections we examine the effects of equivalent osmotic stress in *in vitro* experiments on the electrical activities of the giant axons, from worms taken from sea water, after first describing the ionic dependency of the resting and action potentials.

Ionic dependency of resting and action potentials of the giant axon

In normal artificial sea water (ASW) the axonal resting potentials averaged −66·0 ± 2·2 mV (n = 15) and the overshoot of the action potential +53·9 ± 1·3 mV (n = 15) (see Fig. 10).

The resting potential declined exponentially with decreasing external potassium concentration down to 10 mM (Fig. 2). The slope of the regression line in Fig. 2 is 41·8 mV for decade change in $[K^+]_o$. This indicates a relatively low selectivity of the axon membrane for potassium ions when compared with the 58 mV slope for decade change in external potassium predicted by the Nernst relation for an ideal potassium electrode. The apparent potassium selectivity of the membrane of the *Sabella* giant axon is, in fact, considerably less than that of the serpulid worm, *Mercierella enigmatica*, which shows a 58·8 mV change in potential for decade change in $[K^+]_o$ (Carlson & Treherne, 1977) or than that of the giant axon of the sabellid *Myxic*

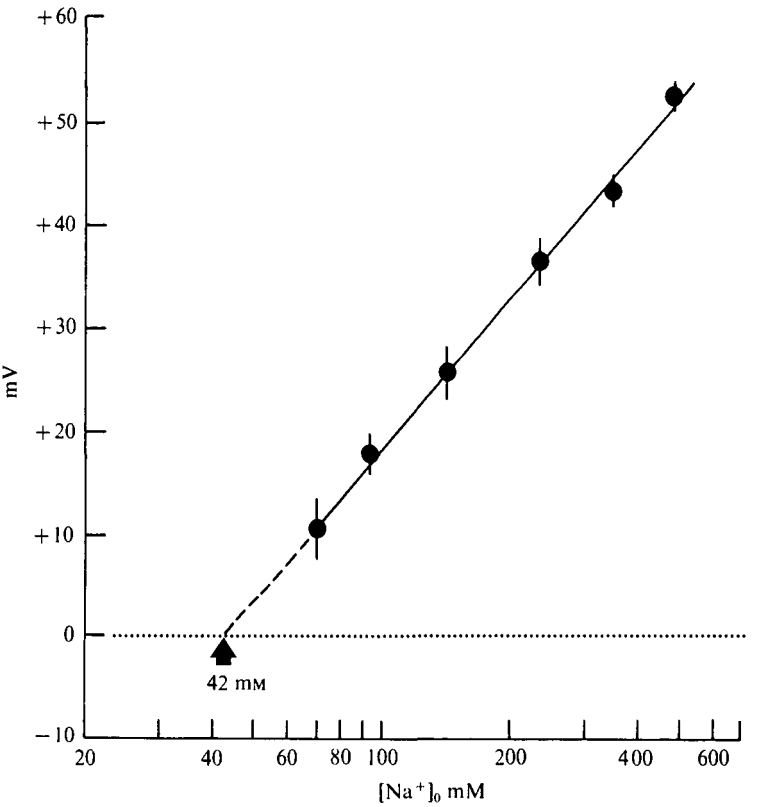

Fig. 3. Relation between external sodium concentration and the extent of the overshoot of the action potentials. The continuous line drawn through the points is the calculated regression line (r = 0·9577) which has a slope of 48·9 mV for decade change in $[Na^+]_o$. The points show the mean of measurements on five different axons. Extrapolation of the exponential relation to zero potential yields a value of 42 mM for $[Na^+]_i$.

infundibulum in which a 54·1 mV slope for decade change in external potassium concentration was observed (Goldman, 1968).

An intracellular potassium concentration of 490 mM is predicted by extrapolation of the experimental portion of the curve, shown in Fig. 2, to zero potential. This intracellular potassium level is much higher than that of 250 mM estimated for the *Mercierella* axon (Carlson & Treherne, 1977) or of 280 mM measured in the extruded axoplasm of the *Myxicola* giant axon (Gilbert & Shaw, 1969).

The overshoot of the action potentials showed a continuous exponential decline of 48·9 mV for decade change in $[Na^+]_o$ with decreasing external sodium concentration (Fig. 3). This indicates a lower apparent relative permeability of the active membrane of *Sabella* for sodium ions than that of the *Mercierella* axon in which 55·8 mV slope for decade change in $[Na^+]$ is recorded (Carlson & Treherne, 1977). Extrapolation of the exponential relation to zero potential yields a value of 42 mM for the effective intracellular sodium concentration of giant axons from sea-water-adapted worms in ASW. The intracellular sodium level in the Sabella giant axon (Fig. 3) thus lies between the value of 20 mM given for the extruded axoplasm in *Myxicola* (Gilbert & Shaw, 1969)

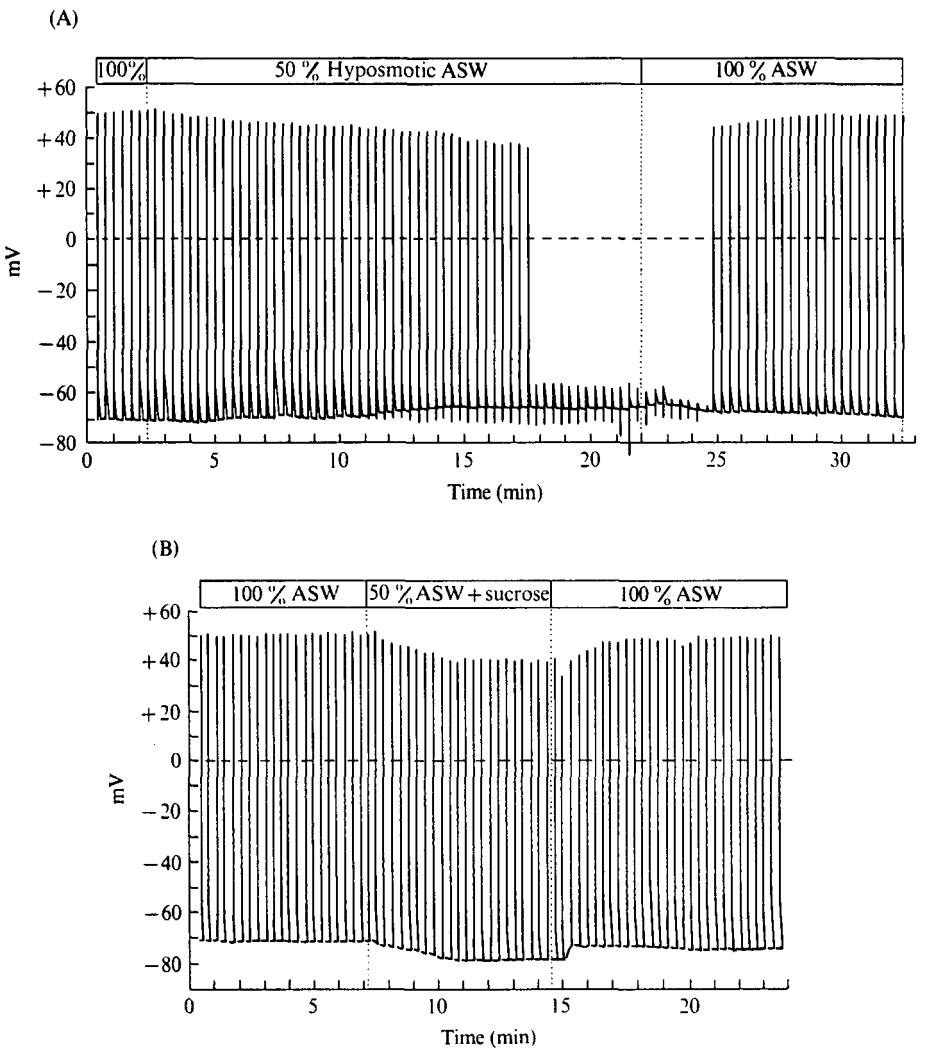

Fig. 4. (A) Effects of exposure to 50%, hyposmotic, ASW on the intracellularly recorded resting and action potentials. (B) Effects of 50%, isosmotic, dilution of ASW on the resting and a worm maintained in normal sea water. (Recorded by a transient signal processor and displayed on a chart recorder).

and the relatively high value of 90 mM for the *Mercierella* axon (Carlson & Treherne, 1977).

Effects of diluted saline on axonal resting and action potentials

Exposure to 50% ASW, diluted with distilled water, resulted in a relative slow decline in the amplitude of the intracellularly recorded action potentials (Fig. 4A). As can be seen from Fig. 4A a slow depolarization developed, which varied, in different preparations, from 8 to 15 mV. Abrupt conduction block developed during the terminal phase of axonal depolarization, in this case after 15 min. On return to 100% ASW there was a gradual repolarization and an abrupt return of overshooting action

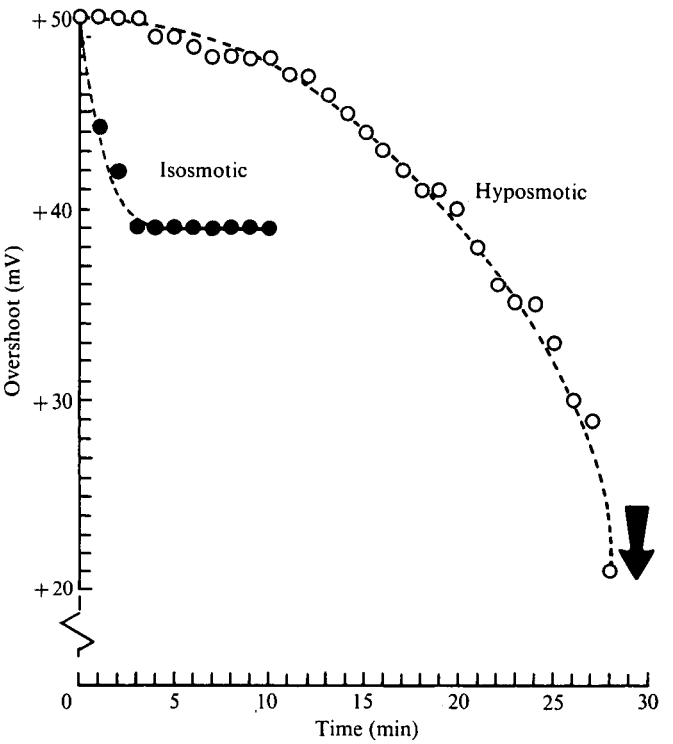

Fig. 5. Comparison of the rates of decline of the overshoot following exposure to 50% hyposmotic and isosmotic (sucrose-substituted) ASW.

potentials (Fig. 4A). Occasionally after relatively long exposure to 50% ASW the axonal depolarization continued on return to normal ASW and action potentials could not be elicited. However, this last effect does not seem to be genuine but rather to result from a permanent axonal damage of the impaled axon by the microelectrode, for normal action potentials could be recorded in the other giant axon.

Exposure to 50% ASW in which isosmicity (1040 m-osmol) was maintained by the addition of sucrose induced a modest hyperpolarization of the resting membrane and a decrease in the overshoot of the action potential (Fig. 4B). The extent of the decrease, from $52{\cdot}9 \pm 2{\cdot}6$ to $35{\cdot}7 \pm 1{\cdot}3$ mV ($n = 9$) was not significantly different from that observed when only $[Na^+]_o$ was reduced from 470 to 255 mM in choline substituted saline (i.e. from $52{\cdot}2 \pm 1{\cdot}9$ to $37{\cdot}0 \pm 5{\cdot}2$ mV ($n = 5$)).

The differences in the effects of hyposmotic and isosmotic dilution of the bathing medium is illustrated in Fig. 5. Exposure to 50% isosmotic dilution (i.e. when constant osmotic concentration was maintained by the addition of sucrose) caused a relatively rapid decline in the extent of the overshoot of the action potential to a steady level. With 50% hyposmotic dilution (i.e. when ASW was diluted with distilled water), on the other hand, there was a relatively slow and complex decline in the extent of the overshoot of the recovered action potentials with an eventual development of conduction block.

Exposure of giant axons to hyposmotic dilutions of more than 50% appeared to

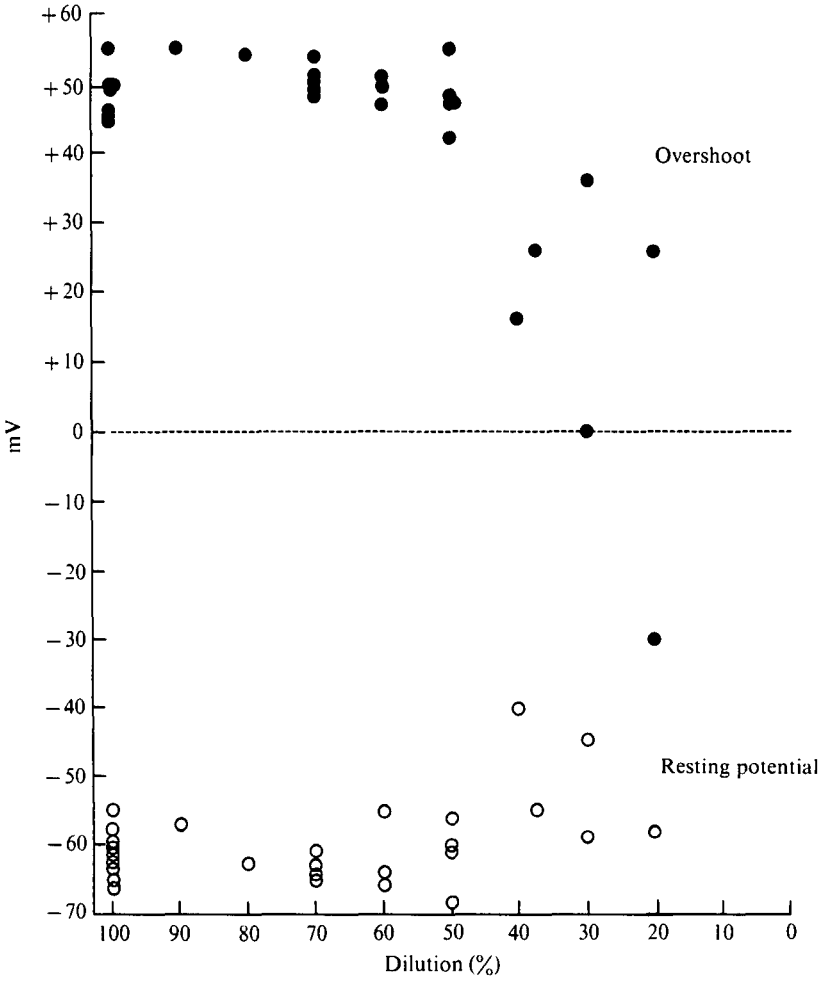

Fig. 6. Effects of exposure to hyposmotic ASW, at various dilutions, on the resting (open circles) and action potentials (closed circles). The points represent the maximum values obtained on return to normal ASW.

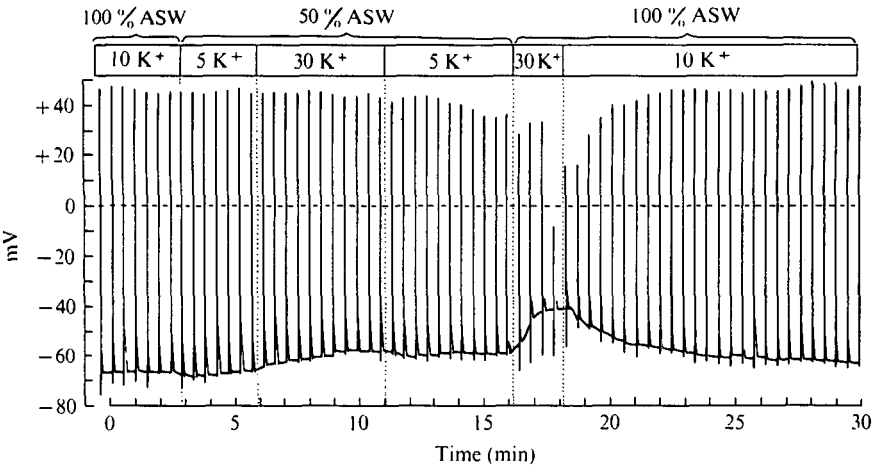

Fig. 7. Effects of elevated potassium concentration (30 mM) on axonal resting and action potentials in 50 % hyposmotic and normal ASW.

cause irreversible damage, for incomplete recovery of the action potentials was observed on return to 100% ASW (Fig. 6).

Potassium depolarization in normal and hyposmotic saline

The effects of elevated external potassium (30 mM-K^+) was observed in giant axons during exposure to 50%, hyposmotic, saline and, subsequently, in 100% ASW. As can be seen in Fig. 7 elevation of the external potassium concentration, from 5 to 30 mM, in hyposmotic conditions (50% ASW) resulted in a relatively slow axonal depolarization. The level of depolarization was maintained on subsequent return to normal 50% ASW (i.e. with 5 mM-K^+). Exposure to 30 mM-$[K^+]_o$ in 100% ASW, on the other hand, resulted in a rapid and much larger depolarization which was reversed on return to normal 100% ASW (i.e. with 10 mM-K^+). As can be seen in Fig. 7 the potassium depolarization in 100% ASW, unlike that in 50% ASW, was accompanied by a rapid decline in the amplitude of the recorded action potentials.

Effects of sodium deficiency in hyposmotic and isosmotic salines

In these experiments giant axons were exposed to 50% isosmotic or hyposmotic saline and were then transferred to equivalent sodium-free salines. With 50% isosmotic saline, sodium deficiency induced a rapid decline in the amplitude of the action potentials and the development of conduction block. These effects were accompanied by a modest depolarization of the axonal membrane (Fig. 8A). In contrast, sodium deficiency in 50%, hyposmotic, saline caused a relatively slow decline in the amplitude of the intracellularly recorded action potentials (Fig. 8B). Conduction block did not occur in the experiment illustrated in Fig. 8B despite the much longer period of exposure to sodium-deficient saline. As in experiments with 100% isosmotic saline, substitution of sodium ions for those of choline invariably resulted in depolarization (Fig 8A, B). The polarity of this response is opposite to that which would be predicted from an increased outward diffusion of intracellular sodium ions and is presumed to result from a specific effect of choline ions on the axonal membrane. With more prolonged exposure to choline substituted saline the membrane repolarized to the normal resting level in normal saline, an effect which could result from de-sensitization of the axonal membrane to choline ions.

Effects of hyposmotic saline on axonal volume

No appreciable increase in axonal diameter was measured on transfer of giant axons from normal to 50% ASW (Fig. 9). The percentage increase in axonal diameter averaged only $3{\cdot}3 \pm 1{\cdot}6$% ($n = 5$) after 30 min exposure to 50% hypotonic saline. As can be seen in Fig. 9 further dilution resulted in only slight increase in axonal diameter, even in distilled water. The lack of effect at the extreme hyposmotic dilutions could result from permanent damage to the axonal membrane as was shown from the electrophysiological data illustrated in Fig. 6.

Effects of hypersalinity on resting and action potentials

Increase in osmotic and ionic concentrations produced little effect on the intracellularly recorded resting and action potentials of giant axons. Fig. 10 illustrates the

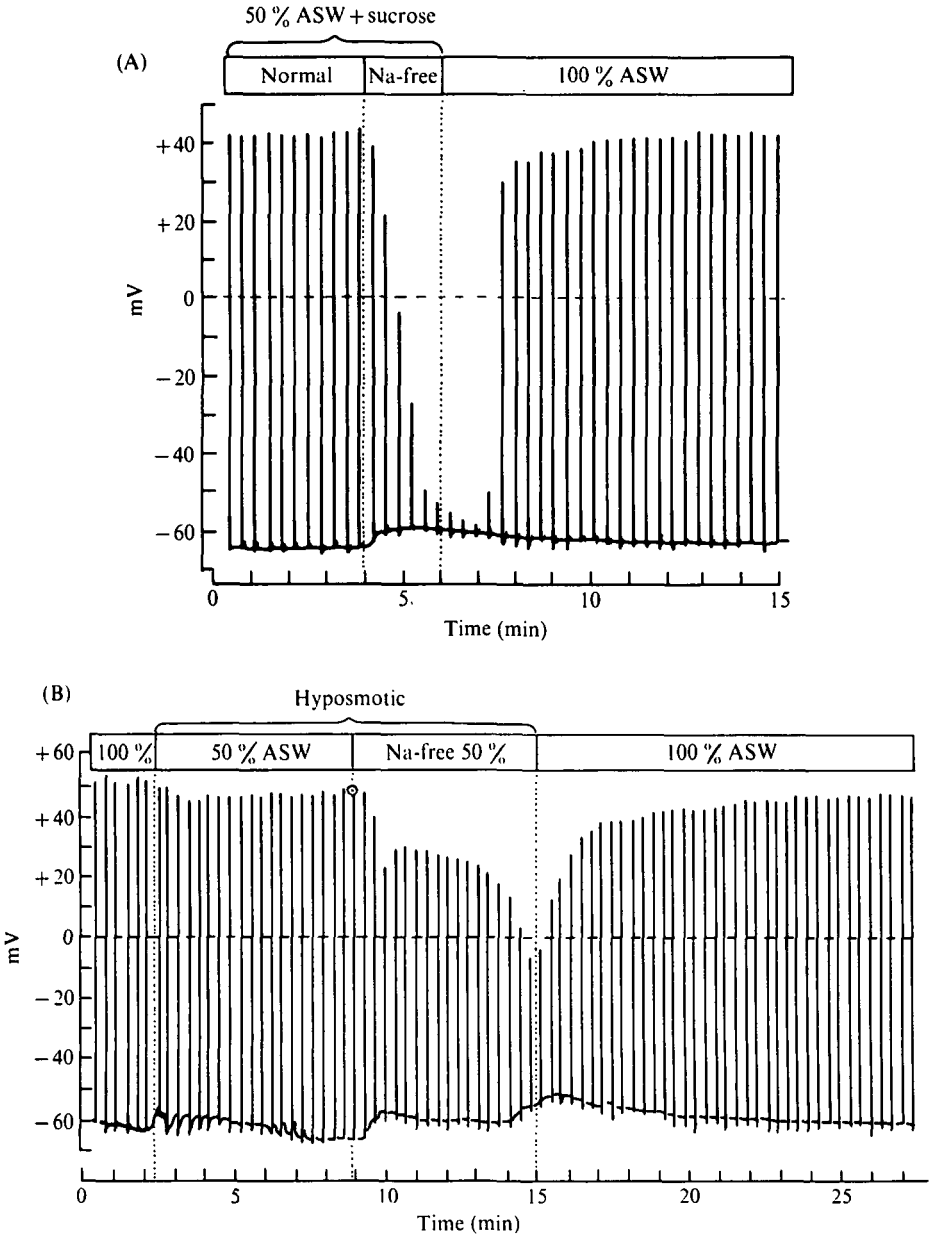

Fig. 8. (A) Effects of exposure to sodium-free conditions (choline substitutes) in a giant axon maintained in 50 %, isosmotic, ASW. (B) Effects of sodium-free conditions during exposure of the giant axon to 50 %, hyposmotic, ASW.

electrical responses of a giant axon following exposure to ASW made hypertonic by the addition of 235 and 470 mM-NaCl. The increased sodium concentration and osmolarity produced no detectable effects on the action potentials.

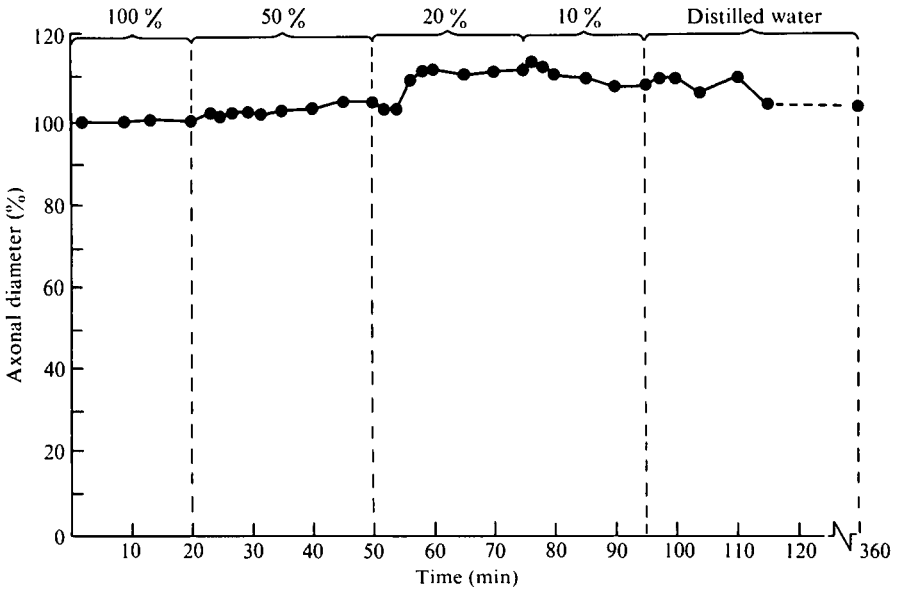

Fig. 9. Effects of successive hyposmotic dilution of ASW on the diameter of a surgically isolated giant axon.

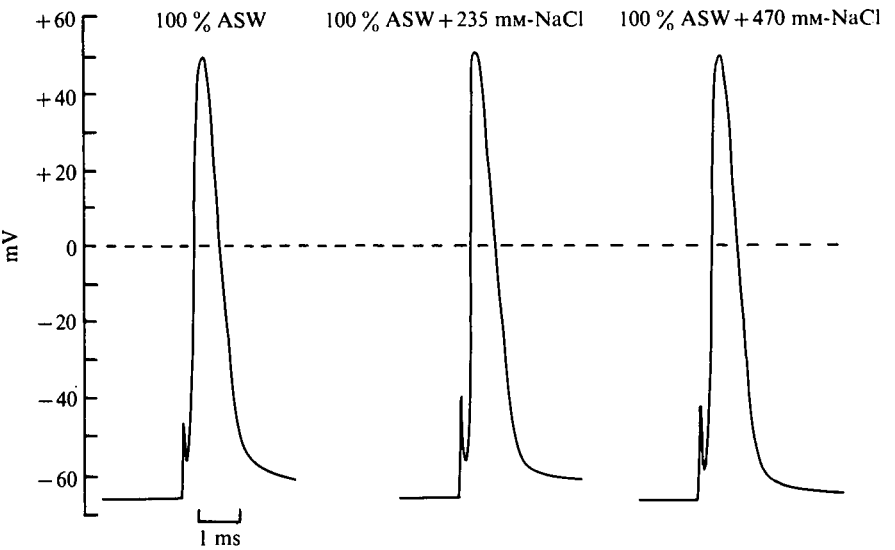

Fig. 10. Effects of hyperosmicity on the action potentials recorded from a giant axon taken from a worm maintained in normal sea water. (Recorded by a transient signal processor and displayed on a chart recorder.)

DISCUSSION

The above results show that *Sabella* is an osmoconformer whose nervous system can be exposed to alterations in blood osmotic concentration, of between 543 and 1236 m-osmol, in response to changing external salinities.

The giant axons can withstand abrupt hyposmotic dilution of the bathing medium of 50% (i.e. from 1040 to 520 m-osmol). This contrasts with the axons of the stenohaline spider crab (*Maia squinado*) which suffer irreversible change to the spike generating system at 70% hyposmotic dilution (665 m-osmol) (Pichon & Treherne, 1976). Unlike the axons of the stenohaline osmoconformer the giant axons of *Sabella* showed only a relatively slow decline in the amplitude of the action potentials and no abrupt hyperpolarization of the resting membrane on exposure to hyposmotic saline (Fig. 4A).

The relatively slow electrical responses of the *Sabella* giant axons to hyposmotic dilution of the bathing medium can be reasonably attributed to reduced intercellular access of ions during hyposmotic stress. This can be inferred from the slow decline in the overshoot of the action potentials observed during exposure to sodium-free hyposmotic saline as compared with the more rapid decline recorded during equivalent isosmotic dilution. It can also be inferred from the slower rate of axonal depolarization, in response to elevated external potassium, observed during hyposmotic as compared with isosmotic dilution of the bathing medium: an effect which is difficult to attribute to a rapid decrease in $[K^+]_I$ or to a reduction in the relative potassium permeability of the axon membrane during hyposmotic stress. In both the latter cases it would be expected that exposure to hyposmotic saline would by itself produce axonal depolarization which is, in fact, not seen except terminally in association with axonal conduction block.

The postulated reduction in intercellular access to the axon surfaces immediately following the onset of hyposmotic stress provides an explanation for the marked difference in the axonal responses to isosmotic and hyposmotic dilution of the external ions. In the former case, when isosmicity was maintained with sucrose, there was a rapid fall in overshoot to a steady value (Fig. 5) which would be expected with a relatively free net outward diffusion of sodium ions from the axon surface. The accompanying axonal hyperpolarization (Fig. 4B) can be reasonably attributed to dilution of the potassium ions in the fluid at the axon surface for the relation between resting potential and $[K^+]_o$ (Fig. 2) predicts a modest hyperpolarization equivalent to that observed during 50% isosmotic dilution of the bathing medium (Fig. 4B).

In contrast, the relatively slow and complex fall in the overshoot of the action potentials during exposure to hyposmotically diluted saline (Fig. 5) can be accounted for by the postulated reduction in the intercellular diffusion of sodium ions away from the axon surface. The abrupt conduction block obtained after prolonged hyposmotic stress (Fig. 4A) could result from the late phase of axonal depolarization which would tend to increase sodium inactivation as well as the leak current.

The postulated restriction in intercellular access to the axon surface during the early stages of hyposmotic can be regarded as a homeostatic mechanism which counteracts short-term fluctuations in the osmotic concentration of the body fluids. In this respect *Sabella* contrasts with the situation in the stenohaline osmoconformer, *Maia squinado* (Pichon & Treherne, 1976) and, contrary to an earlier supposition (Treherne, Carlson & Skaer, 1977), with the extreme euryhaline osmoconformer *Mercierella enigmatica* in which rapid axonal responses have been recorded in response to abrupt changes in the osmotic concentration of the bathing medium (Treherne, Benson & Skaer, 1977). In the latter species the axon itself is, however, able to adapt to

an extreme dilution regime equivalent to that measured in the blood of a colony of *Mercierella* on transfer from sea water to distilled water (i.e. a reduction in blood concentration to 94 m-osmol) (Treherne *et al.* 1977). As is shown in the subsequent paper the *Sabella* giant axon can also adapt to produce action potentials when bathed with solutions of reduced osmotic concentration (Treherne & Pichon, 1978). This adaptation is, however, more limited than that of the *Mercierella* axon (i.e. only to 50% dilution). It appears, therefore, that the apparent reduction in intercellular access induced in the *Sabella* axon by hyposmotic stress may, by reducing the rate of dilution of the fluid in the immediate vicinity of the axon surface, be an adaptation to protect this axon which itself possesses only limited power of adaptation. The structural basis for the hyposmotically induced reduction in intercellular excess remains to be elucidated. An obvious possibility is that osmotic swelling of glial processes reduces the width of intercellular clefts linking the extra-axonal fluid with the bathing medium.

The electrical responses of the *Sabella* axon to hyposmotic stress differ in another respect from those described for other species. In the myelinated nerve fibres of the frog decrease in the osmotic concentration of the Ringer solution (at low $[K^+]_o$) induced a marked hyperpolarization (Schmidt & Stämpfli, 1959). In the stenohaline crustacean *Maia squinado*, exposure to hyposmotic media caused a pronounced hyperpolarization resulting from increased potassium and/or chloride permeability of the axon membrane (Pichon & Treherne, 1976). In the *Sabella* axon, on the other hand, no axonal hyperpolarization was observed during hyposmotic stress. This may be due, in part, to reduced access to the axon surfaces, but at later stages of exposure to hyposmotic saline (when the action potential was approaching conduction block) a modest axonal depolarization occurred. It is difficult to attribute this dilution to intracellular potassium resulting from axonal swelling, for no appreciable increase in axonal diameter was observed during exposure to hyposmotic saline (Fig. 9). Such an effect could result from the membrane becoming more leaky to all ions or from a more specific increased leak of intracellular anions during hyposmotic stress.

The absence of appreciable axonal swelling during exposure to hyposmotic saline has been observed in two other species of annelid. No detectable increase in axonal diameter could be detected in the giant axons of the earth worm, *Lumbricus terrestris*, on exposure to 60%, hyposmotic, saline (Goldman, 1964). This was attributed to the physical restraint afforded by the numerous lamella of the surrounding nerve sheath. A similar absence of swelling of the *Mercierella* giant axon during hyposmotic stress was tentatively attributed to hemidesmosome-like structures which link the axon membrane to a network of neurofilaments within the axon (Skaer *et al.* 1978). It was calculated that the very small effective radii of the areas of axon membrane delimited by adjacent hemidesmosomes could reduce the membrane tension, to the range known to be tolerated by other cells, in the presence of increased internal hydrostatic pressure resulting from excess internal osmotic concentration. It is conceivable, therefore, that the absence of appreciable swelling of the *Sabella* giant axon could result from some structural restraints such as has been proposed in the axons of *Lumbricus* and *Mercierella*, and which are apparently absent in the node of Ranvier (Müller-Mohnssen & Stämpfli, 1958) and crustacean nerves (Gérard & Gilles, 1972) in which appreciable axonal swelling has been observed following hyposmotic dilution of the bathing medium.

Hyperosmotic stress produced no appreciable changes in the action potentials recorded in the *Sabella* giant axon. The absence of effect of excess external sodium ions on the overshoot accords with earlier observations on crab axons (Pichon & Treherne, 1976) and the experiments of Hodgkin & Katz (1949) on squid giant axons. In the latter preparation the overshoot was observed to increase by 11 mV for a 56% increase in external sodium concentration, but only transiently and in well cleaned preparations. In the *Sabella* giant axon, as in that of the squid (Hodgkin & Katz, 1949), no significant increase in the duration of the action potentials was observed as has been described in the node of Ranvier by Meves (1964).

The present results indicate that the giant axon of *Sabella* is able to withstand abrupt changes in external osmotic and ionic concentration in the range of variations in blood concentration experienced by this osmoconformer. Hyposmotic stress is associated with a reduction in intercellular access which appears to provide some short-term protection for the giant axon. Nevertheless, conduction failure occurs after prolonged exposure to hyposmotic solutions in the extreme range of blood dilution (*ca.* 50%) experienced by *Sabella*. This indicates that other, long-term, axonal adaptations to hyposmotic stress must occur. These adaptations are considered in the next paper (Treherne & Pichon, 1978).

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