J. exp. Biol. (1978), **75**, 253–263
With 8 figures
Printed in Great Britain

LONG-TERM ADAPTATIONS OF *SABELLA* GIANT AXONS TO HYPOSMOTIC STRESS

BY J. E. TREHERNE* AND Y. PICHON†

Station Biologique de Roscoff, 29N–Roscoff, France and The Laboratory, Marine Biological Association, Citadel Hill, Plymouth, U.K.

(*Received* 9 *January* 1978)

SUMMARY

Sabella is a euryhaline osmoconformer which is killed by direct transfer to 50% sea water, but can adapt to this salinity with progressive dilution of the sea water. The giant axons were adapted to progressive dilution of the bathing medium (both *in vivo* and *in vitro*) and were able to function at hyposmotic dilutions (down to 50%) sufficient to induce conduction block in unadapted axons.

Hyposmotic adaptation of the giant axon involves a decrease in intracellular potassium concentration which tends to maintain a relatively constant resting potential during adaptation despite the reduction in external potassium concentration. There is no appreciable change in the intracellular sodium concentration, but the relative sodium permeability of the active membrane increases during hyposmotic adaptation. This increase partially compensates for the reduction in sodium gradient across the axon membrane, during dilution of the bathing media, by increasing the overshoot of the action potentials recorded in hyposmotically adapted axons.

INTRODUCTION

The preceding paper (Carlson, Pichon & Treherne, 1978) showed that the polychaete worm, *Sabella penicillus* L., is a euryhaline osmoconformer in which the nervous system can be exposed to variations in osmotic and ionic concentration of the blood sufficient to cause irreversible damage to the axons of a stenohaline osmoconformer (Pichon & Treherne, 1976). The ability of the giant axon of *Sabella* to withstand, *in vitro*, abrupt hyposmotic stress was shown to result from an increased restriction to intercellular ion movements between the axon surface and the external medium (Carlson *et al.* 1978). This restriction appears to be a homeostatic device to protect the axon from short-term fluctuations in osmotic and ionic concentration of the body fluids. Continued exposure to diluted saline in the extreme hyposmotic range experienced by the blood, nevertheless, caused a reversible axonal conduction block. This suggests that *in vivo* the axon must be capable of adapting to changes in

Permanent addresses:
* Department of Zoology, University of Cambridge, U.K.
† Laboratoire de Neurobiologie Cellulaire, Centre Nationale de la Recherche Scientifique, 91190 Gif-sur-Yvette, France.
Reprint requests should be addressed to Dr Treherne.

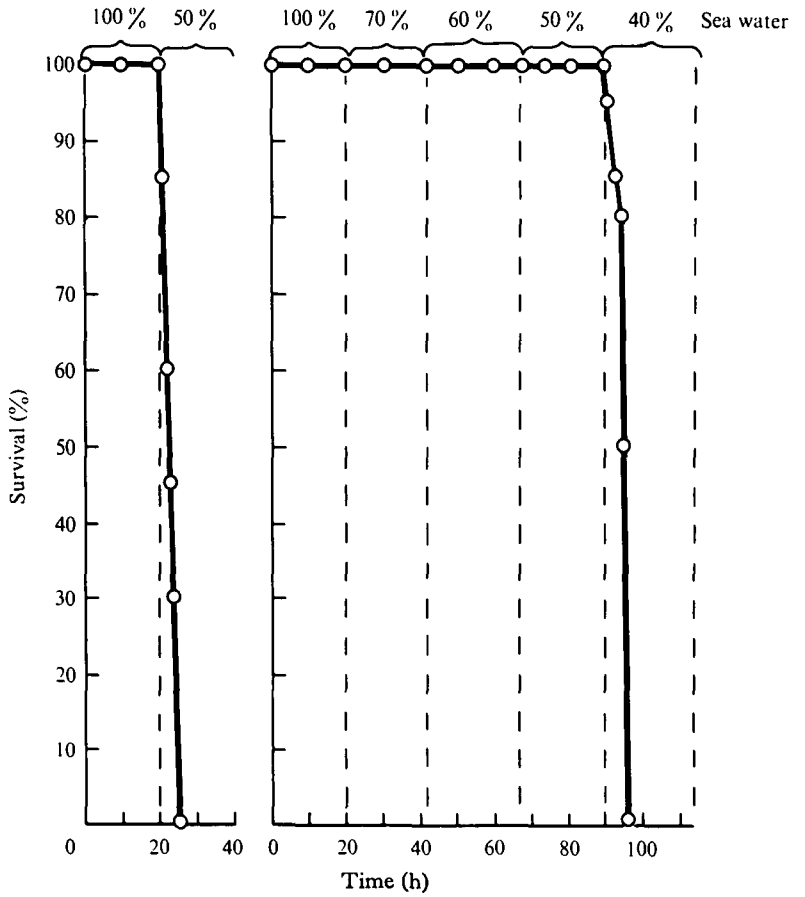

Fig. 1. Effects of direct transfer from normal (1040 m-osmol) to 50% sea water and on successive transfer from normal to 50% sea water on the survival of *S. penicillus*.

the osmotic and ionic concentration of the blood resulting from alterations in external salinity.

In this paper we describe the results of experiments designed to elucidate the physiological mechanisms involved in the hyposmotic adaptation of the *Sabella* giant axon.

METHODS AND MATERIALS

Worms were collected at low tide near Roscoff or trawled in the Tamar estuary near Plymouth, U.K. and maintained in aquaria in fresh, flowing, sea water until used. Hyposmotic adaptation was achieved by exposing freshly collected individuals to progressive dilution by mixing flowing and well-aerated sea water with Roscoff tap water. To adapt to 50% sea water the worms were successively exposed, each day, to 70, 60, 55 and, finally, 50% dilutions of sea water. Increased salinity was achieved by appropriate addition of sodium chloride to freshly collected sea water. The osmotic concentration of the blood, collected from the tentacular crown, was measured using the method of Ramsay & Brown (1955).

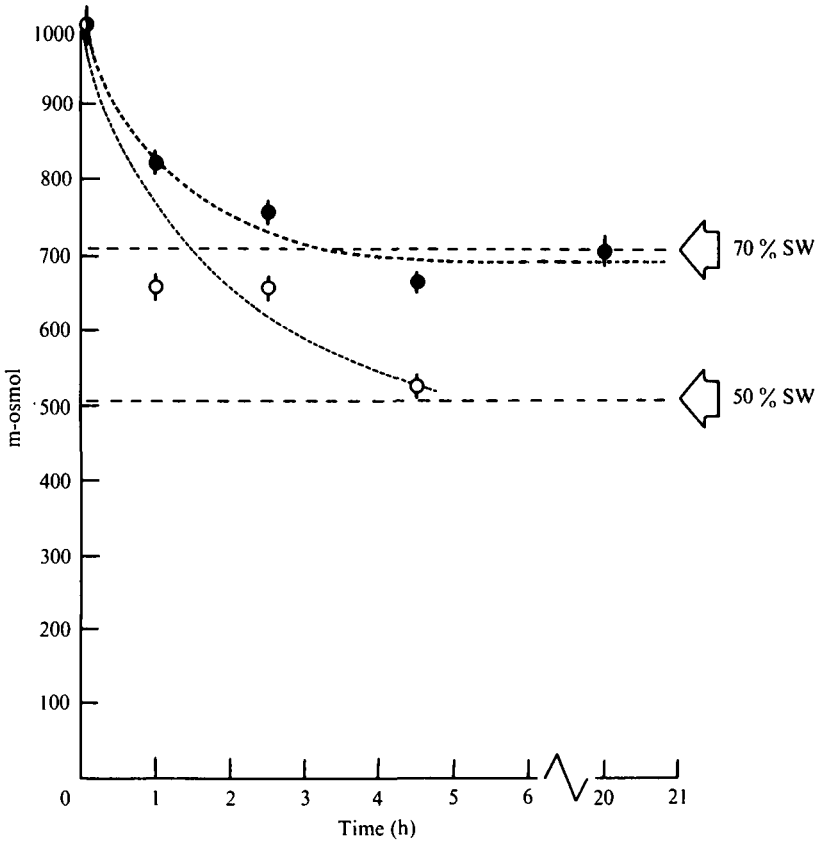

Fig. 2. Effects of exposure to 70 and 50 % sea water on the osmotic concentration of the blood.

The electrophysiological techniques used were essentially similar to those used previously (Carlson *et al.* 1978). The dissection of hyposmotically adapted worms was carried out in appropriately diluted artificial sea water (ASW). *In vitro* hyposmotic adaptation of the axons was achieved by progressive dilution of the ASW in the experimental chamber. This dilution was made by vigorously syringing the diluted saline onto the preparation, in the absence of constant flow through the chamber, to avoid fluid stratification in the immediate vicinity of the axon surfaces (Treherne, Benson & Skaer, 1977).

The experimental saline used in these experiments was an artificial sea water and had the following 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.

RESULTS

Salinity tolerances of Sabella

Freshly caught individuals were rapidly killed by transfer from normal to 50 % sea water (Fig. 1). Progressive daily hyposmotic dilution enabled the animals to survive in 50 % sea water, although subsequent transfer to 40 % caused rapid mortality (Fig. 1).

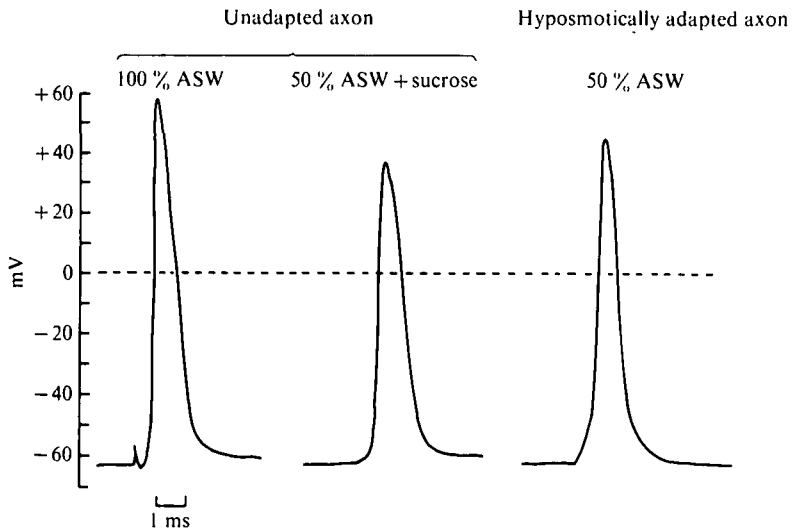

Fig. 3. Action potentials recorded in axons, from sea-water-adapted worms, in 100% ASW (A) and 50% (sucrose-substituted) ASW (B) and in an axon, from a worm adapted to 50% sea water, in 50%, hyposmotic, ASW (C). (Recorded by a transient signal processor and displayed on a chart recorder).

A number of attempts to adapt worms to below 50% dilution were unsuccessful, despite very gradual rates of dilution, which suggests that one half dilution of normal sea water is probably near the physiological limit for this species. Increase in salinity of above about 150% was also found to be lethal for sea-water-adapted individuals.

The osmotic concentrations of the blood declined rapidly following exposure to diluted sea water (Fig. 2). As can be seen from Fig. 2 the half-time for dilution of the blood was 1 h for transfer of sea-water-adapted animals to 70% or 50% sea water.

Electrical activity in axons from hyposmotically adapted individuals

In contrast to the axons of sea-water-adapted individuals (Carlson *et al.* 1978) those of individuals adapted to 50% sea water were able to conduct action potentials during prolonged exposure to 50% ASW (Fig. 3).

The resting potentials measured in the axons of hyposmotically adapted worms in 50% ASW averaged $63{\cdot}9 \pm 5{\cdot}8$ mV ($n = 7$) and were not significantly different from those of sea-water-adapted individuals in 100% ASW ($66{\cdot}0 \pm 2{\cdot}2$ mV, $n = 15$) or in 50%, isosmotic (sucrose-substituted), ASW ($66{\cdot}6 \pm 3{\cdot}7$, $n = 5$).

The overshoot of the action potentials recorded in the axons of hyposmotically adapted individuals in 50% ASW averaged $42{\cdot}3 \pm 2{\cdot}2$ mV ($n = 6$) and were significantly smaller than those recorded in the axons of sea-water-adapted individuals in 100% ASW ($52{\cdot}7 \pm 2{\cdot}6$, $n = 32$). They were, on the other hand, significantly larger than those of sea-water-adapted worms in 50% isosmotic ASW ($35{\cdot}7 \pm 4{\cdot}6$ mV, $n = 12$).

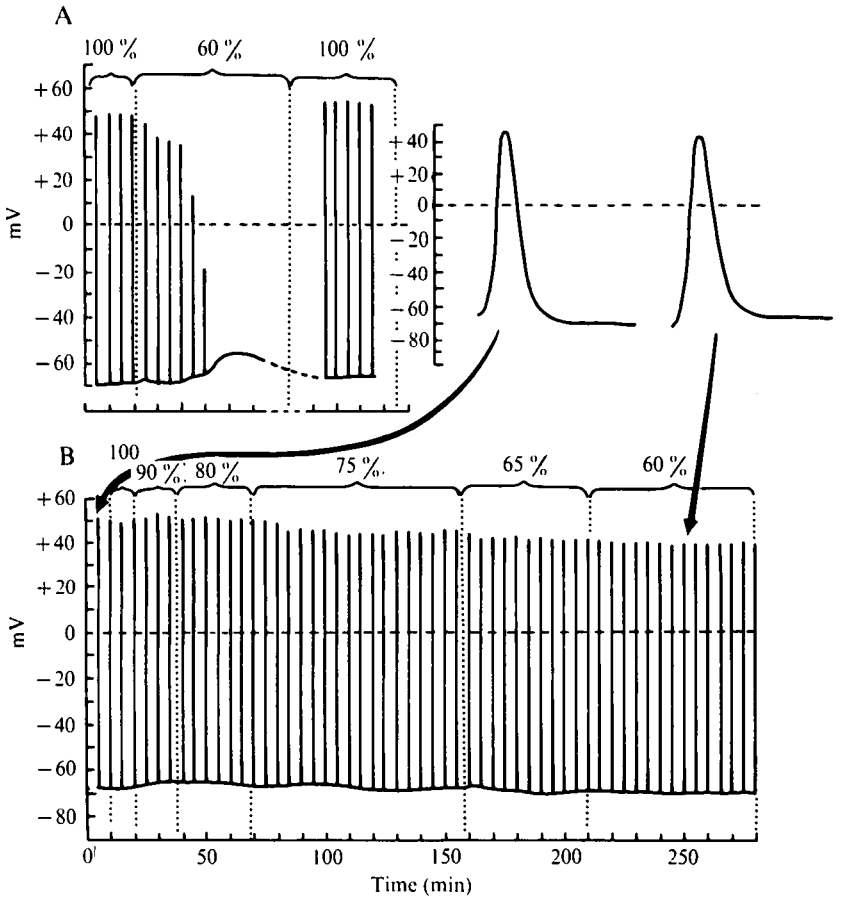

Fig. 4. (A) Effects of abrupt exposure of a giant axon from a sea-water-adapted worm to 60%, hyposmotic, ASW. (B) Effects of progressive dilution of the bathing medium on the resting and action potentials recorded in the giant axon taken from a sea-water-adapted worm.

In vitro *adaptation of axons to hyposmotic saline*

As previously shown (Carlson *et al.* 1978) abrupt exposure to hyposmotic saline (60% ASW) eventually led to reversible conduction block and axonal depolarization (Fig. 4A). In contrast, progressive hyposmotic dilution of the bathing medium resulted in sustained axonal function, with overshooting action potentials persisting at 60% hyposmotic dilution of the bathing medium (Fig. 4B). The maximal rate of rise of action potentials ($\dot{V}_{max}$) declines, in the experiment illustrated in Fig. 4A, from 706·8 V s^{-1}, in the 100% ASW, to $\dot{V}_{max}$ = 385·5 V s^{-1} after hyposmotic adaptation in 60% ASW. The resting potential showed no appreciable overall change during *in vitro* hyposmotic adaptation of the giant axon. Attempts to adapt the giant axon, *in vitro*, to < 60% were unsuccessful.

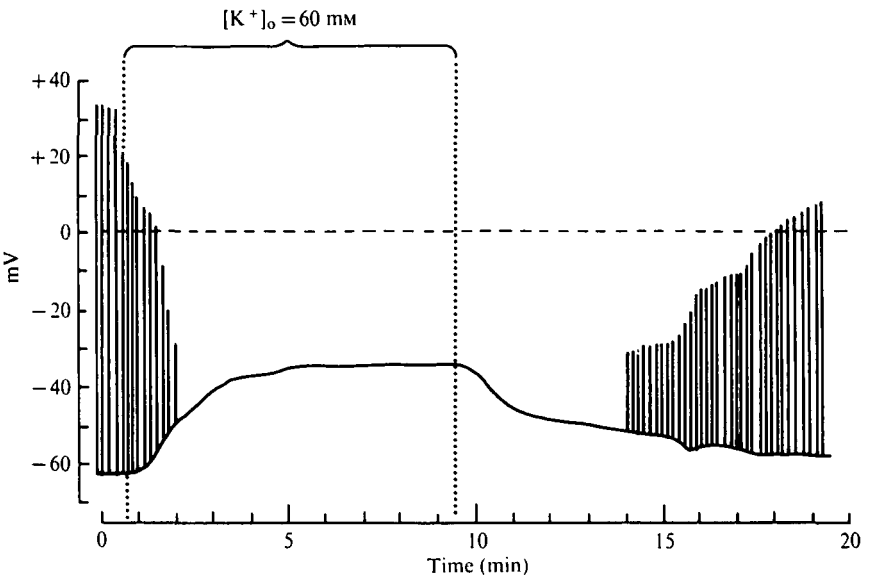

Fig. 5. Effects of exposure to 60 mM-$[K^+]_o$ on the resting and action potentials recorded in an axon which was adapted, *in vitro*, to 60%, hyposmotic, ASW ($[K^+]_o$ = 6 mM).

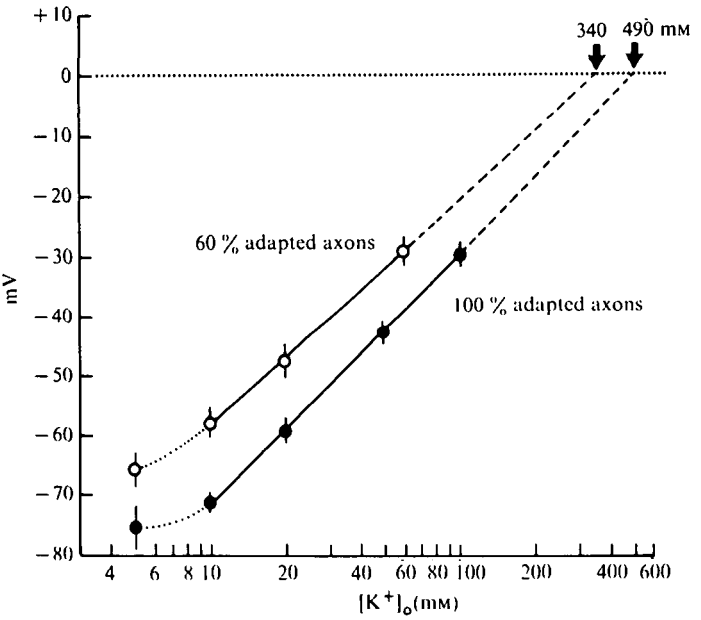

Fig. 6. Relation between the axonal resting potential and external potassium concentration in axons from sea-water-adapted worms, in 100% ASW, and from axons which were adapted, *in vitro*, to 60%, hyposmotic, ASW. The continuous lines show the calculated regression lines (r = 0·9028, for sea-water-adapted axons; r = 0·9884 for the hyposmotically adapted axons). The line for sea-water-adapted axons shows a slope of 41·8 mV for decade change in $[K^+]_o$ and for 60% adapted axons a slope of 36·8 mV. $[K^+]_i$ was estimated by extrapolation of the regression line to zero potential.

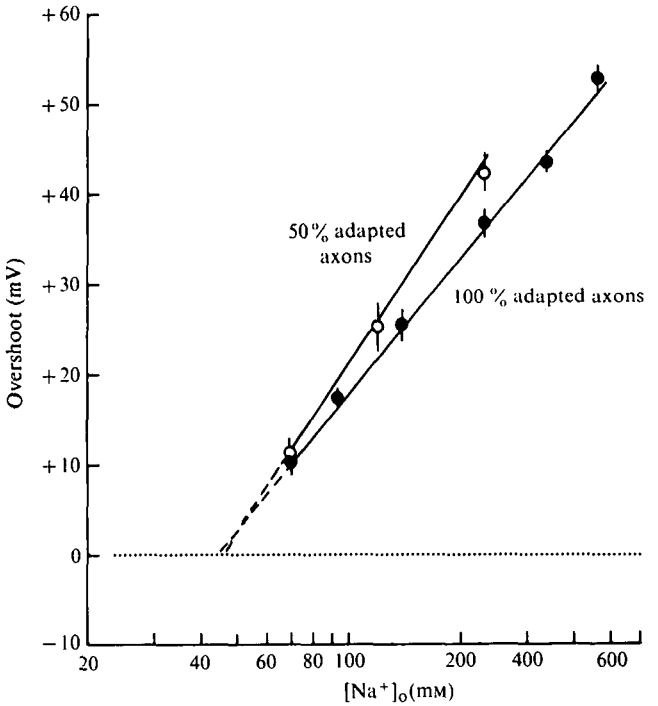

Fig. 7. Relation between the extent of the overshoot of the action potentials in axons from sea-water-adapted worms, in 100% ASW, and from axons taken from animals adapted to 50% sea water, in 50%, hyposmotic, ASW. The continuous lines are the calculated regression lines (r = 0·9517 for sea-water-adapted axons; r = 0·9234 for 50% adapted axons). The mean slopes of the regression lines are 48·8 ± 2·6 mV, for decade change in $[Na^+]_i$, for the 100% adapted axons and 59·8 ± 6·4 mV for the 60% adapted axons.

Effects of $[K^+]_o$ *on the resting potentials of hyposmotically adapted axons*

Elevated external potassium concentrations resulted in relatively rapid depolarization and subsequent repolarization on return to the normal (6 mM) external potassium level in axons adapted, *in vitro*, to 60% hyposmotic ASW (Fig. 5). In this respect the hyposmotically adapted axons were similar to those of sea-water-adapted individuals in 100% ASW (Carlson *et al.* 1978). They differ, however, from normal axons exposed to abrupt hyposmotic dilution in which a supposed reduced intercellular access resulted in relatively slow potassium depolarization of the axonal membrane (Carlson *et al.* 1978).

Fig. 6 illustrates the relation between membrane potential and external potassium concentration in axons from sea-water-adapted individuals, in 100% ASW, and in axons which were adapted *in vitro* (Fig. 4B) to 60%, hyposmotic, ASW. The slope relating membrane potentials to $[K^+]_o$ for the hyposmotically adapted axons showed 36·8 mV alteration (for decade change in external potassium) which was not significantly different from that of 41·8 mV for the axons from sea-water animals (Fig. 6). Extrapolation of the exponential relations shown in Fig. 6 to zero potential indicates that the estimated intracellular potassium concentration had decreased from 490 to 340 nM during *in vitro* hyposmotic adaptation.

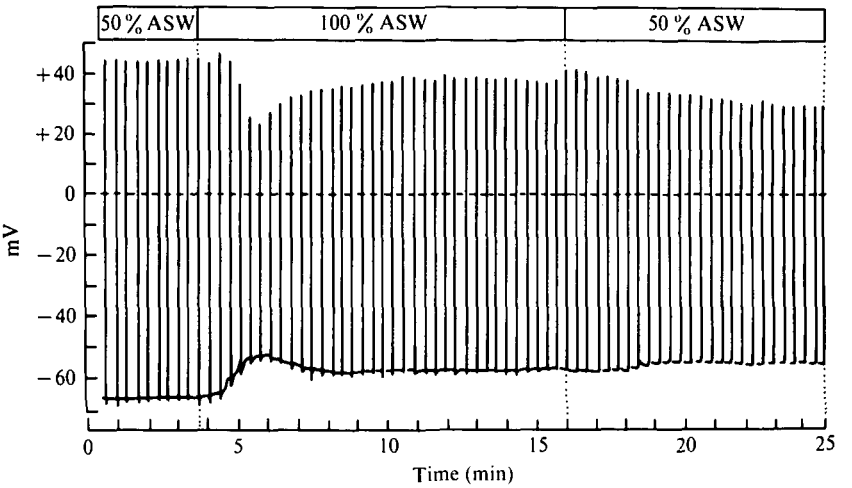

Fig. 8. Effects of exposure of 50 %-adapted axons to 100 % ASW on the intracellularly recorded resting and action potentials.

Effects of $[Na^+]_o$ *on the overshoot of the action potentials hyposmotically adapted axons*

The effects of variation in external sodium concentration on the intracellularly recorded action potentials was measured in the axons of individuals taken from 100 % sea water and from individuals which were adapted to 50 % sea water (Fig. 7). The slope of the calculated regression line for the axons from hyposmotically adapted animals shown in Fig. 7 was $59{\cdot}8 \pm 6{\cdot}4$ mV for decade change in $[Na^+]_o$ as compared with that of $48{\cdot}8 \pm 2{\cdot}6$ mV for the axons of sea-water-adapted worms. The significant difference between the Nernst slopes in Fig. 7 ($t = 3{\cdot}595$, $P < 0{\cdot}01$ for 8 degrees of freedom) indicates that the relative sodium permeability of the active nerve membrane had increased during hyposmotic adaptation. The intracellular sodium concentrations (estimated by extrapolation of the exponentials to zero potential) were, however, similar in the axons from sea water and hyposmotically adapted individuals (Fig. 7). In both cases $[Na^+]_o$ can be estimated to be *ca.* 45 mM.

Effects of increased salinity on hyposmotically adapted axons

Transfer of hyposmotically adapted axons (taken from individuals adapted to 50 % sea water) from 50 % to normal ASW caused axonal depolarization and, subsequently, incomplete repolarization (Fig. 8). The initial depolarization was accompanied by a rapid decline in the overshoot and was followed by partial recovery. No significant repolarization or increase in overshoot occurred on return to 50 % ASW. The overshoot, in fact, showed a progressive decline in 50 % ASW presumably as a result of irreversible damage caused by the abrupt exposure of the hyposmotically adapted axon to the osmotic concentration of normal ASW (i.e. from 520 to 1040 m-osmol).

DISCUSSION

The giant axons of this polychaete osmoconformer are able to adapt to progressive dilution of the bathing medium (both *in vivo* and *in vitro*) and to function at hyposmotic concentrations (down to 50%) sufficient to induce conduction block in unadapted axons.

Unlike the extreme osmoconformer, *Mercierella*, hyposmotic adaptation of the *Sabella* giant axon is not accompanied by appreciable hyperpolarization of the axonal membrane. In *Mercierella* this axonal hyperpolarization results from dilution of $[K^+]_o$ and from the fact that the intracellular potassium is not proportionally diluted during hyposmotic adaptation (i.e. one quarter dilution of the external medium producing only a halving of $[K^+]_i$) (Benson & Treherne, 1978*b*). In the *Sabella* giant axon, on the other hand, the intracellular potassium shows a proportionally greater dilution (i.e. 69% dilution of $[K^+]_i$ for 60% dilution of external medium) which compensates for the reduction in $[K^+]_o$.

The decrease in intracellular potassium concentration during hyposmotic adaptation of the giant axon cannot be attributed merely to dilution of this cation due to axonal swelling, for there was no equivalent reduction in the estimated intracellular sodium concentration. Furthermore, it has been shown by direct microscopical measurements that there is no appreciable increase in axonal diameter during progressive dilution of the bathing medium (Carlson *et al.* 1978). It appears, therefore, that the observed decrease in $[K^+]_i$ during hyposmotic adaptation must have resulted from a net loss of this major intracellular cation from within the giant axon: a strategy which is employed by a variety of cells to reduce their internal osmotic concentrations during hyposmotic stress (cf. Hoffman, 1977).

The average overshoot of the action potential recorded in hyposmotically adapted axons in 50% hyposmotic ASW ($42{\cdot}3 \pm 2{\cdot}2$ mV) was significantly larger than that for the action potentials recorded in unadapted axons bathed with 50%, isosmotic, ASW ($35{\cdot}3 \pm 4{\cdot}6$ mV). This effect could not result from an increased sodium gradient across the membrane of hyposmotically adapted axons, since in both sets of experiments $[Na^+]_o$ was the same and similar values for $[Na^+]_i$ (*ca.* 45 mM) were estimated (from the relation between $[Na^+]_o$ and the peak of the overshoot) in adapted and unadapted axons. The larger overshoot measured in hyposmotically adapted axons, in 50% ASW, appears, however, to result from an increase in the relative sodium permeability of the active membrane. This was shown by the 59·8 mV alteration for decade change in $[Na^+]_o$ (which approximates to the relation predicted by the Nernst equation for an ideal sodium electrode) measured in hyposmotically adapted axons as compared with the 48·8 mV slope obtained with unadapted axons (Fig. 7).

It appears then that hyposmotic adaptation of the *Sabella* giant axon involves a decrease in intracellular potassium concentration which tends to maintain a relatively constant resting potential, despite dilution of external potassium ions, during hyposmotic adaptation. This is accompanied by an increase in the relative sodium permeability of the active membrane in hyposmotically adapted axons so as to increase the extent of the overshoot without appreciable change in the effective sodium gradient across the membrane.

The responses to progressive hyposmotic dilution of the bathing medium differ in

a number of ways from those observed in the axons of the extreme euryhaline osmoconformer *Mercierella enigmatica* (Treherne, Benson & Skaer, 1977). As has already been emphasized, the absence of appreciable axonal hyperpolarization in the *Sabella* giant axon is an obvious difference from the physiological adaptations of the *Mercierella* axon to extreme hyposmotic stress (Benson & Treherne, 1978*a*, *b*). In the *Mercierella* axon the hyperpolarization tends to compensate for the reduction in overshoot resulting from the decrease in E_{Na} during dilution of the external ions, so as to maintain the amplitude of the action potentials. This hyperpolarization also tends to reduce the degree of inactivation of the sodium channels. These effects are reinforced in the *Mercierella* axon by a progressive decrease in intracellular sodium concentration during hyposmotic adaptation so as to maintain the relative sodium gradient across the axon membrane. There is, however, no change in the apparent relative sodium permeability of the active axonal membrane which still approximates to an ideal sodium electrode during hyposmotic adaptation. In contrast the *Sabella* giant axon shows no change in intracellular sodium concentration but does exhibit an increase in the relative sodium permeability of the axon membrane so as to increase the extent of the overshoot despite the similarity of the sodium gradients across the axon membranes of unadapted and adapted axons.

The physiological responses of the *Sabella* giant axon can be regarded as effective adaptations to the limited, osmotic stress, experienced by this axon as compared with that of *Mercierella*, the most extreme osmoconformer known (Skaer, 1974). First, the *Sabella* giant axon appears to receive short-term protection by a reduction in intercellular accessibility during exposure to abrupt hyposmotic dilution of the bathing medium (Carlson *et al.* 1978). Despite an earlier belief to the contrary (Treherne, Carlson & Skaer, 1977) the *Mercierella* axon does not appear to receive appreciable short-term protection from the immediate effects of abrupt hyposmotic stress (Treherne, Benson & Skaer, 1977). The *Mercierella* axon can, however, adapt more rapidly, and to a much lower degree of hyposmicity (down to 7·5%) (Benson & Treherne, 1978*b*), than the *Sabella* axon. Secondly, the long-term adaptation of the *Sabella* axon is associated with a proportionally larger reduction of intracellular potassium, which is present at the high concentration of 490 mM in the axons of sea-water-adapted individuals (Carlson *et al.* 1978). The reduction in the concentration of this major internal cation during hyposmotic adaptation, which is presumably accompanied by an equivalent loss of anions, must therefore contribute substantially to achieving osmotic equilibrium with the external medium. In the *Mercierella* axon there is a proportionally smaller reduction in intracellular potassium during hyposmotic adaptation (Benson & Treherne, 1978*b*). This enables hyperpolarization of the axonal membrane to occur, despite reduction in $[K^+]_o$, and to reduce apparent sodium inactivation during hyposmotic adaptation (Benson & Treherne, 1978*a*). However, the membrane of the *Mercierella* axon possesses structural specializations (in the form of hemidesmosome-like structures linked to a system of intracellular filaments) which could enable it to withstand excess internal hydrostatic pressure resulting from osmotic imbalance with the bathing medium (Skaer *et al.* 1978).

The present results show that abrupt exposure of hyposmotically adapted axons to solutions of similar concentration to the blood of sea-water-adapted individuals eventually causes irreversible axonal damage. It appears, therefore, that long-term

physiological adjustments are necessary to enable the giant axon to adapt to changes in blood ionic and osmotic concentration associated with increases in external salinity from dilute to normal sea water.

It should be emphasized that *Sabella penicillus* can experience in natural conditions changes in salinity sufficient to produce alterations in the ionic and osmotic concentration of the blood of equivalent magnitude to those employed in this investigation. This is shown, for example, in the occurrence of *S. penicillus* in Stonehouse Pool, Plymouth, U.K. in 1967 (P. E. Gibbs, personal communication). During that year the salinity of the water varied between approximately 35 and 17‰ (Gibbs, 1971). The physiological adaptations described in this paper are, thus, likely to be employed in natural conditions and to be of adaptive value for this modest euryhaline osmoconformer.

This research was supported in part by grants from the U.S. European Research Office and the Royal Society.

REFERENCES

BENSON, J. A. & TREHERNE, J. E. (1978*a*). Axonal adaptations to osmotic and ionic stress in an invertebrate osmoconformer (*Mercierella enigmatica* Fauvel). II. Effects of ionic dilution on resting and action potentials. *J. exp. Biol.* (in the Press).

BENSON, J. A. & TREHERNE, J. E. (1978*b*). Axonal adaptations to osmotic and ionic stress in an invertebrate osmoconformer (*Mercierella enigmatica* Fauvel). III. Adaptations to hyposmotic dilution *J. exp. Biol.* (in the Press).

CARLSON, A. D., PICHON, Y. & TREHERNE, J. E. (1978). Effects of osmotic stress on the electrical activities of the giant axon of a marine osmoconformer, *Sabella penicillus*. *J. exp. Biol.* **75**, 237–251.

GIBBS, P. E. (1971). A comparative study of reproductive cycles in four polychaete species belonging to the family Cirratulidae. *J. mar. Biol. Ass. U.K.* **51**, 745–769.

HOFFMAN, E. K. (1977). Control of cell volume. In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oschman and B. J. Wall), pp. 285–332. London: Academic Press.

PICHON, Y. & TREHERNE, J. E. (1976). The effects of osmotic stress on the electrical properties of the axon of a marine osmoconformer (*Maia squinado*. Brachyura: Crustacea). *J. exp. Biol.* **65**, 553–563.

RAMSAY, J. A. & BROWN, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. Sci. Instrum.* **32**, 372–375.

SKAER, H. LE B. (1974). The water balance of a serpulid polychaete, *Mercierella enigmatica* (Fauvel). I. Osmotic concentration and volume regulation. *J. exp. Biol.* **60**, 321–330.

SKAER, H. LE B., TREHERNE, J. E., BENSON, J. & MORETON, R. B. (1978). Axonal adaptations to osmotic and ionic stress in an extreme invertebrate osmoconformer (*Mercierella enigmatica* Fauvel). I. Ultrastructural and electrophysiological observations on axonal accessibility. *J. exp. Biol.* (in the Press).

TREHERNE, J. E., BENSON, J. A. & SKAER, H. LE B. (1977). Axonal accessibility and adaptation to osmotic stress in an extreme osmoconformer. *Nature* **269**, 430–431.

TREHERNE, J. E., CARLSON, A. D. & SKAER, H. LE B. (1977). Facultative blood-brain barrier and neuronal adaptation to osmotic stress in a marine osmoconformer. *Nature* **265**, 550–553.