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

## II. EFFECTS OF IONIC DILUTION ON THE RESTING AND ACTION POTENTIALS

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SUMMARY

The giant axon of this extreme euryhaline osmoconformer possess an unusual ability to produce action potentials of large amplitude over a wide range of ionic dilution when constant osmotic concentration is maintained by the addition of mannitol to the bathing medium. Ionic dilution under these circumstances causes a decline in the overshoot of the action potential (resulting largely from reduction in $[Na^+]_o$) and an appreciable axonal hyperpolarization (primarily as a result of decrease in $[K^+]_o$). This hyperpolarization tends to compensate for the reduction in the extent of the overshoot and so maintains the amplitude of the sodium-mediated action potentials during isosmotic dilution of the bathing medium. The axonal hyperpolarization also appears to reduce sodium inactivation so as to maintain a rapid rate of rise of the action potential despite drastic reduction in the ionic concentration of the bathing medium. Prolonged exposure to reduced ionic concentrations appears to induce a ouabain sensitive reduction in intracellular sodium concentration which increases the sodium gradient across the axon membrane during isosmotic dilution of the external medium.

## INTRODUCTION

The nervous system of the serpulid worm, *Mercierella enigmatica* (Fauvel), is able to function effectively during extreme osmotic stress (Treherne, Carlson & Skaer, 1977; Treherne, Benson & Skaer, 1977). This ability is not associated with any novel specialization of the ionic mechanisms which determine axonal excitability. The tetrodotoxin-sensitive action potentials are sodium-mediated and the resting medium approximates to a potassium selective electrode (Carlson & Treherne, 1977). Axonal function during hyposmotic stress is not, as had been supposed previously (Treherne, Carlson & Skaer, 1977), associated with a restricted access of ions and molecules to the axon surfaces (Treherne, Benson & Skaer, 1977; Skaer *et al.* 1978). It appears, then, that the axons in this euryhaline osmoconformer are able to adapt,

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relatively rapidly, to massive changes in the ionic and osmotic concentrations of the body fluids. In this paper we examine the electrical responses of the giant axon of *Mercierella enigmatica* to ionic dilution by studying the effects of reduced ion concentrations in isosmotic conditions.

## MATERIALS AND METHODS

The electrophysiological recording techniques were similar to those previously used (Carlson & Treherne, 1977) except that in some experiments the action potentials were differentiated using an operational amplifier connected as a differentiating circuit (with a band width of 0·08–150 Hz). This differentiator was connected to the output of a transient-recorder and signal processor and the differentiated signals, together with the action potentials, were displayed on a Tekman chart recorder.

To record action and resting potentials in axons bathed with normal extracellular fluid, sea-water-adapted worms were dissected and pinned out beneath a layer of silicone oil.

The experimental chamber and the method of solution change was as previously described (Skaer *et al.* 1978). The normal physiological saline was based on the blood composition of sea water adapted individuals (Carlson & Treherne, 1977) and 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). Isosmotic dilution of the saline was achieved by appropriate addition of mannitol. In some experiments reductions in potassium concentration (< 30 mM) were compensated by the addition of choline. Elevated potassium concentrations were made by substitution for magnesium ions.

## RESULTS

### *Axonal membrane potentials in saline and beneath silicone oil*

Axonal membrane potentials were measured, in as near normal physiological conditions as possible, by impaling giant axons in preparations which were dissected and maintained under a layer of silicone fluid. Successful impalement under these conditions proved difficult, but, nevertheless, the maximal resting and action potentials recorded were of similar magnitude to those obtained in preparations exposed to physiological saline (Fig. 1). These observations suggest that the ionic composition of the normal saline, devised by Carlson & Treherne (1977) on the basis of the measured blood composition of sea-water-adapted individuals (Skaer, 1974), is a reasonable approximation to that of the body fluids of sea-water-adapted individuals.

### *Effects of isosmotic dilution on axonal membrane potentials*

The effects of successive isosmotic dilutions on axonal function were observed in individuals which had been maintained in full strength sea water. In these experiments the physiological saline (equivalent in ionic and osmotic concentration to the blood of these sea-water-adapted animals) was successfully diluted, constant osmotic concentration (1024 m-Osmol) being maintained by the addition of mannitol. The axons were returned to normal saline between isosmotic dilutions.

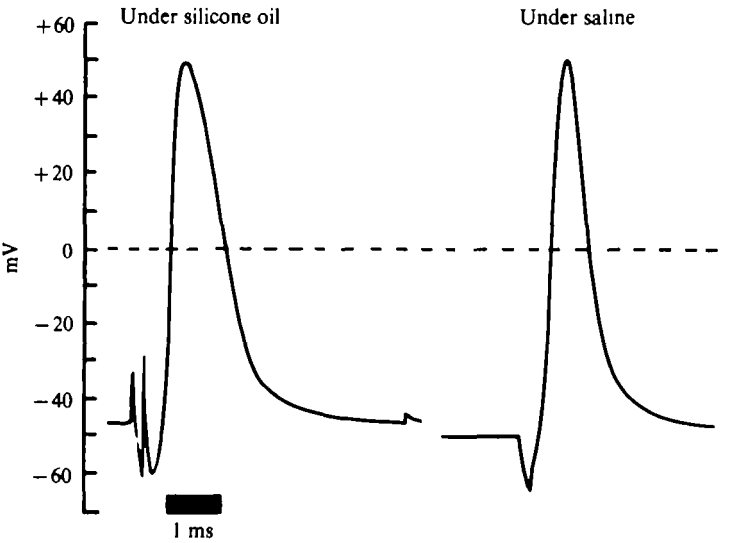


Fig. 1. Action potentials recorded from the axon of a sea-water-adapted individual beneath silicone oil (A) and in a preparation bathed in normal physiological saline (B). (Chart recordings displayed using a transient signal processor.)

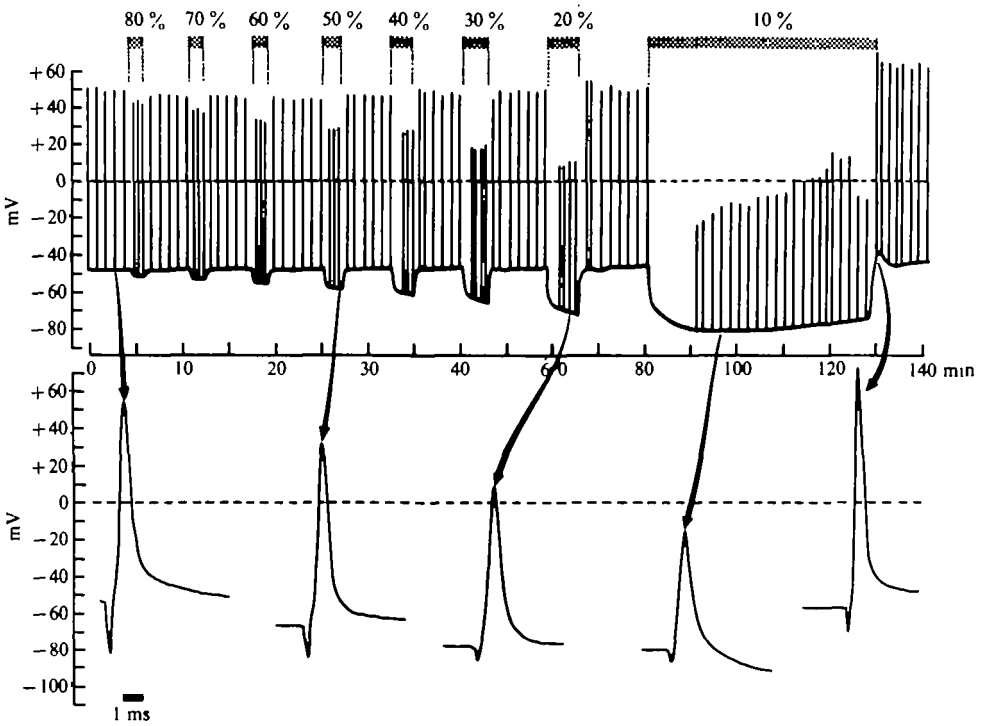


*Fig. 2. The effects of successive isosmotic dilutions of the bathing medium on the resting* and action potentials recorded from the axon of a sea-water-adapted animal. In this and subsequent experiments isosmocity was maintained during dilution of the inorganic ions by appropriate addition of mannitol to the bathing medium. The changes in membrane potential were displayed as chart recordings using a transient signal processor.

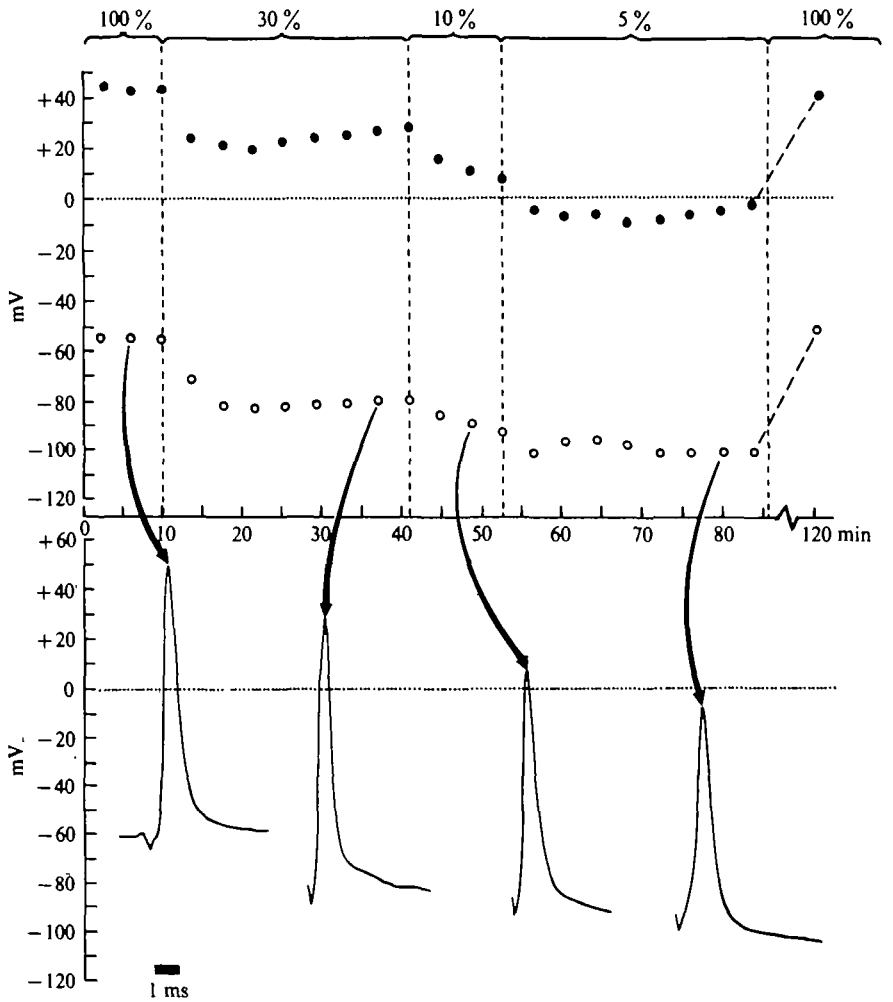


Fig. 3. The effects of progressive isosmotic dilution of the bathing medium on the resting (open circles) and action potentials (closed circles) of an axon from a sea-water-adapted individual.

Dilution of the inorganic ions, at constant osmotic concentration, produced two immediate effects: hyperpolarization of the resting membrane and reduction in the overshoot of the action potentials (Fig. 2). The extent of these changes increased with increasing ionic dilution of the bathing medium. Isosmotic dilution from 100 to 10% frequently resulted in conduction block and subsequent recovery of the action potentials.

With progressive isosmotic dilution of the physiological saline the axonal hyperpolarization which accompanied the decline in overshoot resulted in the maintenance of action potentials of relatively constant amplitude at dilutions as low as 5% (Fig. 3).

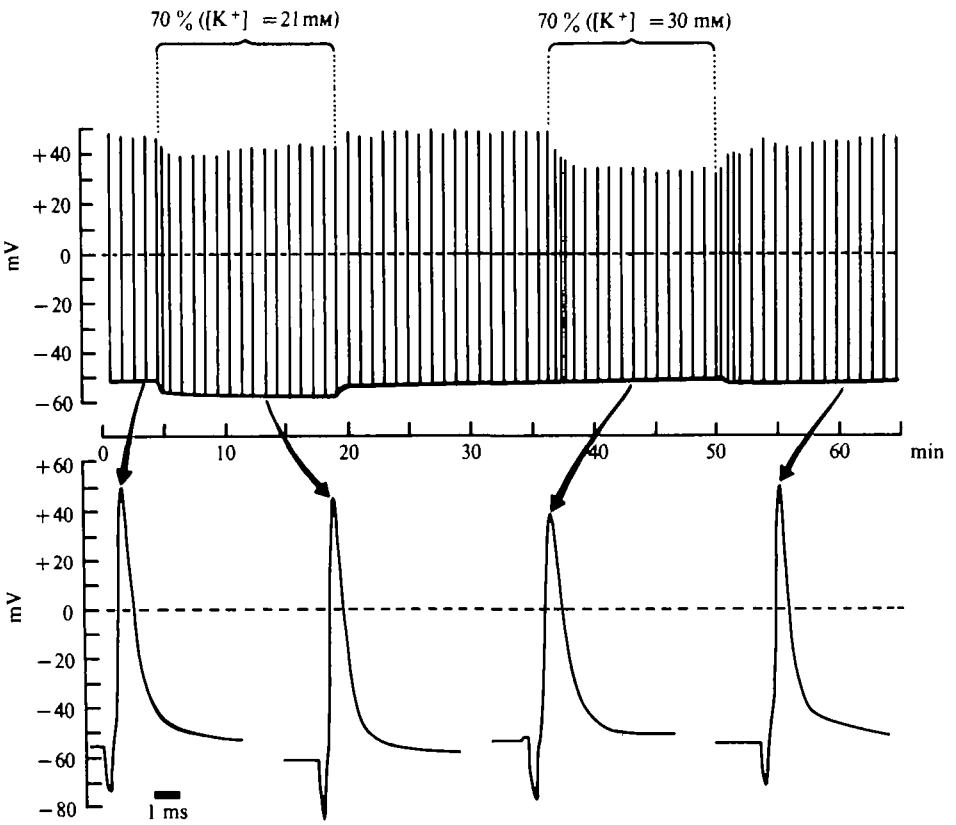


Fig. 4. The effects of external potassium concentration on the resting and action potentials of axons from sea-water-adapted axons during isosmotic dilution of the bathing medium. In the first exposure the potassium ions were proportionally diluted; in the second, the potassium concentration was maintained constant (30 mM) during dilution of the other ions in the bathing medium.

### *The contribution of potassium dilution to axonal hyperpolarization*

The hyperpolarization produced by isosmotic dilution can be largely related to the reduction in potassium concentration of the bathing medium. This is shown by the absence of appreciable hyperpolarization when the potassium concentration of the bathing medium was maintained at a constant concentration of 30 mM during isosmotic dilution (Fig. 4). Isosmotic dilution, at constant $[K^+]_o$, invariably resulted in action potentials of smaller overshoot than when the potassium ions were also diluted at constant osmotic concentration.

As previously shown (Carlson & Treherne, 1977), the axonal resting potential at the potassium concentration of the blood of sea-water-adapted individuals (30 mM-$K^+$) lies in the exponential portion of the slope relating membrane potential to $[K^+]_o$ (Fig. 5). As illustrated in Fig. 5, the changes in axonal resting potential were essentially similar during isosmotic dilution or when only the potassium concentration was reduced, in normal saline, by substitution with choline chloride. The exponential

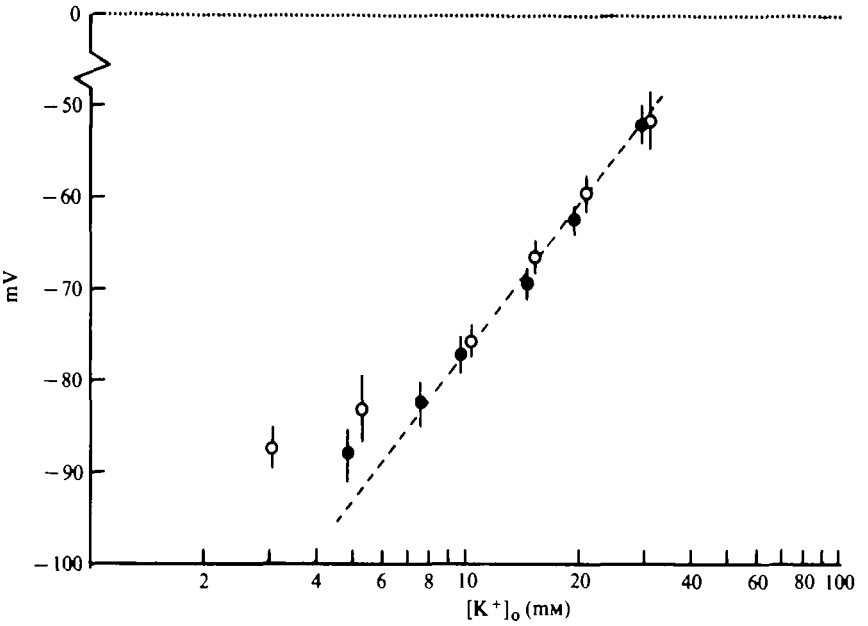


Fig. 5. Relation between external potassium concentration and resting potential during progressive ionic dilution when the osmotic concentration of the bathing medium was maintained constant by the addition of mannitol (open circles). These data are compared with the relation obtained when only potassium concentration was reduced, by substitution with choline ions (closed circles). The broken line indicates the 58 mV slope for decade change in $[K^+]_o$. The vertical lines represent the extent of twice the standard error of the mean ($n$ = 5–10).

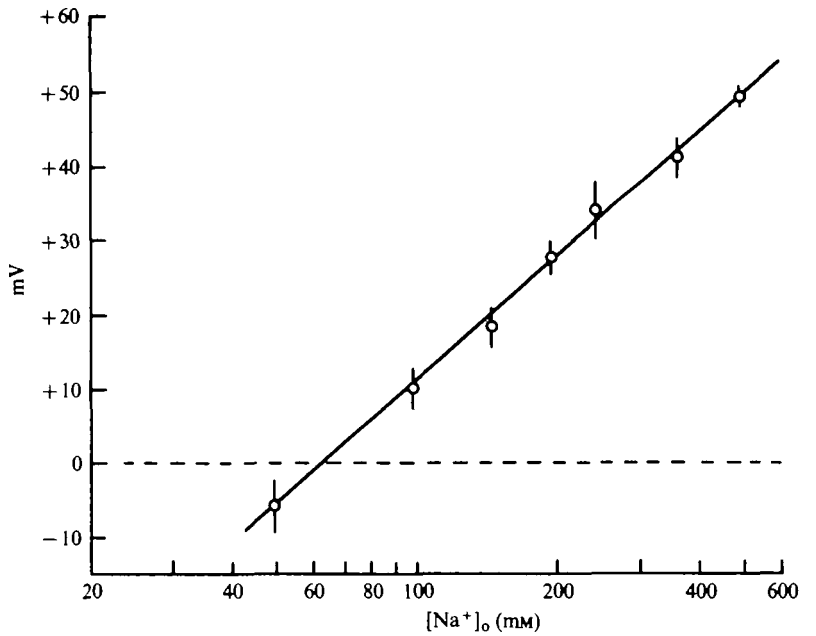


Fig. 6. Relation between external sodium concentration and the extent of the overshoot of the action potentials recorded during dilution of the ions in the bathing medium in isosmotic conditions (i.e. when the osmotic concentration was maintained constant by addition of mannitol) as illustrated in Fig. 2. The calculated regression line has a slope of 55·7 mV for decade change in $[Na^+]_o$ ($r$ = 0·9787: $n$ = 47).

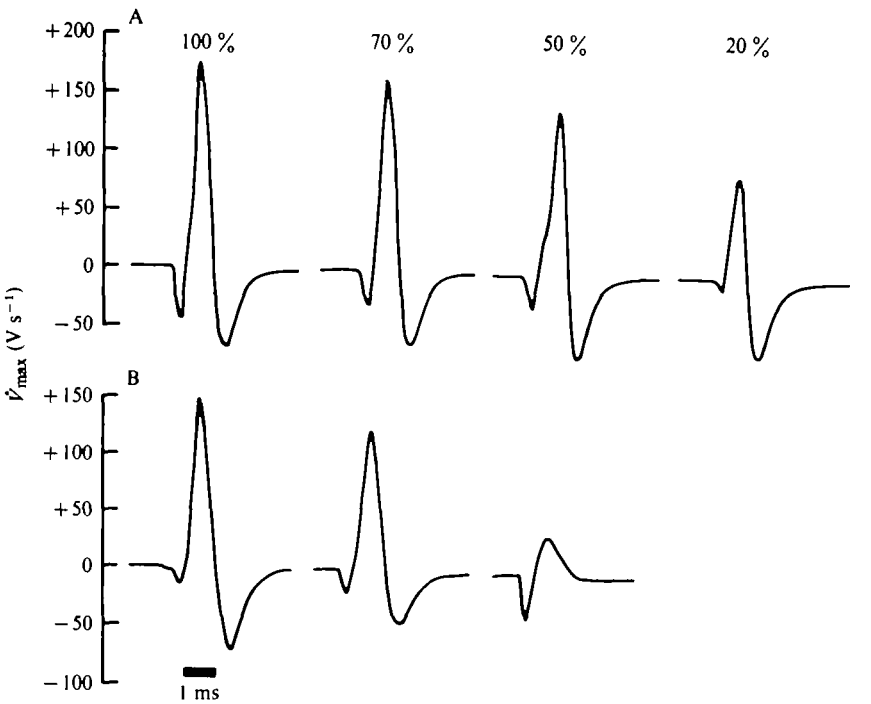


Fig. 7. (A) Effects of normal isosmotic ion dilution (i.e. when the osmotic concentration of the bathing medium was maintained by the addition of mannitol) on the rates of change of membrane potential ($\dot{V}_{max}$) during action potentials recorded in the axon of a sea-water-adapted individual. (B) Effects of isosmotic ion dilution when $[K^+]_o$ was maintained constant at 30 mM. (Chart recording displayed using a transient signal processor and differentiator.)

portion of both sets of data lie close to the 58 mV slope for decade change in $K^+]_o$ predicted by the Nernst relation.

### *Effects of reduction in $[Na^+]_o$ during isosmotic dilution*

The peak of the action potential recorded during isosmotic dilution can be related to the concentration of sodium ions in the bathing medium. The data illustrated in Fig. 6 yield a slope of 55·7 mV for decade change in $[Na^+]_o$ which is close to that of 55·8 mV obtained when only sodium ions were diluted in the bathing medium (Carlson & Treherne, 1977).

### *Measurement of $\dot{V}_{max}$ during isosmotic dilution*

The maximal rate of rise of the action potential ($\dot{V}_{max}$) was used as a measure of the net inward ionic current across the axon membrane (cf. Hodgkin & Katz, 1949). As $\dot{V}_{max}$ can also include contributions from potassium and leak conductances (cf. Cohen & Strichartz, 1977) it will be used as a minimum estimate of $I_{Na}$. In these experiments the effects of $\dot{V}_{max}$ were studied during normal isosmotic dilution (i.e. when all the ions were diluted) and when $[K^+]_o$ was maintained constant (30 mM), to eliminate the hyperpolarizing effects of potassium dilution of the bathing medium (cf. Figs. 3, 4). When all of the concentrations of all the inorganic ions were reduced

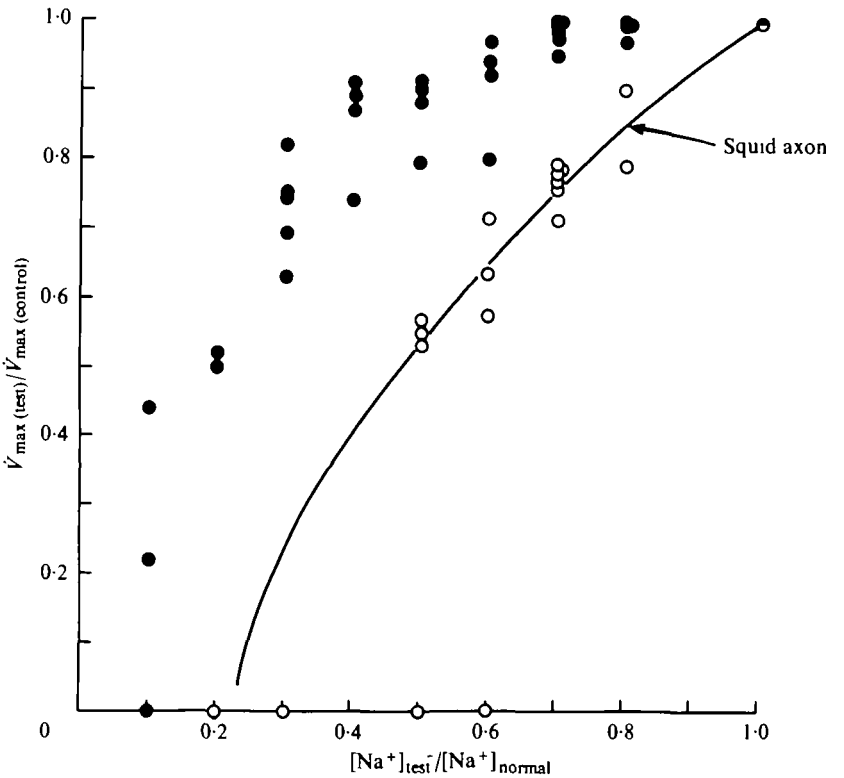


Fig. 8. Relation between the relative maximum rate of rise of the action potential ($\dot{V}_{max\,(test)}$ $\dot{V}_{max\,(normal)}$) and the relative external sodium concentration ($[Na^+]_{o(test)}/[Na^+]_{(normal)}$) during isosmotic dilution of the bathing medium. The closed circles represent measurements made during normal isosmotic dilution (i.e. when all ions were diluted at constant osmotic concentration) and the open ones those observed during isosmotic dilution at constant potassium concentration ($[K^+]_o$ = 30 mM). The curved line shows the relation for the squid giant axon based on data from Hodgkin & Katz (1949).

(i.e. by normal isosmotic dilution) the $\dot{V}_{max}$ showed a relatively modest decline (Fig. 7A) as compared with that observed, in the absence of appreciable hyperpolarization, when $[K^+]_o$ was maintained constant (Fig. 7B).

The effects of ionic dilution, but with constant $[K^+]_o$, were strikingly similar to those observed in the squid axon when $\dot{V}_{max}$ is related to the relative sodium concentration of the external medium (Fig. 8). The abrupt conduction failure in the *Mercierella* axon occurred at 50% $[Na^+]_o$ which is higher than observed in the squid axon. This difference in the blocking concentration could result from a variety of factors, for example: different current thresholds, differences in the axonal geometries of the two preparations or from the lower resting potential of the *Mercierella* axon at the relatively high $[K^+]_o$ of sea-water-adapted animals.

The above results contrast with those observed when $[K^+]_o$ was also reduced (i.e. during isosmotic dilution of all ion species). In this case the axonal hyperpolarization resulting from the reduced $[K^+]_o$ was correlated with a less pronounced decline in relative $\dot{V}_{max}$ during progressive ionic dilution (Fig. 8).

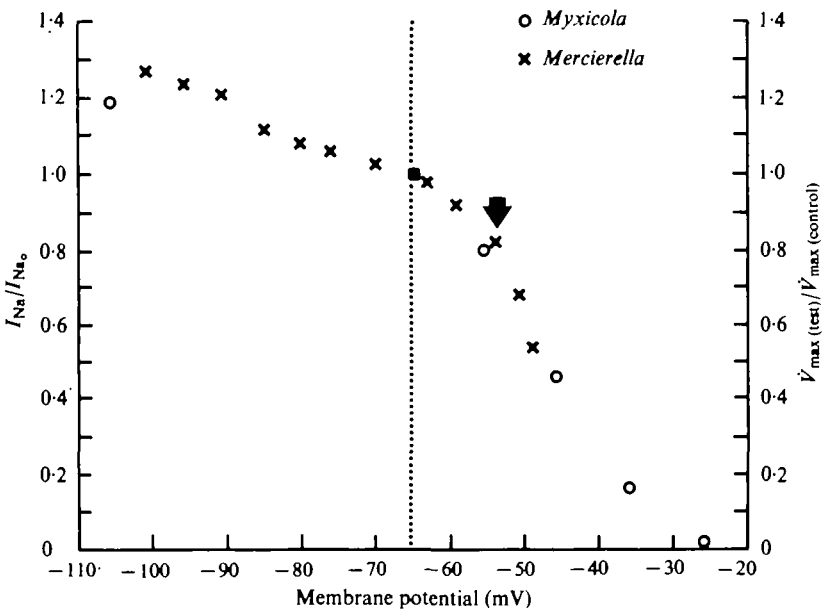


Fig. 9. Comparison of the relations between the relative inward sodium current ($I_{Na}/I_{Na_o}$), for the *Myxicola* giant axon, and the maximum rate of rise of the action potential ($\dot{V}_{max\,(test)}/\dot{V}_{max\,(control)}$), for the *Mercierella* axon, with axonal membrane potential. The values for both species are plotted relative to the normal resting potential for the *Myxicola* axon (i.e. 65 mV), which is indicated by the vertical broken line. The black arrow indicates the resting potential of the *Mercierella* axon in normal saline (i.e. at $[K^+]_o$ = 30 mM). The resting potential and values for the relative inward sodium current for the *Myxicola* axon are taken from the voltage clamp studies of Goldman & Schauf (1972) in which the resting membrane potential was altered by prepulses of long duration (80 ms).

### *Voltage dependence of* $\dot{V}_{max}$

The influence of the resting potential on $\dot{V}_{max}$ was measured, at constant ionic concentration, by changing $[K^+]_o$ in these experiments isosmicity was maintained, with reduced $[K^+]_o$ by proportional addition of choline chloride. Increased potassium concentration (i.e. > 30 mM) was achieved by equivalent reduction in the concentration of magnesium ions in the bathing medium.

In Fig. 9 the data for the *Mercierella* axon are compared with those of Goldman & Schauf (1972) for the *Myxicola* giant axon. For the *Mercierella* axon $\dot{V}_{max}$ at various resting potential ($\dot{V}_{max\,(test)}$) is plotted relative to that at −65 mV ($\dot{V}_{max\,(control)}$), the resting potential of the *Myxicola* giant axon (Goldman & Schauf, 1972). This is compared with the relative sodium current ($I_{Na}/I_{Na_o}$) measured in the voltage-clamped *Myxicola* axon immediately after long (i.e. 80 ms) hyperpolarizing and depolarizing potential changes.

The rather similar voltage-dependency of the sodium current, in the *Myxicola* axon, with the net inward ionic current (estimated from $\dot{V}_{max}$) in the *Mercierella* axon, indicates a similarity in the sodium inactivating mechanisms in these two annelid species. However, at the normal resting potential of sea water-adapted individuals of *M. enigmatica* (i.e. 54 mV at $[K^+]_o$ = 30 mM) the net inward ionic

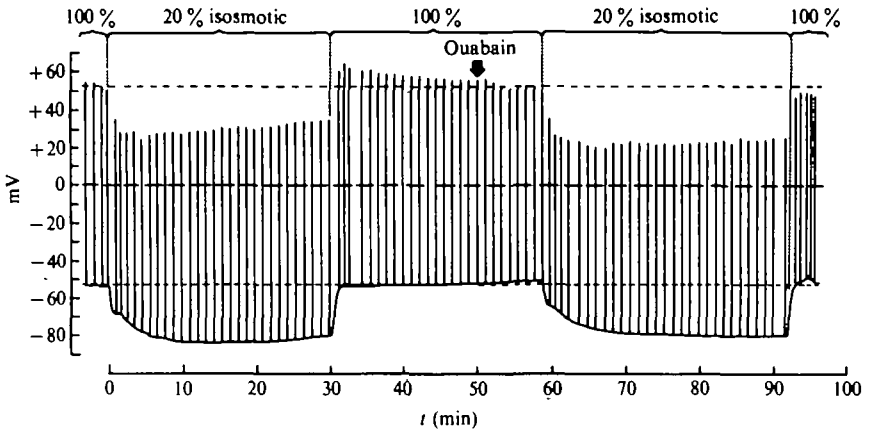


Fig. 10. Effects of prolonged exposure to 20% isosmotically diluted saline on the axonal resting and action potentials in normal conditions and in the presence of $10^{-3}$ M ouabain.

current is close to the steep portion of the curve relating this current to membrane potential (i.e. close to the point where net inward ionic current declines rapidly with depolarization). In these experiments the conducted action potentials were blocked by > 36 mV depolarization from the normal resting potential of sea-water-adapted animals.

*Effects of prolonged isosmotic dilution*

After prolonged exposure to solutions of reduced ionic concentration, the overshoot of the action potentials often showed an appreciable increase on return to normal physiological saline. This can be seen in Fig. 10, where the overshoot was some 10 mV larger than that recorded before exposure to 20% (isosmotic) physiological saline. In this experiment the resting potential showed a relatively rapid return to a value very close to that initially recorded in 100% saline. It is difficult, therefore, to attribute the increase in spike amplitude to an alteration in potential-dependent sodium inactivation. Furthermore, relatively large changes in resting potential, in this range, although affecting the rise time do not appreciably effect the overshoot of the action potential of the *Mercierella* axon (Carlson & Treherne, 1977). As can be seen from Fig. 10, the amplitude of the action potentials also showed a tendency to increase towards the end of the period of exposure to 20% (isosmotic) saline, even though the resting potential had decreased slightly.

Continued exposure to normal saline (after prolonged exposure to isosmotically diluted saline) was associated with a decline in the overshoot of the recorded action potentials to a level close to that initially observed in normal saline (Fig. 10). As shown in Fig. 10, a subsequent exposure to 20% (isosmotic) saline in the presence of a sodium transport inhibitor ($10^{-3}$ M ouabain) was not followed by an increase in the extent of the overshoot on return to normal physiological saline.

One explanation of the above results would be that the increase in overshoot (observed after exposure to isosmotically diluted saline) resulted from a reduction in $[Na^+]_i$ during ionic dilution of the external medium, a process which was inhibited in the presence of ouabain. The subsequent decline in overshoot observed in normal

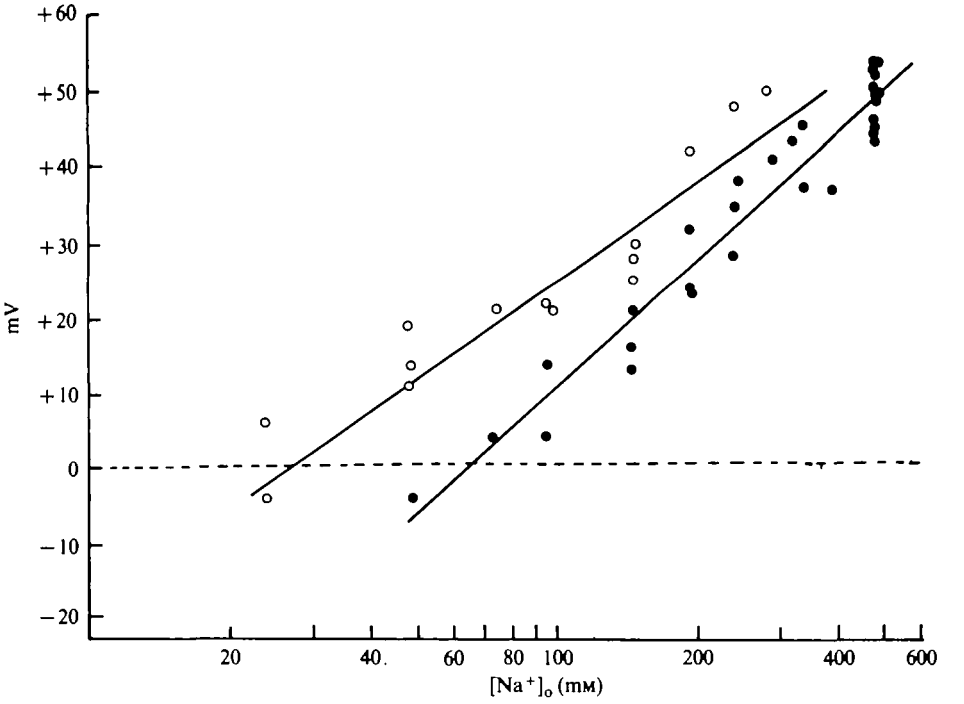


Fig. 11. Relation between external sodium concentration and the extent of the overshoot of axonal action potentials recorded after short (< 5 min) (closed circles) and prolonged (> 60 min) (open circles) exposures to isosmotically diluted salines. The lines drawn through the points are the calculated regression lines. The slope of the line for the briefly exposed axon has a 55·0 mV slope ($r$ = 0·9634; $n$ = 29) and after prolonged exposure to isosmotic saline a 43·5 mV slope for decade change in $[Na^+]_o$ ($r$ = 0·9439; $n$ = 14).

saline could, thus, be attributed to restablishment of the normal sodium gradient across the axon membrane.

An apparent change in intracellular sodium concentration during prolonged exposure of the axons to isosmotic solutions of reduced ionic concentration can also be deduced from the data illustrated in Fig. 11. These show a significant shift in the experimental relationship between external sodium concentration and the extent of the overshoot of the action potential after brief (< 5 min) and prolonged (> 60 min) exposure to isosmotic dilution of the ionic concentration of the bathing medium. Furthermore, slope of the line after prolonged exposure was 43·5 mV for decade change in $[Na^+]_o$ as compared with 55·0 mV after brief stabilization following isosmotic dilution. This suggests that a decline in $[Na^+]_i$ was associated with a reduction in sodium selectivity of the axon membrane during prolonged exposure to solutions of reduced ionic concentration in isosmotic conditions.

## DISCUSSION

The axons of sea-water-adapted individuals showed an unusual ability to maintain action potentials of large amplitude following massive isosmotic dilution (down to 5%) of the inorganic ions in the bathing medium. These results from the axon of

this euryhaline osmoconformer (cf. Fig. 3) contrast, for example, with those obtained from the isolated squid giant axon in which exposure to 33% sea water (containing 67% isotonic dextrose) caused a large decrease in amplitude (> 40 mV) and drastic reduction in the rate of rise of the action potential (Hodgkin & Katz, 1949).

The maintenance, in the *Mercierella* axon, of action potentials of relatively large amplitude and rate of rise following isosmotic dilution is associated with a pronounced hyperpolarization of the axon membrane. This hyperpolarization appears to result largely from the dilution of external potassium ions which in the blood of sea-water-adapted animals are at relatively high concentration equivalent to that of 30 mM used in the normal saline in the present experiments. This unusually high potassium concentration, for a marine invertebrate, can be regarded as a reasonable approximation to the activity of this cation in the blood of sea-water-adapted individuals, because the resting potentials of muscles directly exposed to this saline were closely similar to those recorded in intact individuals in which the muscle fibres were bathed by the blood (Skaer, 1974). This supposition is also supported by the present observations that action and resting potentials can be recorded in axons bathed with extracellular fluid (beneath silicone fluid) which are of similar magnitude to those recorded in preparations with normal saline.

It was previously shown that the axonal membrane of *Mercierella* approximates to an ideal potassium electrode, with a 58·8 mV slope for decade change in $[K^+]_o$ above 10 mM (Carlson & Treherne, 1977). At a concentration of 30 mM-$K^+$ (equivalent to that of the blood $[K^+]$ of sea-water-adapted individuals) the resting potential ($53{\cdot}6 \pm 1{\cdot}4$ mV) is on a steep portion of the slope relating potential to external potassium concentration. These observations are confirmed here, the dominant role of potassium dilution in determining the axonal hyperpolarization being indicated by the close similarity of the Nernst slopes obtained during normal isosmotic dilution (i.e. when the concentrations of all ion species were reduced) and when only $[K^+]_o$ was reduced (Fig. 5).

The reduction in the overshoot of the action potential in the *Mercierella* axon following isosmotic dilution of the external ions appears to be determined mainly by the changes in external sodium concentration. This is shown by the similarities of the slopes relating the extent of the overshoot to $[Na^+]_o$, for sea-water-adapted axons, when all of the ion species were diluted (i.e. during normal isosmotic dilution) and when only $[Na^+]_o$ was changed (Fig. 6). The 55·7 mV slope (for decade change in $[Na^+]_o$) observed during isosmotic dilution is close to the theoretical one of 58 mV predicted by the Nernst relationship and suggests that sodium ions are largely involved in carrying the inward current of the action potential in the concentration range used in these experiments.

It appears, then, that the peak of the action potential, in the axon of sea-water-adapted animals, approaches the sodium equilibrium potential ($E_{Na}$) over a wide range of isosmotic dilutions of the bathing medium. The maintenance of action potentials of large amplitude under these conditions is therefore a consequence of the axonal hyperpolarization associated with the reduction of $[K^+]_o$. This hyperpolarization seems to be an unusual specialization compared with the axons of other invertebrates which have been investigated. This specialization is a consequence of the

unusually high potassium concentration of the blood of sea-water-adapted individuals which maintains the resting potential in a steep portion of the semi-logarithmic slope relating external potassium concentration to membrane potential (Fig. 5). At the lower blood potassium concentration, characteristic of most marine invertebrates (*ca.* 10 mM-$K^+$) the axonal resting potential is relatively insensitive to changes in $[K^+]_o$. In the marine sabellid, *Myxicola infundibulum*, for example, the resting potential follows a steep exponential relation with $[K^+]_o$ only at concentrations > 50 mM (Goldman, 1968). Furthermore, the *Mercierella* axon membrane is more sensitive to changes in potassium concentration in the steep exponential portion of the slope relating resting potential to $[K^+]_o$ exhibiting a 58·8 mV slope (Carlson & Treherne, 1977) as compared with 54·1 mV, for decade change of $[K^+]_o$, in the *Myxicola* giant axon (Goldman, 1968). Thus in the axon of *Myxicola*, an apparently stenohaline sabellid, ionic dilution will result in only a relatively small hyperpolarization, due to a reduction in $[K^+]_o$, as compared with that observed in the *Mercierella* axon.

The rapid axonal hyperpolarization associated with ionic dilution of the external medium has an important effect in reducing the apparent inactivation of the sodium channels. The voltage dependency of the net inward ionic current of the action potential in *Mercierella* (Fig. 9) estimated from $\dot{V}_{max}$ (cf. Hodgkin & Katz, 1949; Cohen & Strichartz, 1977) was shown to approximate to that of the sodium current measured in the voltage-clamped giant axon of *Myxicola* following long 'prepulse potentials' (Goldman & Schauf, 1972). However, at the relatively low resting potential of sea-water-adapted axons (*ca.* 54 mV) as compared with the *Myxicola* giant axon (*ca.* 65 mV) there appears to be a larger degree of sodium inactivation. A consequence of this is that the hyperpolarization (resulting from reduced $[K^+]_o$ during isosmotic dilution in the *Mercierella* axon) induces an appreciable reduction in apparent sodium inactivation. The effect of this on the inward ionic current can be judged by comparison of relative maximum rate of rise of the action potential ($\dot{V}_{max}$) measured during normal isosmotic dilution (when all ionic concentrations are reduced) and when $[K^+]_o$ is maintained constant (but all other ions are diluted) (Fig. 8). In the latter case, in which there is no appreciable hyperpolarization, the values of relative $\dot{V}_{max}$ show a similar relation to external sodium concentration to that observed in the squid giant axon by Hodgkin & Katz (1949). In the former case (i.e. when reduction in $[K^+]_o$ produced appreciable axonal hyperpolarization) $\dot{V}_{max}$ showed a much smaller decline when related to the reduced external sodium concentrations.

The preceding results indicate that the ability of the axons in sea-water-adapted *Mercierella* to conduct action potentials of large amplitude following massive isosmotic dilution of the bathing medium is a consequence of the hyperpolarization of the resting membrane resulting from the dilution of the external potassium ions. Thus, although overshoot is reduced with declining $E_{Na}$ the accompanying increase in resting potential maintains the action potential at large amplitude. Furthermore, the reduction in apparent sodium inactivation, which accompanies axonal hyperpolarization, ensures that a rapid rate of rise of the action potential is maintained during isosmotic dilution of the external medium. This further ensures that conduction velocity, which is dependent upon the rate of rise of the action potential (cf. Hodgkin, 1954), will also be maintained. It may even increase, because the space constant

would also tend to be reduced as a consequence of the increase in extracellular resistance resulting from isosmotic ionic dilution of the bathing medium (cf. Katz, 1947).

An additional axonal response to ionic dilution involves the apparent reduction of the intracellular sodium concentration on prolonged exposure to isosmotic solutions of reduced ion concentration. This is deduced from the increase in the extent of the overshoot seen on return to normal saline after exposure of the axons to isosmotically diluted saline, an effect which is abolished in the presence of ouabain (Fig. 10) and from change in the Nernst slope for sodium produced by prolonged exposure to isosmotically diluted media (Fig. 11). This apparent reduction in $[Na^+]_i$ could result from a constant rate of extrusion of sodium ions, by the sodium pump, in the face of reduced passive inward leak of sodium ions through the axonal membrane. Alternatively, if the passive sodium leak is small, it could result from an increase in the rate of active extrusion of sodium ions from within the axon in the conditions of ionic dilution used in these experiments. An increase in sodium efflux has been shown to occur from squid giant axons in sodium-deficient saline (Hodgkin & Keynes, 1955). This increased efflux seems not to be passively mediated, for it was inhibited by dilute dinitrophenol, and appears to be accompanied by an outward movement of an intracellular anion. The possibility exists, therefore, that the apparent changes in intracellular sodium seen in the experiments on the *Mercierella* axon could be mediated by increased activity of the sodium pump in response to dilution of external sodium ions. However, whatever mechanism is involved, a reduction in $[Na^+]_i$ (to maintain the relative sodium gradient across the axon membrane) is an appropriate response to external ionic dilution by the axon of an animal which is subjected to large fluctuations in blood composition.

The results presented indicate the mechanisms by which the axons of this extreme euryhaline osmoconformer are able to function relatively normally over a wide range of isosmotic dilutions of the bathing medium. In the succeeding paper (Benson & Treherne, 1978) an account is given of the processes involved in axonal adaptations to the additional physiological stress involved in hyposmotic dilution, such as the animal might encounter in its natural environment.

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