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IONIC BASIS OF AXONAL EXCITABILITY IN AN EXTREME EURYHALINE OSMOCONFORMER, THE SERPULID WORM *MERCIERELLA ENIGMATICA* (FAUVEL)

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SUMMARY

Despite the extreme fluctuations in blood concentration experienced by this marine osmoconformer, essentially 'conventional' ionic mechanisms are involved in conduction by the giant axons in isosmotic conditions. The resting axonal membrane approximates to an ideal potassium electrode, with a 58·8 mV slope for decade change in $[K^+]_o$ above 10 mM. The action potential overshoot shows a 55·8 mV decline with decade reduction in $[Na^+]_o$ and the action potentials are blocked by tetrodotoxin at around 5×10^{-7} M.

The rising phase and overshoot of the action potential remain constant at potassium concentrations up to the relatively high level of 30 mM found in the blood, indicating an unusual absence of sodium inactivation over a wide range of resting potentials. Relatively rapid, symmetrical movement of potassium ions between the bathing medium and the axon surface is deduced from the potential changes induced by alterations in $[K^+]_o$. Outward movement of sodium ions ($t_{0.5}$ = 33·5 s) occurs at a similar rate to that of potassium, but inward movement of Na^+ is relatively slow and complex. It is concluded that the ability of axons to function in dilute media must involve specific adaptations to osmotic and ionic stress.

INTRODUCTION

Unlike most nerve cells which have been studied, those of marine osmoconformers may experience large fluctuations in osmotic and ionic concentration. In a stenohaline species, the spider crab (*Maia squinado*), the axonal resting potential is relatively resistant to osmotic stress. The spike-generating mechanism is, however, irreversibly damaged by relatively modest hyposmicity (below 665 m-osmoles), which probably contributes to the lethal effects of osmotic stress in this crustacean osmoconformer (Pichon & Treherne, 1976). In euryhaline osmoconformers, on the other hand, the nervous system must withstand extreme fluctuations in osmotic concentration. The most spectacular example of this is the serpulid worm, *Mercierella enigmatica*, which lives in an unusually wide range of salinities (< 1–55 ‰) (Seurat, 1927; Heldt, 1944; Tebble, 1953) and which can tolerate relatively rapid changes in the osmotic con-

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centration of the blood, in the range of 43–1620 m-osmoles (Skaer, 1974*a*). Nothing is known of the physiological mechanisms which enable nerve cells to cope with such extreme changes of blood composition. It is conceivable, for example, that the axons function by 'conventional' ionic mechanisms and are protected from the adverse effects of osmotic stress or, alternatively, that novel ionic mechanisms are involved in excitation and conduction. In this paper an account will be given of the ionic mechanisms involved in maintaining axonal excitability and of the accessibility of the axon surfaces, in isosmotic conditions, in this euryhaline osmoconformer.

METHODS

M. enigmatica were obtained from Weymouth Harbour, England, and maintained in aerated sea water. Each animal was opened posterior to the prostomium along the dorsal midline and a section of gut was removed exposing the paired giant axons. These axons are approximately 30 μm in diameter and can be seen running the length of the worm 60 μm on either side of the midline (see text-fig. 1, Skaer, 1974*c*). The preparation was pinned out flat, ventral side down, on Sylgard and surrounded by a perspex wall (20 × 10 × 4 mm) which formed the experimental chamber. Solutions flowed continuously through this chamber at a rate of approximately 2·0 ml min^{-1} from a gravity-fed system, described by Treherne *et al.* (1970) and incorporating a multiway non-return valve (Holder & Sattelle, 1972). Rapid solution changes (4·0 ml min^{-1}) were also made by directly syringing solutions into the chamber. Solutions were removed by continuous aspiration.

Giant axons were stimulated by insulated silver electrodes using rectangular pulses of 0·2 ms duration via an R.F. isolating unit from a Farnell stimulator. The integrity of the nerve and the stimulating parameters were monitored using extracellular electrodes of insulated copper wire. Intracellular recording was by glass microelectrodes filled with 3·0 M-KCl which had resistances of between 10 and 20 MΩ. They were used in conjunction with a high impedance, negative capacitance (W. P. Instruments, Inc.) preamplifier with a gain of 5, the output of which was monitored on a Tektronix 561 oscilloscope. Photographs of action potentials were taken using a Nihon Kohden PC2A oscilloscope camera. The output was also fed into a transient-recorder and signal processor which allowed continuous potentiometric recordings (Tekman) of both action and d.c. potentials.

The normal physiological solution employed in these experiments was based on that of Skaer (1974*d*) and approximated in cation concentration to the blood of seawater-adapted animals (Skaer, 1974*b*): 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; O.P. 1024 m-osmoles). This solution was prepared using the following recipe: NaCl, 274·55 g/l; KCl, 2·236 g/l; $MgCl_26H_2O$, 10·368 g/l; $MgSO_47H_2O$, 6·409 g/l; $CaCl_2$, 6·79 g/l; 12·5 mM-NaOH + 7·5 mM Pipes.

Variations in sodium concentration were achieved by appropriate substitutions with tris or sucrose. The osmolarity of dilute potassium solutions (< 30 mM) was maintained by the addition of tris. Elevated potassium concentrations (> 30 mM) were achieved by appropriate omission of sodium ions. Chloride-deficient saline was prepared using sodium and potassium isethionate, calcium acetate and magnesium

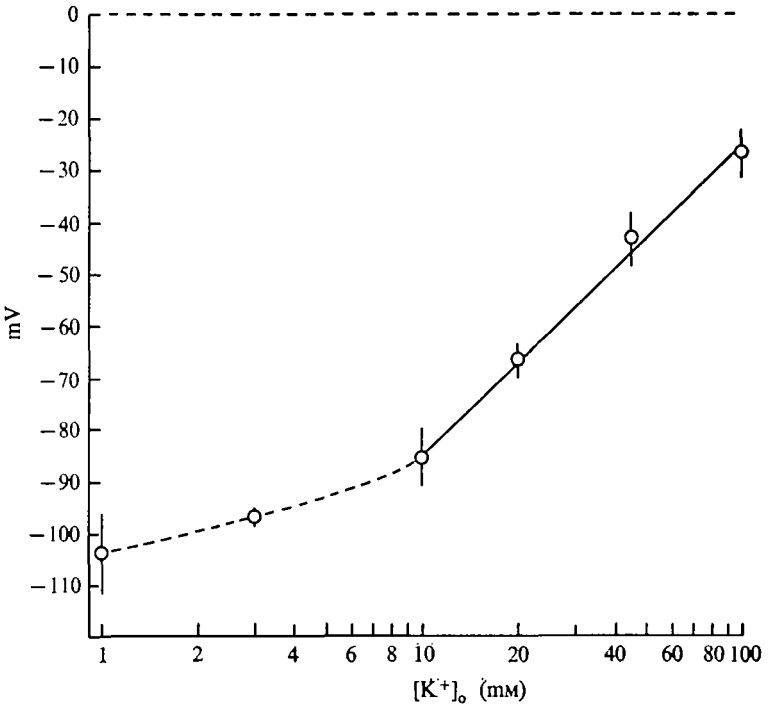

Fig. 1. Relation between axonal resting potential and external potassium concentration. The calculated regression line (continuous line) has a slope of 58·8 mV for decade change in $[K^+]_o$ ($r = 0{\cdot}965$, $n = 20$). Each point is the mean of five measurements, the vertical lines illustrate the extent of twice the standard error of the mean.

sulphate. For comparison, recordings were also made in sea water (10 mM-K^+, 470 mM-Na^+). All numerical results are quoted as mean $\pm 2 \times$ S.E. (number of observations).

RESULTS

Effects of external potassium concentration on axonal membrane potentials

The resting potentials in the giant axons averaged $53{\cdot}6 \pm 1{\cdot}4$ ($n = 20$) mV in 'blood saline' (30 mM-K^+). For potassium concentrations above 10 mM, the resting potential showed an exponential relationship with a slope of 58·8 mV for decade change in $[K^+]_o$ (Fig. 1), which indicates that the axon membrane approximates to an ideal potassium electrode. Extrapolation according to the Nernst equation yields a value for $[K^+]_i$ of approximately 250 mM. In sea water (10 mM-K^+), the average resting potential was $74{\cdot}7 \pm 1{\cdot}1$ ($n = 20$) mV.

Fig. 2 shows action potentials recorded in normal blood saline (30 mM-K^+) and in saline of reduced potassium concentration (10 mM). The average overshoot with $[K^+]_o = 30$ mM was $34{\cdot}5 \pm 1{\cdot}1$ ($n = 16$) mV, while that at $[K^+]_o = 10$ mM was $38{\cdot}1 \pm 1{\cdot}1$ ($n = 16$) mV. The substantial independence of external potassium shown by the overshoot and the rising phase over the range 10–30 mM, despite the large change in resting potential, suggests that at the blood concentration of 30 mM-K^+, the action potential is not affected by significant sodium inactivation.

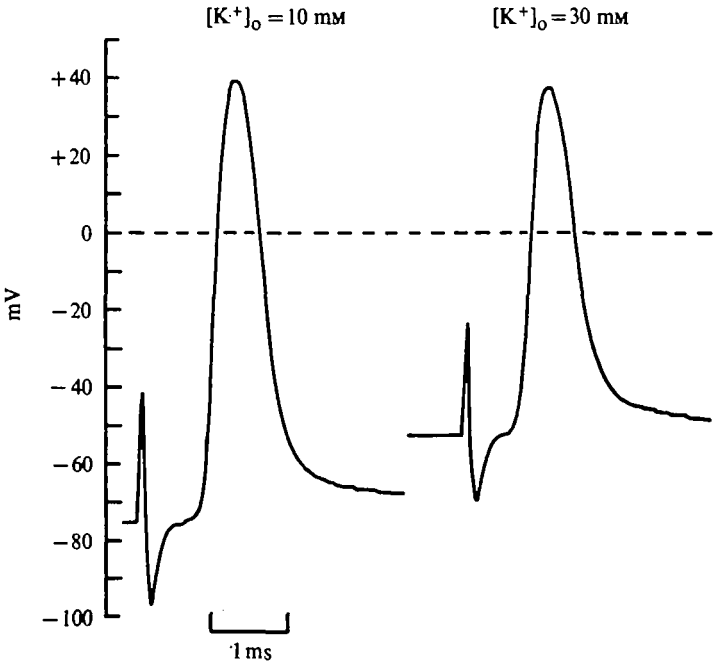

Fig. 2. The effect of reduced (10 mM) and normal external potassium concentration (30 mM) on axonal resting and action potentials. (Chart recordings using transient signal processer.)

Effects of external sodium concentration on axonal membrane potentials

The overshoot of the action potentials decline exponentially with decreasing external sodium concentration, with a 55·8 mV slope for decade change in $[Na^+]_o$ (Fig. 3). The approximation to the theoretical slope of 58 mV, predicted by the Nernst equation, suggests a relatively high selectivity for sodium ions in carrying the inward current of the action potential. Extrapolation according to the Nernst equation suggests that $[Na^+]_i$ is around 90 mM.

The data illustrated in Fig. 3 were obtained by substituting NaCl with sucrose. Essentially similar results were obtained when sodium ions were replaced by those of tris. For example, reduction of $[Na^+]_o$ from 482 to 300 mM, in tris-substituted saline, caused a reduction in the overshoot of $11{\cdot}7 \pm 0{\cdot}4$ ($n = 6$) mV which is not significantly different from that of $11{\cdot}9 \pm 0{\cdot}8$ ($n = 5$) mV obtained with sucrose-substituted saline.

Alteration in the external sodium concentration produced no appreciable effects on the resting potential (Fig. 3).

Effects of TTX

When applied in normal saline, tetrodotoxin at concentrations of 10^{-7} to 10^{-6} M caused a rapid reduction in the rising phase and the overshoot of the action potential and eventual conduction block (Fig. 4). The action potential showed a rapid, but incomplete, recovery on return to normal saline.

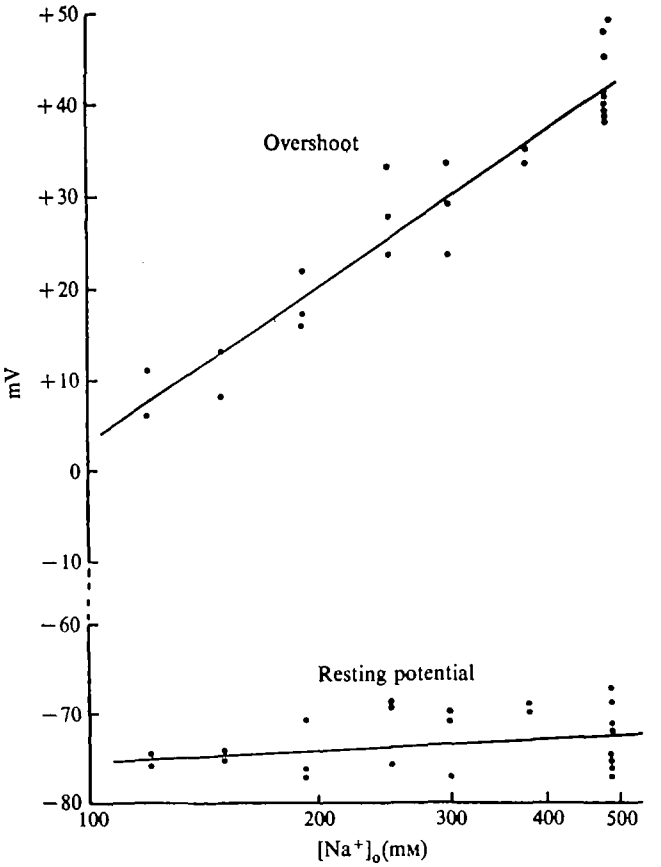

Fig. 3. Effects of variation of external sodium concentration on the overshoot of the action potential and on the resting potential. The continuous lines are the calculated regression lines for the overshoot ($r = 0{\cdot}950$, $n = 23$) and the resting potential ($r = 0{\cdot}345$, $n = 23$). The line for the overshoot shows a 55·8 mV slope for decade change in $[Na^+]_o$.

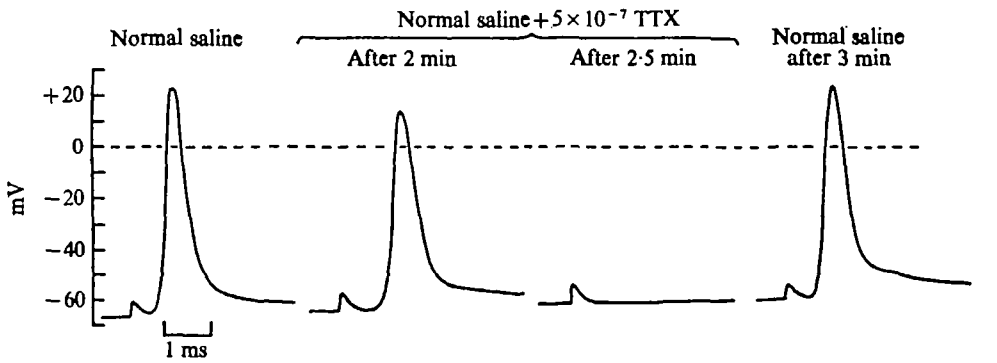

Fig. 4. Effects of 5×10^{-7} M tetrodotoxin on axonal action potentials.

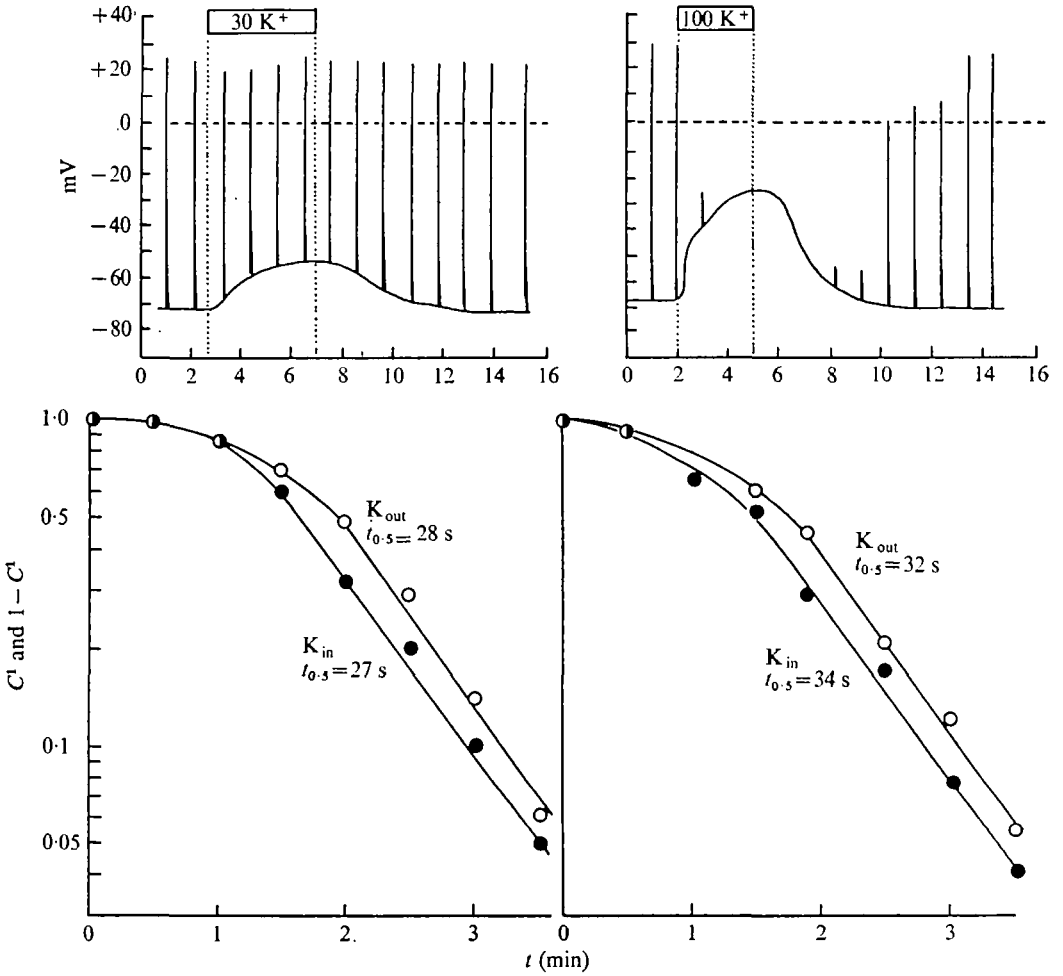

Fig. 5. Effects of changing $[K^+]_o$ from 10 mM to 30 and 100 mM on axonal resting and action potentials (upper figures). The lower figures illustrate the relative change of extra-axonal potassium concentration (calculated from the Nernst relation for the resting potentials of those axons) following elevation (closed circles) and subsequent reduction (open circles) of $[K^+]_o$ to 10 mM.

Accessibility of the axon surfaces to potassium ions

The kinetics of axonal depolarization and repolarization were used to calculate the rates of change of concentration of potassium ions at the axon surfaces following elevation and subsequent reduction of the concentration of this cation in the bathing medium. In these experiments the extra-axonal potassium concentrations were estimated from the 'steady state' Nernst slopes for the individual axons. The estimated concentrations changes were initially complex and then became exponential (Fig. 5), as would be expected from a first-order diffusion process. Movements of potassium ions between the bathing medium and the axon surfaces were symmetrical, the half-times for inward and outward diffusion being, respectively, $31{\cdot}0 \pm 3{\cdot}1$ ($n = 6$) and $32{\cdot}2 \pm 3{\cdot}1$ ($n = 6$) s.

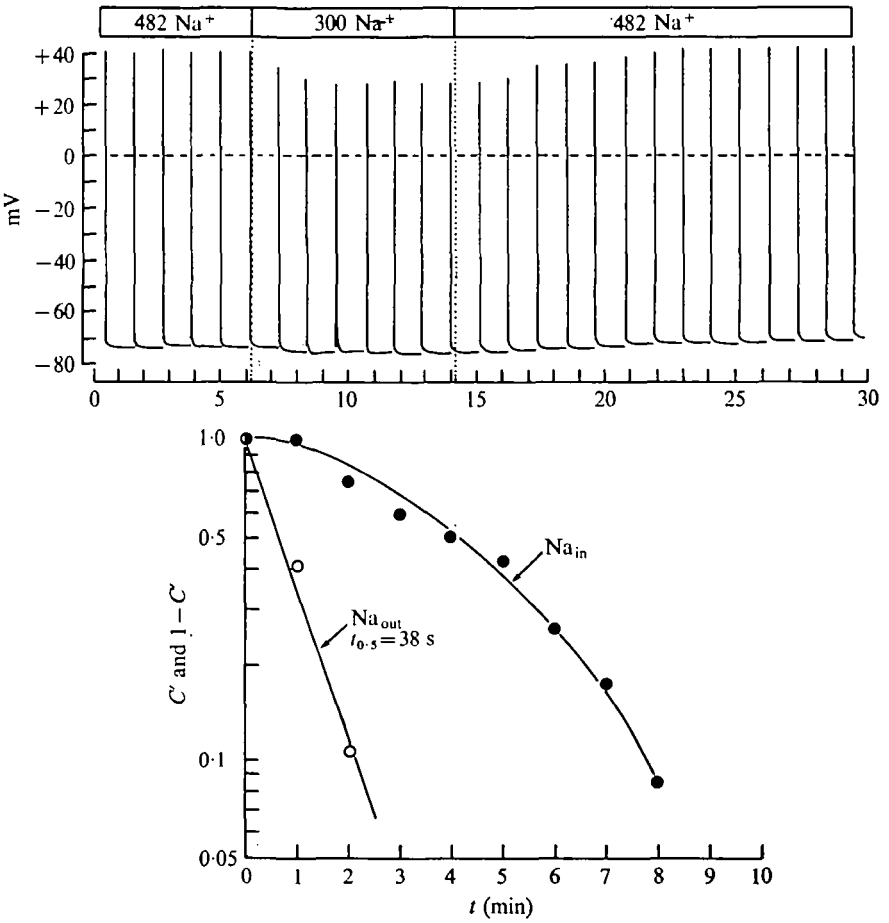

Fig. 6. Effects of reduced external sodium concentration (from 482 to 300 mM-Na^+) on the axonal action potentials (upper figure). The lower figure illustrates the rates of change in extra-axonal relative sodium concentration (calculated from the Nernst relation with the overshoot) following reduction (open circles) and return to 482 mM Na^+ (closed circles).

Accessibility of the axon surfaces to sodium ions

Reduction of $[Na^+]_o$ caused a decline in the overshoot of the action potential which occurred more rapidly than the recovery observed on return to normal saline (Fig. 6). The fall in the estimated extra-axonal sodium concentration following reduction in $[Na^+]_o$, occurred exponentially ($t_{0.5} = 33{\cdot}5 \pm 3{\cdot}0$ ($n = 8$)) as would be expected from a first-order diffusion process. The accumulation of sodium at the axon surface, on return to normal saline, was complex and slower (Fig. 6).

DISCUSSION

In many respects the ionic mechanisms determining excitability in the axon of this euryhaline osmoconformer are similar to those in the nerve cells of two other annelids which have been investigated. The intracellular potassium concentration of 250 mM (estimated from the Nernst relation for the membrane potentials) is similar to that of

280 mM in the extruded axoplasm of another marine annelid, *Myxicola infundibulum* (Gilbert & Shaw, 1969). The estimated intracellular sodium concentration (90 mM) is, however, higher than in *Myxicola* axoplasm (20 mM-Na^+).

As with leech neurones (Nicholls & Kuffler, 1964) the resting membrane approximates to an ideal potassium electrode, but shows marked deviation from the 58 mV slope predicted by the Nernst equation at low external potassium concentrations (below 10 mM, as compared with 20 mM in the neurones of *Hirudo*). The potassium selectivity in *Mercierella* is, however, somewhat greater than in the giant axon of *Myxicola* in which a 54·1 mV slope, for decade change of $[K^+]_o$, is recorded and in which there is a marked departure from the exponential relationship at external concentrations below 50 mM (Goldman, 1968).

As with the *Myxicola* giant axon (Gilbert & Shaw, 1969; Goldman & Binstock, 1969) the action potentials in that of *Mercierella* are sodium-dependent and are abolished by tetrodotoxin at a concentration of around 10^{-7} M (Binstock & Goldman, 1969). Similarly, as in *Myxicola* (Goldman & Binstock, 1969), the active membrane approximates to an ideal sodium electrode, while the resting potential is not significantly affected by $[Na^+]_o$.

The blood of *M. enigmatica* appears to be characterized by an unusually high potassium concentration for a marine invertebrate, which in seawater-adapted animals can be as high as 44·9 mM (Skaer, 1974*b*). The concentration of 30 mM-K^+ used in the normal saline in these experiments probably represents a reasonable approximation to the activity of this cation in the blood, for the resting potentials of muscle cells exposed to this saline were similar to those bathed with blood and impaled in intact individuals (Skaer, 1974*d*). This concentration might be expected to produce a depolarization sufficient to induce significant inactivation of the inward current of the action potential (cf. Hodgkin & Huxley, 1952). For example, in the squid giant axon a potassium concentration equivalent to that in the blood of *M. enigmatica* abolished the action potential (Curtis & Cole, 1942) while in the crayfish giant axon a modest depolarization of 10–15 mV, resulting from an increase of 10–15 mM-K^+, reduced the action potential to zero (Dalton, 1959). The present results with *M. enigmatica* show, however, that changing the external potassium concentration from 10 to 30 mM resulted in only a small decline in the overshoot, although the resting membrane was depolarized by some 20 mV. The lack of effect of a 20 mV depolarization could result from (*a*) the fact that the resting potential is 11 mV larger, in normal sea water, than that of the squid giant axon (Hodgkin & Katz, 1949) and (*b*) the possibility that the h_∞ (inactivation) curve is shifted to the left with respect to potential as compared with the squid giant axon (Hodgkin & Huxley, 1952). It is conceivable that such a shift in the inactivation curve could result from the relatively high calcium level (31 mM) in the blood of *M. enigmatica*, which (by analogy with the squid axon) would reduce Na inactivation in depolarized axons (Frankenhaeuser & Hodgkin, 1957). However, exposure to Ca-deficient (EGTA) saline (at 30 mM-K^+) did not produce appreciable effects on the action potential (Carlson & Treherne, 1977). This lack of effect of Ca-deficient saline on *Mercierella* axons could, however, result from a retention of extracellular calcium, or a relatively slow leakage of calcium ions from the vicinity of the axon surfaces.

The relatively rapid electrical responses of the axons to alterations in the potassium

and sodium concentrations of the bathing medium indicate that the axonal surfaces are, as in other non-insect invertebrates (cf. Treherne & Moreton, 1970; Abbott & Treherne, 1977), accessible to these monovalent cations in isosmotic conditions. The changes in extra-axonal potassium concentration, following alteration in $[K^+]_o$, were initially complex and then exponential, as would be expected from a first-order diffusion process. The apparent inward and outward movements of potassium ions were reasonably symmetrical ($t_{0.5}$ for inward diffusion being 31·0 s, and for outward diffusion, 32·2 s). The outward movement of sodium ions, following reduction in $[Na^+]_o$, also appeared to approximate to a first-order diffusion process ($t_{0.5}$ = 33·5 s The similar half-times for outward movements of the two ions are in accord with their diffusivities in free solution, which differ by only about 19%.

It appears then that despite the extreme fluctuations in blood composition experienced by *M. enigmatica* there is no appreciable restriction in accessibility of potassium and sodium ions to the axon surfaces in isosmotic conditions. *Mercierella* is similar, in this respect, to a freshwater annelid (the leech) and to a marine species (*Myxicola*), although considerable quantitative differences exist between the three species, (e.g. $t_{0.5}$ for K_{in} being 3·7 s in leech ganglion cells (Nicholls & Kuffler, 1964), 31 s in *Mercierella* and 87 s in *Myxicola* giant axon (calculated from the data of Goldman, 1968)). The situation also appears to be more complex in the *Myxicola* preparation where an involvement of fixed negative charges in the vicinity of the axon surfaces has been tentatively postulated to account for a terminal slow phase of repolarization which apparently produces a marked asymmetry in inward and outward potassium movements (Goldman, 1968).

Although the outward movement of sodium ions from the axon surface, following reduction in $[Na^+]_o$, appeared to approximate to a first-order diffusion process (and to be comparable to both the inward and outward movements of K^+) the inward movements of sodium, following elevation of $[Na^+]_o$, were relatively slow and complex. There is no obvious or convincing explanation for this asymmetry. It is conceivable that it could result from a change in intercellular cation accessibility resulting from a reduction in $[Na^+]_o$. However, rapid outward movements of Na^+ were still observed following a second exposure to a lower external sodium concentration. Furthermore, the symmetrical potassium movements (following elevation of $[K^+]_o$ from 10 to 100 mM and subsequent reduction to 10 mM) occurred in the event of relatively large changes in $[Na^+]_o$, which were made to accommodate the changes in potassium concentration of the bathing medium. The asymmetry in Na^+ movements could result from active transport across some glial components, although it is difficult to see what physiological advantage would be conferred by a more rapid Na_{out} as compared with Na_{in}. Alternatively, it could be that sodium ions are involved in interactions with an extracellular anion matrix which could, by its ion-selective properties, impose an asymmetry in the movements of this cation between the axon surfaces and the bathing medium. Such mechanisms have been tentatively proposed to explain asymmetrical cation movements in between the bathing medium and the axon surface, for example, in *Myxicola* (Goldman, 1968) and a crustacean species (Abbott, Pichon & Lane, 1977), although there is, at present, little concrete evidence for such a mechanism.

The results of this investigation are surprising, in that the axons of such an extreme osmoconformer function in the absence of a permanent blood–brain barrier, using

'conventional' ionic mechanisms, the only special features being an absence of appreciable sodium inactivation at the relatively high potassium concentration of the blood, and an anomalous asymmetry in the movements of sodium ions between the bathing medium and the axon surface. It seems clear, therefore, that the ability of the axons to withstand and to function during extreme osmotic stress must involve specific adaptations to massive changes in ionic and osmotic concentration. These adaptations will be described in succeeding papers.

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