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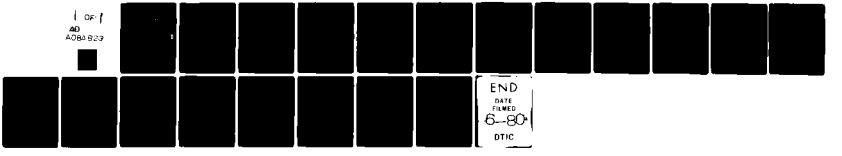

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This investigation has used invertebrate nervous systems to elucidate two basic aspects of central nervous ionic homeostasis: neuronal adaptations to ionic and osmotic stress and ionic homeostasis of the brain microenvironment.

The research on the giant axons of polychaetes has established the important principle that some nerve cells can adapt to very large →

Continuation

20. changes in the composition of their immediate fluid environment. These adaptations involve structural modification and changes in the cellular mechanisms which mediate excitation and conduction.

The results of the investigation on the insect central nervous system has shed light on the permeability properties of the blood-brain interface, which shares some features with the functional organization of the mamalian central nervous system. The physiological information obtained has enabled a physiological model to be erected which explains all of the available experimental information and should be susceptible to further experimental tests.

SUMMARY

This investigation has used invertebrate nervous systems to elucidate two basic aspects of central nervous ionic homeostasis: neuronal adaptations to ionic and osmotic stress and ionic homeostasis of the brain microenvironment.

The research on the giant axons of polychaetes has established the important principle that some nerve cells can adapt to very large changes in the composition of their immediate fluid environment. These adaptations involve structural modification and changes in the cellular mechanisms which mediate excitation and conduction.

The results of the investigation on the insect central nervous system has shed light on the permeability properties of the blood-brain interface, which shares some features with the functional organization of the mammalian central nervous system. The physiological information obtained has enabled a physiological model to be erected which explains all of the available experimental information and should be susceptible to further experimental tests.

KEY WORDS

Neuronal environment - blood brain interface - giant axons - neuroglia - ionic homeostasis

TABLE OF CONTENTS

I. INTRODUCTION

The research carried out during the period of the grant was concerned with an important and relatively unexplored aspect of neural function: central nervous ionic homeostasis. This research has exploited some unique advantages provided by some invertebrate animals by studying the physiological adaptations of nervous systems which are naturally exposed to very large fluctuations in blood composition, namely the giant axons of two estuarine polychaetes (which are directly exposed to massive changes in the ionic and osmotic concentration of the extra-axonal fluid) and the nervous connectives of an insect species (in which ionic homeostasis is achieved by extremely effective regulation of the extracellular fluid environment of the nerve cells).

The results of the investigations on the polychaete axons have shown that nerve cells are not necessarily at the mercy of the composition of their body fluids and establish the important physiological principle that neurones may adapt relatively rapidly to massive changes in the osmotic and ionic composition of their immediate fluid environment.

The results of the studies on the insect preparation have elucidated and alternative physiological strategy to the problem of body fluids of fluctuating composition: the combination of an effective peripheral blood-brain barrier system and local glial-mediated regulation of the composition of the brain microenvironment. This strategy is similar, in a number of basic features, to that of the mammalian central nervous system and, for this reason, is a valuable model system for the quantitative studies of the physiology of central nervous homeostasis. Such studies are of more than fundamental physiological importance, for an understanding of the mode of action and the mechanisms of resistance to insecticidal compounds requires knowledge of the permeability properties of the blood-brain interface and, also, of the associated underlying regulatory processes.

In this report account is given of the major findings and conclusions of the research carried out during the tenure of the grant. First for the studies of neuronal adaptability and then for the homeostatic mechanisms in the regulation of the ionic composition of the brain microenvironment.

More detailed accounts of this work can be found in the papers which have already been published. These are referred to in the text and are also listed separately.

II. NEURONAL ADAPTATIONS TO IONIC AND OSMOTIC STRESS

Two species of estuarine polychaete worms were used for these studies, both were shown to be euryhaline osmoconformers. The nervous system of Sabella penicillus was found to experience changes in the osmotic concentration of the blood, of between 543 and 1236 m-osmol, in response to changing external salinities (Carlson, Pichon & Treherne, 1978). More extreme fluctuations occur in the blood of the serpulid, Mercierella enigmatica, the range (between 84 and 2304 m-osmol) being the most extreme known for any animal species (Skaer, 1974a; Benson & Treherne, 1978b).

a) Experiments on Sabella giant axons

The giant axons of Sabella can withstand abrupt hyposmotic dilution of the bathing medium from 1040 to 520 mOsm, equivalent to 50% dilution (Carlson, Pichon & Treherne, 1978). This is in marked contrast to the irreversible damage incurred in the spike generating mechanism of the stenohaline osmoconformers. Furthermore, the axons of Sabella are unusual in showing only relatively slow electrical responses to abrupt dilutions of the surrounding fluids (Carlson et al., 1978). Thus the overshoot of the intracellularly recorded action potential declines slowly and with a rather complex time course in hyposmotic media, yet the axons are conventionally dependent upon sodium and the spike is rapidly diminished in low sodium conditions when the osmotic concentration is maintained with sucrose (Fig. 1). This effect, together with the slower rate of potassium depolarization observed during hyposmotic stress as compared with equivalent isosmotic treatments, suggests that there is a reduced intracellular access to the axon surfaces when the osmotic concentration around the nerve is reduced. This could arise from osmotically-induced swelling of the surrounding glial processes, and would presumably provide limited short-term, protection from the adverse effects of fluctuations in blood osmolality.

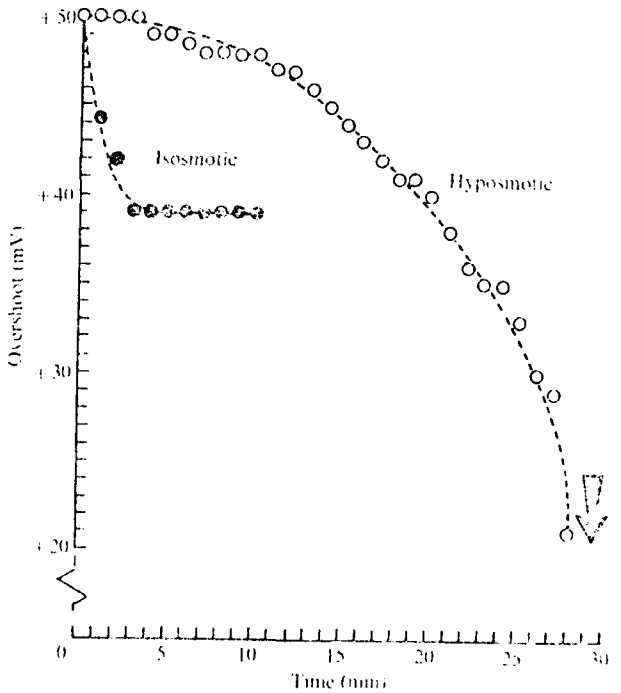

Fig. 1. Comparison of the rates of decline of the overshoot following exposure to 50% hyposmotic and isosmotic (sucrose-substituted) ASW.

In spite of this short-term limitation of access, the giant axons of Sabella nevertheless develop a reversible conduction block after prolonged exposure to 60% hyposmotic solutions (Fig. 2A). But the axons are able to adapt to this situation if the dilution is gradually imposed, and will then continue to conduct action potentials (Fig. 2B).

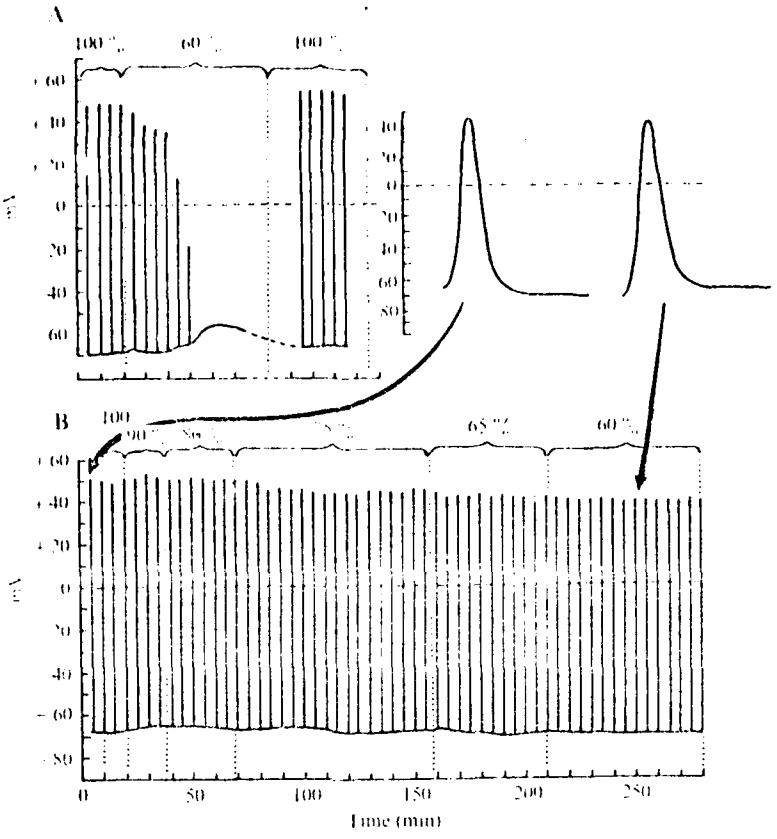

Fig. 2. (A) Effects of abrupt exposure of a giant axon from a sea-water-adapted worm to 60 %, hyposmotic, ASW. (B) Effects of progressive dilution of the bathing medium on the resting and action potentials recorded in the giant axon taken from a sea-water-adapted worm.

This hyposmotic adaptation in the giant axons involves an appreciable reduction in intracellular potassium concentration, as revealed by a comparison of the changes in resting potential with varying $[K^+]_o$ before and after adaptation (Treherne & Pichon, 1978). This reduction is approximately proportional to the potassium dilution in the external medium, so that a 60% reduction of $[K^+]_o$ is associated with a 69% decline in $[K^+]_i$, from 490 mM to 340 mM. This effect ensures that there is no marked change in axonal resting potential, in contrast with the situation in a more extreme conformer, Mercierella, in which intracellular potassium is not proportionately reduced and a marked hyperpolarization results.

The decline in intracellular potassium observed in Sabella axons during progressive dilution of the external medium does not appear to result from axonal swelling and cell dilution (Carlson et al., 1978). The recorded decline in $[K^+]_i$ during adaptation must, therefore, result from a net loss of this major intracellular cation from the axoplasm, an apparently common strategy used by cells to reduce the internal osmotic concentration during hyposmotic stress (cf. Hoffman, 1977).

The active membrane in axons from seawater-adapted Sabella is not completely selective for sodium ions. This is shown by the significant departure in the slope of the line relating overshoot to internal sodium concentration from the 58 mV per decade change in $[Na^+]_o$ predicted by the Nernst relation for an ideal sodium electrode (Fig. 3). However, in hyposmotically adapted axons the sodium

selectivity increases (Fig. 3), a factor which partially compensates for the reduction in sodium gradient across the axon membranes as the external fluids are diluted. The overshoot of the action potential is thereby increased in hyposmotically-adapted as compared with seawater-adapted axons at equivalent external sodium concentrations (Treherne & Pichon, 1978).

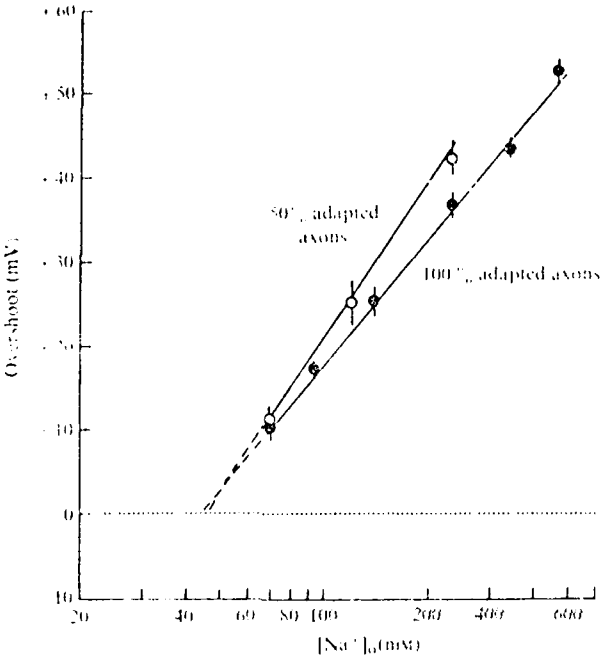

Fig. 3. Relation between the extent of the overshoot of the action potentials in axons from sea-water-adapted worms, in 100% ASW, and from axons taken from animals adapted to 50% sea water, in 50% hyposmotic, ASW. The continuous lines are the calculated regression lines ($r = 0{\cdot}9517$ for sea-water-adapted axons; $r = 0{\cdot}9234$ for 50% adapted axons). The mean slopes of the regression lines are 48·8 ± 2·6 mV, for decade change in $[Na^+]_o$ for the 100% adapted axons and 59·8 ± 6·4 mV for the 60% adapted axons.

The physiological adaptations of Sabella giant axons are thus adequate to permit a maintenance of excitability under the regimes of limited osmotic stress experienced by a modest osmoconformer. Firstly, the axons receive short-term protection by restriction of the intercellular access to their surfaces during abrupt hypoosmotic stress. Secondly, there is a substantial reduction in intracellular potassium, which is presumably accompanied by an equivalent loss of intracellular anions (cf. Gilles, 1979), contributing to the achievement of osmotic equilibrium with the blood or bathing medium. Thirdly, the relative sodium permeability of the active membrane increases during hyposmotic adaptation so as to partially compensate for the reduction in external sodium concentrations.

b) Experiments on Mercierella giant axons

Despite the very large fluctuations in osmotic and ionic concentration of the blood experienced by this extreme osmoconformer the giant axons appear to be unprotected by a recognizable barrier system. The giant axons are overlaid only by narrow glial processes which provide an incomplete covering of

axonal surfaces. Where more complete covering occurs the intercellular clefts are not sealed by junctional complexes and ionic lanthanum penetrates to the surfaces of axons of both sea water-adapted individuals (in normal saline and during initial hyposmotic stress) and in those which are hyposmotically-adapted (Skaer et al., 1978; Treherne, 1978).

Although unprotected from the extreme fluctuations in osmotic concentration of the blood the Mercierella axons nevertheless exhibit structural specializations which, it is proposed, enable them to withstand an appreciable excess of intracellular hydrostatic pressure resulting from osmotic imbalance during hyposmotic stress. These are hemidesmosome-like structures, associated with the axon membrane, which are connected to a network of neurofilaments within the axon. The provision of regularly, closely-spaced, supports for the axonal membrane appears to be a highly effective way of limiting the tension on it by reducing the radius of curvature (Skaer et al., 1978a). For example, a notional excess of internal concentration of 100 mOsM will produce an internal pressure of 2.42×10^5 Pa (2.42×10^6 dyn cm^2) within a giant axon of 15 μm radius. Under these circumstances it can be calculated that an increase in area of only 10% of the membrane between the hemidesmosomes will be sufficient to reduce the membrane tension to about 0.03 N m^{-1}, which is less than one hundredth of the value for the unstretched axon and roughly equivalent to the tension which the membrane of a human erythrocyte can withstand before haemolysis occurs (Rand, 1964).

The giant axons of Mercierella utilize the apparently common strategy of reducing the intracellular electrolyte concentrates during hyposmotic adaptation. As with Mytilus axons (Willmer, 1978a), but unlike the giant axons of Sabella (Treherne & Pichon, 1978), there is non-proportional retention of both sodium and potassium during progressive dilution of the external medium (Benson & Treherne, 1978b; Treherne, 1978). For example, reduction of the external ion concentrations to 25% resulted in only an approximate halving of $[Na^+]_i$ and $[K^+]_i$ (i.e. from 310 to 145 mM for potassium and 87 to 44 mM for sodium ions).

The non-proportional retention of intracellular potassium ions is of critical importance in the hyposmotic adaptation of the Mercierella giant axons. This is correlated with the unusually high potassium concentration (30 mM) of the blood of sea water-adapted individuals (Skaer, 1974b). Now at the normal blood potassium concentration the resting potential of the Mercierella axon lies on a steep portion of the Nernst slope, which relates the axonal resting potential to external potassium concentration (Carlson & Treherne, 1977; Benson & Treherne, 1978a).

The net effect of the sensitivity of the resting potential to changes in $[K^+]_o$ and non-proportional retention of $[K^+]_i$ is that there is a proportional increase in the outwardly-directed gradient of potassium ions during hyposmotic dilution (Benson & Treherne, 1978b). This accounts for the appreciable hyperpolarization observed during adaptation of the Mercierella giant axons to dilution of the external medium (Fig. 4).

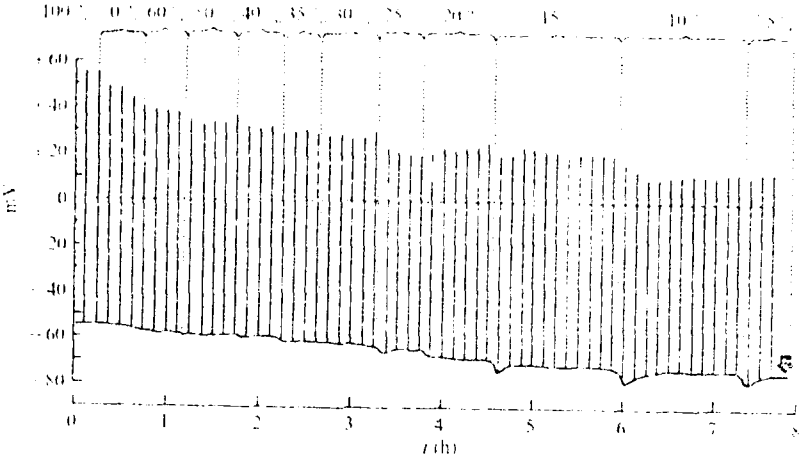

Fig. 4. Continuous recording of the resting and action potentials during gradual hyposmotic dilution of the bathing medium.

The hyperpolarization of the axon membrane during hyposmotic adaptation has two important consequences. First, it tends to compensate for the reduction in the overshoot of the action potential, resulting from decrease in $[Na^+]_o$, and, thus contributes to the maintenance of the amplitude of the action potential. Secondly, the increased resting potential reduces sodium inactivation and, consequently, maintains a rapid rate of rise of the action potential during extreme dilution of the bathing medium.

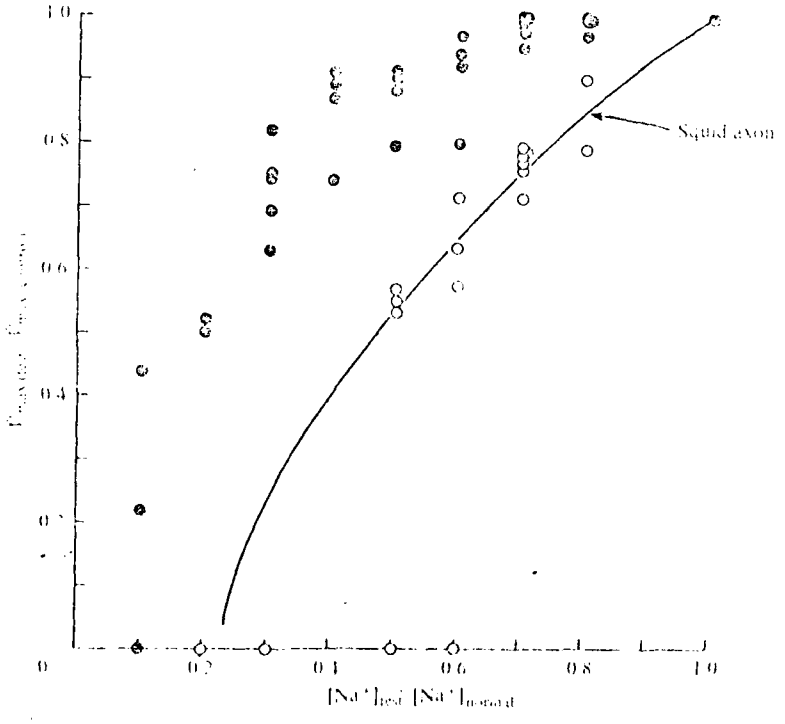

Fig. 5. Relation between the relative maximum rate of rise of the action potential ($P'_{max(test)}/P'_{max(normal)}$) and the relative external sodium concentration ($[Na^+]_{test}/[Na^+]_{normal}$) during isosmotic dilution of the bathing medium. The closed circles represent measurements made during normal isosmotic dilution (i.e. when all ions were diluted at constant osmotic concentration) and the open ones those observed during hyposmotic 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).

The amplitude of the action potentials are also maintained during hyposmotic dilution by the reduction in intracellular sodium concentration (Fig. 6) (Benson & Treherne, 1978b). This reduction appears to be a specific response to reduced external ionic concentration, for it occurs (in the absence of the equivalent reduction in $[K^+]_i$) during isosmotic dilution of the bathing medium (Benson & Treherne, 1978a). The reduction in $[Na^+]_i$ is abolished in the presence of ouabain and could, therefore, result from an increase in net sodium efflux such as occurs in the squid giant axon in sodium-deficient saline (Hodgkin & Keynes, 1955). Theincreased sodium efflux from the cephalopod giant axon appears not to be passively mediated, for it was found to be inhibited by dilute dinitrophenol. It is suggested that the increased sodium efflux is accompanied by an outward movement of an intracellular anion.

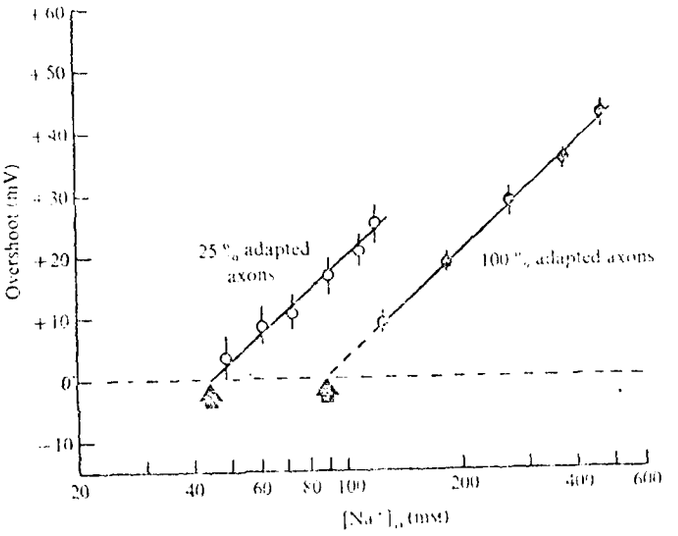

Fig. 6. The relation between $[Na^+]_o$ the extent of the overshoot measured in the axons of sea-water-adapted animals in normal saline in axons adapted, *in vitro*, to 25 %, hyposmotic saline. The calculated regression line for 100 % adapted axons had a slope of 56·3 mV ($r = 0{\cdot}9741$; $n = 27$) and for 25 % adapted axons a slope of 52·2 mV for decade change in $[Na^+]_o$ ($r = 0{\cdot}9450$; $n = 37$). The values of $[Na^+]_o$ were estimated by extrapolating the regression lines to zero potential. Variation in $[Na^+]_o$ were made by substitution with choline chloride. The symbols represent the mean and extent of twice the standard error of the mean.

III. IONIC HOMEOSTASIS OF THE BRAIN MICROENVIRONMENT IN INSECTS

The research carried out during the tenure of this grant has enabled a model to be devised which accounts for the available information obtained using electrophysiological, ultrastructural, radioisotopic and flame photometric techniques.

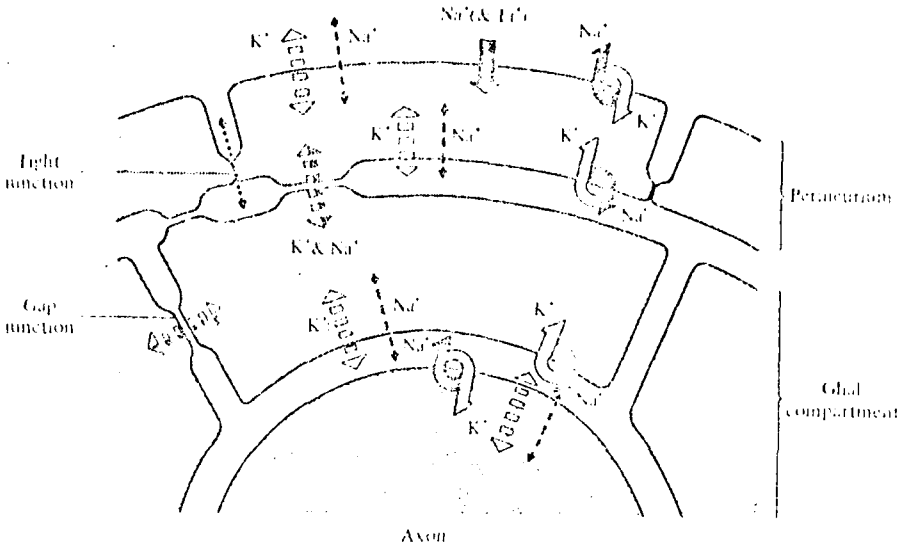

Fig. 7. Model for regulation of extracellular sodium concentration in the cockroach nerve cord. Intercellular passage of sodium between the extracellular fluid and the *bathing medium* (dotted arrow) is limited by tight junctions at the inner ends of the clefts between the perineurial cells. Passage from extracellular routes, through the perineurial and glial cells, involves diffusion across the cell membranes (broken arrows) and conventional Na/K pumps extruding sodium from the cells (linked arrows). Entry of sodium (and lithium) at the outer perineurial membranes could be carrier-mediated (single solid arrow) or be by diffusion down an electrochemical gradient. The presence of both inward and outward transfer of sodium provides dynamic control of its extracellular concentration. [The neural lamella over the perineurium is not incorporated in the model since it is relatively leaky to small water soluble ions and molecules (cf. Treherne, 1974).]

According to the model (Fig. 7) intercellular diffusion between the blood and axonal surfaces is restricted at the inner ends of the tortuous clefts that traverse the superficial layer of specialized neuroglia, the perineurium (Lane, 1974). This accounts for the observation that altered external ion concentrations result in extra-neuronal potential changes, originating at the outer perineurial membranes, before any effects are measured in the axons (Treherne et al., 1970; Pichon & Treherne, 1970; Pichon, Moreton & Treherne, 1971). The restriction is suggested to result from the tight junctions, at the inner ends of the perineurial clefts, and which appear to limit the penetration of ultrastructural tracers along the clefts (Lane & Treherne, 1972).

Recent unpublished research has confirmed the peripheral localization of the intercellular diffusion barrier to inorganic ions. These experiments involved the use of pronase in treated connectives which facilitate penetration of the superficial neuroglia and extracellular channels using fine tipped, high-resistance, microelectrodes. Our observations show that high-potassium pulses induce depolarizing responses (similar to those measured at deeper levels in the CNS) at depths of only 5 μm beneath the neural lamella. The superficial localization of the potassium-induced extraneuronal potentials provides circumstantial evidence for intercellular restriction of inward ion movements in the perineurial clefts such as is incorporated in the model illustrated in Fig. 7.

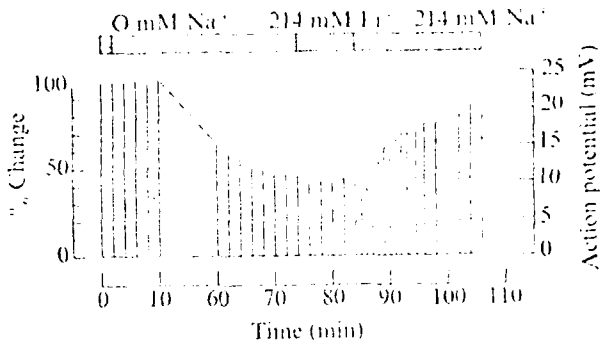

Fig. 8. Effect of exposure to lithium Ringer, following a sodium depletion in the sodium-deficient (Tris) Ringer. Subsequent exposure to the original sodium concentration results in a relatively slow recovery of action potential amplitude.

The perineurial neuroglia and the underlying glial cells are represented, in the model, as confluent compartments which allows intracellular movements of sodium ions, and other small water-soluble ions, to occur via the gap junctions that link adjacent perineurial and glial membranes (Lane, Skaer & Swales, 1977; Lane & Swales, 1978).

According to the model the sodium content of the perineurial cells will be largely determined by the uptake of sodium from the blood and its extrusion into the extracellular fluid and the blood by the Na/K pumps. In the absence of external sodium ions there will be a net outward movement of this cation across the outer perineurial membrane largely mediated by the Na/K pumps. Because of the postulated linkage with the underlying glia the changes in intracellular sodium concentration in the perineurial cytoplasm will be accompanied by equivalent changes within the deeper glial elements.

The above model also accounts for the inability of external lithium ions to gain access to the axon surfaces (Schofield & Treherne, 1978), despite a substantial accumulation of this cation within the central nervous tissues (Bennett, Buchan & Treherne, 1975). The inability of lithium to restore the action potentials in sodium-depleted connectives implies that this cation (which can substitute for sodium in maintaining the inward current of the axonal action potential) must be excluded from the extracellular fluid and, consequently, contained in the glial cytoplasm. This is accounted for in the model by the presence of Na/K pumps on the glial membranes for it is known that linked sodium pumps, in frog muscle (Keynes & Swan, 1959) and crab neurones (Baker, 1965), will not accept lithium ions. The retention of lithium ions within the central nervous tissues, after prolonged washing in normal saline (Bennett et al., 1975) is explained by the outwardly-directed linked sodium pumps on the outer perineurial membranes which, similarly, are presumed not to accept lithium ions.

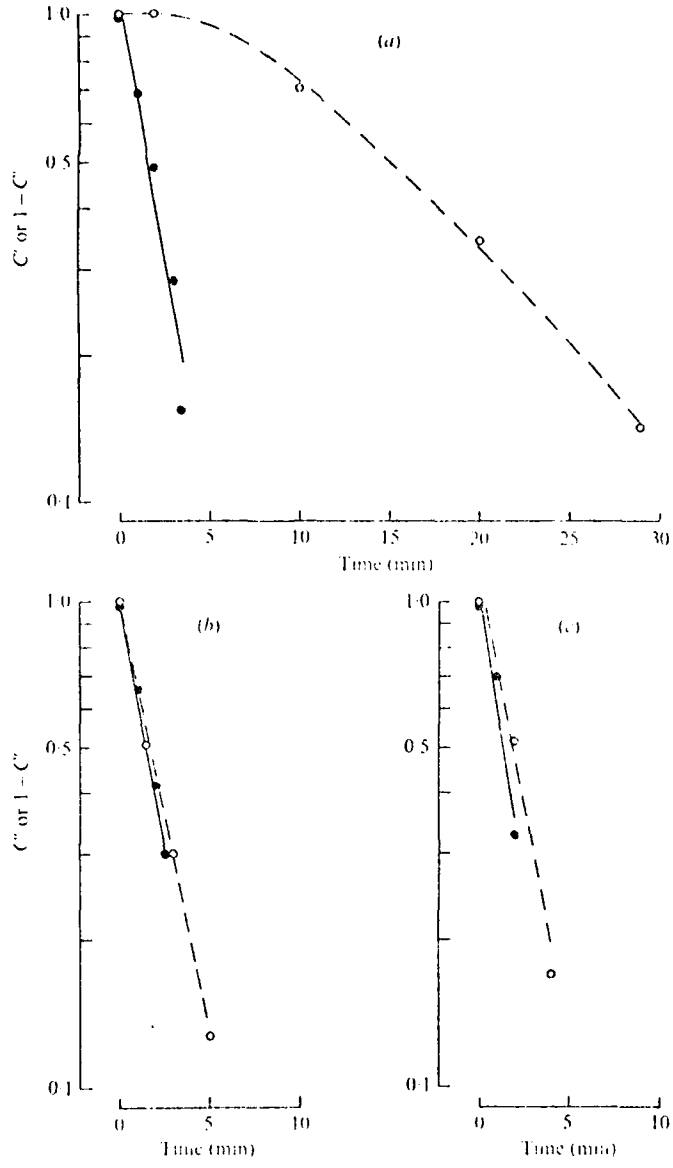

Fig. 9. . Changes in extra-axonal sodium concentration in an intact preparation, resulting from three successive exposures to sodium-free (sucrose-substituted) Ringer. 5 min in basic Ringer was allowed between exposures (*a*) and (*b*); 40 min between exposures (*b*) and (*c*). Estimation from a sucrose-gap recording: depletion of Na expressed as C″ values (○); recovery expressed as 1 − C″ values (●) to simplify comparison with depletion. Half-times for decline: (*b*), 98 s; (*c*), 113 s. Half-times for recovery: (*a*), 95 s; (*b*), 90 s; (*c*), 81 s. Lines fitted by linear regression on rectangular co-ordinates except for the broken line in (*a*) which was fitted by eye.

We have shown that initial exposure of connectives to sodium-deficient saline results in an extremely slow decline in amplitude of the axonal action potentials (Schofield & Treherne, 1978). Relatively rapid recovery of the action potentials occur on return to normal, high-sodium, saline (Fig. 9a). Subsequent exposure to sodium-deficient and normal saline becomes rapid and symmetrical (Fig. 9b). These observations cannot be accounted for by increased intercellular leakage of sodium ions for, as mentioned above, the action potentials in sodium-depleted preparations cannot be restored by prolonged exposure to lithium ions (a cation which can subtitute for sodium in carrying the inward current of the action potential).

The results shown in Fig. 9 can be explained according to our model by a postulated intracellular sodium reservoir which functions to maintain elevated extracellular sodium concentrations during prolonged exposure to sodium-deficient saline. This reservoir can be recharged only relatively slowly and, thus, when uncharged movements of sodium ions between the external medium and the axon surfaces are relatively rapid, and symmetrical, but are nevertheless slowed by a metabolic inhibitor, DNP, and a sodium-transport inhibitor, ethacrynic acid.

The postulated intracellular sodium reservoir is, according to the model, localized in the linked perineurial-glial cytoplasm, although it is possible to erect alternative localization, for example, in the axoplasm itself. Experiments are currently in progress to identify the proposed intracellular sodium reservoir. These experiments will involve the use of radiosodium localization in detergent-treated preparations, in non-electrolyte solution, and also, the use of sodium-selective microelectrodes.

We are currently using radiosodium to study the exchanges of this cation across the blood-brain interface (J.E. Treherne & P.K. Schofield). Our results confirm earlier observations (cf. Treherne, 1962; Pichon & Tucker, 1972) that rapid radiosodium fluxes occur between cockroach central nervous tissues and the bathing medium. ^{22}Na efflux occurs as a two-stage process with half-times of 50 and 1000 sec respectively. Extra-axonal sodium regulation is, therefore, a dynamic process which involves rapid ion fluxes across the blood-brain interface. These fluxes do not appear to involve significant sodium-sodium exchange as is known in some epithelial transport systems. Metabolic and sodium-transport inhibitors have little effect on the rapidly-exchanging component but slow the slowly-exchanging component of radiosodium efflux. Further experiments will be directed towards identification of the rapidly-exchanging sodium fraction.

Recent research has indicated an involvement of the aminergic system in ionic homeostasis in the cockroach nervous system. We have shown that externally-applied octopamine produces marked changes in extraneuronal potentials and also on the amplitude of the action potentials in preparations exposed to sodium-deficient salines and on the rate of axonal depolarization in connectives in high-potassium saline. Our preliminary observations suggest that octopamine may affect both the potassium permeability of the superficial neuroglia and linked sodium-potassium movements in the underlying glial elements.

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